The Relationship Between Caffeine Intake and Immunological and Virological Markers of HIV Disease Progression in Miami Adult Studies on HIV Cohort

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Abstract

Although there are many studies on adverse health effects of substance use and HIV disease progression, similar studies about caffeine consumption are few. In this study, we investigated the effects of caffeine on immunological and virological markers of HIV disease progression. A convenience sample of 130 clinically stable people living with HIV/AIDS on antiretroviral therapy (65 consuming ≤ 250 mg/day and 65 consuming >250 mg/day of caffeine) were recruited from the Miami Adult Studies on HIV (MASH) cohort. This study included a baseline and 3-month follow-up visit. Demographics, body composition measures, substance use, Modified Caffeine Consumption Questionnaire (MCCQ), and CD4 count and HIV viral load were obtained for all participants. Multivariable linear regression and Linear Mixed Models (LMMs) were used to understand the effect of caffeine consumption on CD4 count and HIV viral load. The mean age of the cohort was 47.9 ± 6.4 years, 60.8% were men and 75.4% were African Americans. All participants were on ART during both the visits. Mean caffeine intake at baseline was 337.6 ± 305.0 mg/day and did not change significantly at the 3-month follow-up visit. Multivariable linear regressions after adjustment for covariates showed significant association between caffeine consumption and higher CD4 count ($\beta = 1.532$, $p = 0.049$) and lower HIV viral load ($\beta = -1.067$, $p = 0.048$). LMM after adjustment for covariates showed that the relationship between caffeine and CD4 count ($\beta = 1.720$, $p = 0.042$) and HIV viral load ($\beta = -1.389$, $p = 0.033$) continued over time in a dose-response manner. Higher caffeine consumption was associated with higher CD4 cell counts and lower HIV viral loads indicating beneficial effects on HIV disease progression. Further studies examining biochemical effects of caffeine on CD4 cell counts and viral replication need to be done in the future.

Keywords: HIV/AIDS, caffeine, immunological markers, virological markers, HIV disease progression

Introduction

ANTIRETROVIRAL THERAPY (ART) HAS significantly improved survival rates and decreased morbidity and mortality in people living with HIV/AIDS (PLWH) (20). Due to recent improvements in availability and accessibility of combination ART medications, currently, HIV/AIDS constitutes a chronic disease with decreasing incidence, stable prevalence, as well as significantly decreasing disease pro-

gression rates (24). Irrespective of this laudable progress, many factors such as homelessness, commercial sex work, substance use, and stigmatization of the disease act as barriers to ART compliance and contribute to disease progression (5,11). Most of the PLWH live in marginalized neighborhoods with limited access to healthy and nutritious foods and supplements (27). Although the majority of the Miami Adult Studies on HIV (MASH) cohort, from which our participants were recruited, received social security benefits and some

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forms of food assistance, many of them experienced food insecurity (13). Food insecurity is associated with higher levels of substance use as well as caffeine consumption (32). These factors could adversely affect the health status of the PLWH (13). There is a large body of literature associating substance use with a variety of health-related outcomes in this population (28). However, there are very few studies that relate caffeine consumption to health-related outcomes. A study done among PLWH showed that CD4 cells were significantly lower in consumers of moderate amounts of caffeine (<250 mg/day) compared to nonconsumers (0 mg/day) (26). Similarly, another study showed that administration of safe levels of caffeine (<250 mg/day) decreased CD4 counts by 54% and CD8 counts by 55% compared to placebo (0 mg/day) (3). In contrast, two *in vitro* studies showed that caffeine suppressed replication of infectious HIV-1 strains in cultures of human immune cells (8,23). In a study done on human neuroblastoma cells, it was observed that caffeine significantly decreased the production as well as secreted levels of amyloid beta (A β) protein by neuronal cells (29). This study also showed that caffeine blocked HIV-1 Tat-induced increase in cellular levels of phosphorylated tau, thereby decreasing the levels of phosphorylated tau proteins in the neuronal cells. Thus, this study postulates that caffeine could be a valuable adjuvant in HIV-positive patients showing symptoms of Alzheimer's disease and HIV-1-associated neurocognitive disorders.

Studies done among HIV-seronegative populations have implicated caffeine use to a variety of health problems. High caffeine intake (>400 mg/day) adversely affected the quality of sleep and general well-being scores in healthy subjects, and precipitated anxiety disorders (10,22). High caffeine intake (480 mg/day) adversely affected gabaminergic (inhibitory) neurotransmission, exacerbated mental stress, and produced irreversible psychological derangements in participants receiving medications for anxiety disorders (15). Caffeine in doses of 300 mg/day was also associated with irregularities in blood pressure tracings and cardiac function tests (12). Excessive caffeine intakes were also associated with adverse effects on inflammatory markers. In a cross-sectional survey conducted in 3,042 participants, those who consumed more than 200 mg/day of caffeine had higher levels of interleukin 6, C-reactive protein, tumor necrosis factor- α , and white blood cell counts (35). Such adverse effects on inflammatory markers could be detrimental to disease progression. Caffeine (<250 mg/day) was associated with moderate weight loss in many groups of healthy volunteers (2). This could be detrimental to HIV-infected patients because lower body weight and lower fat mass are associated with faster disease progression (4).

Since studies have found associations between excessive caffeine consumption and many adverse effects in non-HIV-infected populations; it is possible that some of these effects may be more pronounced in HIV-infected subjects who are already compromised in a variety of health-related parameters. Furthermore, some studies show adverse associations between caffeine and immunological markers (CD4 counts), but potentially beneficial associations with virological markers (HIV viral load). Hence, the specific aim of this study was to investigate the net effects of caffeine consumption on immunological and virological measures associated with disease progression in a group of PLWH on stable ART.

Materials and Methods

Study design and setting

This study recruited a convenience sample of 130 participants (65 consuming $\leq$ 250 mg/day and 65 consuming >250 mg/day of caffeine) from the MASH cohort studies, which consisted of 803 PLWH, followed for more than 10 years. The recruitment for this study took place from February to July 2014. This study included a baseline and 3-month follow-up visits, and participants received \$5 as partial reimbursement for their time and efforts, for each of the two visits. The initial screening included detailed explanation of the study as well as obtaining informed voluntary written consent for participation. To obtain a study sample of 128 participants, as estimated in our sample size calculations, we screened a total of 150 subjects, enrolled 130 participants, and excluded 20 ineligible participants. The inclusion criteria for this study were as follows: (1) enrollment in the MASH cohort studies; (2) history of ART; and (3) willingness to participate in this study consisting of two visits. The exclusion criteria were as follows: (1) cardiovascular abnormalities or implanted pacemakers; and (2) morbid medical conditions such as uncontrolled hypertension, anemia, chronic inflammatory diseases, or malabsorption syndromes. The Institutional Review Board at Florida International University approved the study protocol and research procedures.

Measures

Demographic characteristics and substance use. Demographic and socioeconomic information used in this study were collected by the MASH cohort studies. Information used for this study included age, gender, ethnicity, education, and income levels. A number of substance use characteristics such as tobacco use, alcohol consumption, and illicit drug use were also gathered from the MASH data repository. All these variables were adjusted in our analysis based on studies associating these variables with HIV disease progression (32).

Caffeine consumption questionnaire. We used the Modified Caffeine Consumption Questionnaire (MCCQ) developed by Preston *et al.* in the year 1998 (25). This questionnaire is easy to understand, compact, organized, and reports adequate compliance, validity, and reliability. The questionnaire includes a total of 21 sources of caffeine classified in to three groups: beverages; over-the-counter medications; and prescription medications. This questionnaire also included "total caffeine consumption per day by adding these individual sources." For both naturally occurring and artificially added caffeine sources, FDA considers 400 mg/day to be generally safe and not associated with any dangerous side effects (31). The World Health Organization (WHO) defines caffeine overuse as daily consumption exceeding 500 mg/day (34). Beyond this level, caffeine produces visible adverse effects collectively known as "caffeinism." The World Health Organization (WHO) has also cautioned that these adverse effects could be precipitated at much lower doses depending on the physiological profiles of individuals as well as the genetic makeup of the population (34). The International Classification of

Diseases (ICD) manual considers caffeine consumption above 250 mg/day to be associated with many psychological and behavioral side effects, although it is inconclusive whether such effects would be uniformly applicable to the general population (9). The Diagnostic and Statistical Manual of Mental Disorders (DSM-5) considers consumption below 250 mg/day to be generally safe and above this level to be associated with many adverse effects such as withdrawal, addiction, irritability, and craving (1). Summarizing the lower cutoffs of these organizations, we concluded that caffeine consumption below 250 mg/day, irrespective of the sources, is generally safe and not associated with adverse effects. In this study, caffeine categories included low (≤ 250 mg/day, within safe levels) and high (> 250 mg/day, beyond safe levels) based on these recommendations.

CD4 counts and HIV viral load. The MASH cohort studies collected and recorded the laboratory reports for CD4 counts (FACSCanto II Flow Cytometer; BD Biosciences, San Jose, CA) and HIV viral load (RealTime HIV-1 PCR; Abbott Molecular, Des Plaines, IL) after appropriate medical release forms signed by the participants. In our analyses, CD4 counts and HIV viral load were considered continuous variables and transformed into CD4 square root and $\log_{10}$ HIV viral load to ensure normal distribution and better model fit criteria.

Bioimpedance analysis. In the MASH cohort studies, height was recorded to the nearest 0.5 inch using a stadiometer. Height was recorded only once to ensure that it was uniform and there were no minor variations during the follow-up visit. Weight was measured every visit to the nearest 0.1 lbs. using a standard weighing machine, which was calibrated and maintained throughout the MASH cohort studies. The data so obtained along with age were entered into the bioimpedance machine to estimate body cell mass, extracellular mass, lean body mass (LBM), fat mass, body mass index (BMI), and intracellular and extracellular fluid levels. In addition, we also obtained the waist-to-hip ratio using a nonstretchable tape to measure the waist and hip circumference following standard procedures. While estimating the effects of caffeine on immunological and virological markers of HIV disease progression, we adjusted for BMI and fat mass based on studies associating these variables with better immunological and virological profiles (16,18).

Statistical analysis

Field data were collected in paper-based questionnaires and later entered into RedCap database, imported into SPSS version 21 for Windows (IBM Corp, Armonk, NY), and merged into a single dataset for analysis. Descriptive statistics were used to understand the population characteristics and were expressed in terms of means, standard deviations, and percentages. The characteristics of the population were shown for all the participants and separated based on low and high intakes of caffeine. *T*-tests and chi-square tests were used to describe differences in demographic and substance use characteristics with respect to low and high levels of caffeine consumption. Paired sample *t*-tests were used to

measure differences in levels of caffeine consumption between baseline and the follow-up visit for major sources, as well as total levels of caffeine consumption. Multivariable linear regression analyses were used to estimate the strength of association between caffeine consumption and markers of HIV disease progression, CD4 counts and HIV viral load. Assumptions of linear regression were followed before the analyses. Linear mixed models (LMMs) were used to determine overtime changes in the strength of these associations. For parameter estimation in LMM models, Restricted Maximum Likelihood was used. For linear regression and LMM analyses, we initially used bivariate models and subsequently adjusted for associated covariates. These covariates were also tested for significant associations with the outcome variables using crude analysis before being incorporated into the models. To increase the validity of the models, multicollinearity among covariates was tested before the analyses. Statistical significance was set at $p < 0.05$ for all the analyses.

Results

Demographic and socioeconomic characteristics

A total of 130 participants were included in the study and 79 (60.8%) were men (Table 1). The average age was 47.9 ± 6.4 (mean $\pm$ SD) years. Most of the participants were African Americans (75.4%), Hispanics constituted 16.9%, Whites constituted 5.4%, and other races constituted 2.3%. About 24% of the participants had college education, while 45.4% were high school graduates and 30.8% had some form of primary education. All participants had incomes under the level of poverty and the mean income was $\$495.9 \pm \465.9 per month. There were no significant differences in any sociodemographic variable between low and high levels of caffeine consumption in both baseline and follow-up visits.

Substance use

Sixty-one percent of the participants reported smoking within the past 6 weeks of their scheduled baseline visits, 57.9% reported alcohol use, and 48.2% tested positive for illicit drug use. Similarly, 63.2% reported smoking, 60.3% reported alcohol use, and 65.8% tested positive for illicit drug use during the follow-up visit. There were no significant differences in substance use characteristics between baseline and follow-up visits.

Caffeine consumption

Total caffeine consumption was 337.6 ± 305.0 mg/day at baseline and 281.0 ± 260.9 mg/day at the 3-month follow-up. Coffee constituted the largest source of caffeine and mean consumption levels were 161.3 ± 195.0 mg/day at baseline and 190.4 ± 205.0 mg/day at follow-up. Caffeinated soft drinks constituted the second largest source of caffeine and mean consumption levels were 63.2 ± 93.0 mg/day at baseline and 29.7 ± 83.8 mg/day at follow-up. There were no significant differences between baseline and follow-up visits with respect to total caffeine consumption or caffeine from most of the sources. However, there was a significant decrease in consumption of caffeinated soft drinks (63.2 ± 93.0 vs. 29.7 ± 83.9 , $p < 0.001$), energy drinks (46.3 ± 134.4 vs.

TABLE 1. BASELINE DEMOGRAPHIC CHARACTERISTICS OF THE PARTICIPANTS (N=130)

Characteristics	Low caffeine intake (≤ 250 mg/day)	High caffeine intake (> 250 mg/day)	Total	p
Age in years (mean $\pm$ SD)	47.57 $\pm$ 6.81	48.00 $\pm$ 6.15	47.89 $\pm$ 6.37	0.706
Gender, n (%)				0.857
Male	40 (61.5)	39 (60.0)	79 (60.8)	
Female	25 (38.5)	26 (40.0)	51 (39.2)	
Ethnicity, n (%)				0.057
White	3 (4.6)	4 (6.2)	7 (5.4)	
Hispanic	7 (10.8)	15 (23.1)	22 (16.9)	
African Americans	55 (84.6)	43 (66.2)	98 (75.4)	
Other	0 (0.0)	3 (4.6)	3 (2.3)	
Education in years, n (%)				0.202
< 12	24 (36.9)	16 (24.6)	40 (30.8)	
12	29 (44.6)	30 (46.2)	59 (45.4)	
> 12	12 (18.5)	19 (29.2)	31 (23.8)	
Income (\$/month)	464.00 $\pm$ 419.67	527.34 $\pm$ 508.64	495.91 $\pm$ 465.89	0.442

p-Values correspond to χ^2 tests for categorical variables and t-test for continuous variables.

9.6 $\pm$ 65.3, $p=0.005$), and hot cocoa (1.0 $\pm$ 5.0 vs. 0.0 $\pm$ 0.0, $p=0.020$) across the baseline and follow-up visits (Table 2).

CD4 count and HIV viral load

The mean CD4 cell counts of the participants were 500.8 $\pm$ 297.5 cells/mm³ at baseline and 509.9 $\pm$ 299.4 cells/mm³ during follow-up visit ($p=0.739$). Among participants with low caffeine consumption, mean CD4 cell counts were 476.7 $\pm$ 285.7 cells/mm³ at baseline and 488.9 $\pm$ 296.3 cells/mm³ at follow-up ($p=0.071$). Among participants with high caffeine consumption, mean CD4 cell counts were 525.4 $\pm$ 309.6 cells/mm³ at baseline and 530.1 $\pm$ 303.6 cells/mm³ at follow-up ($p=0.064$). There were no significant differences between low versus high caffeine consumers with respect to CD4 cell counts, both during the baseline ($p=0.383$) and follow-up visits ($p=0.474$).

The mean HIV viral loads were 32324.3 $\pm$ 82965.1 copies/mL at baseline and 37395.4 $\pm$ 80785.7 copies/mL at follow-up ($p=0.754$). Among participants with low caffeine consumption, mean HIV viral loads were 34409.1 $\pm$ 71854.8 copies/mL at baseline and 64186.6 $\pm$ 332746.7 copies/mL at follow-up ($p=0.865$). Among participants with high caffeine consumption, mean HIV viral loads were 30239.4 $\pm$

93378.7 copies/mL at baseline and 10348.9 $\pm$ 23388.0 copies/mL at follow-up ($p=0.065$). There were no significant differences between low versus high caffeine consumers with respect to mean HIV viral loads, both during the baseline ($p=0.087$) and follow-up visits ($p=0.065$).

Association between caffeine consumption and immunological and virological markers of HIV disease progression

Crude linear regression analyses at baseline did not show any significant association between caffeine intake and CD4 cell count and log₁₀ HIV viral load. However, caffeine intake was significantly associated with CD4 cell count ($\beta=1.532$, $p=0.049$) and log₁₀ HIV viral load ($\beta=-1.067$, $p=0.048$) after adjusting for covariates (Table 3). Similarly, crude LMM did not show any significant changes in relationship between caffeine intake and CD4 cell count and log₁₀ HIV viral load overtime. However, LMM for overtime effects showed that caffeine consumption significantly predicted higher CD4 cell count ($\beta=1.720$, $p=0.042$) and lower log₁₀ HIV viral load ($\beta=-1.389$, $p=0.033$) in a dose-response manner, after adjusting for the covariates (Table 4).

TABLE 2. CAFFEINE INTAKE OF PARTICIPANTS AT BASELINE AND FOLLOW-UP VISIT (N=130)

Food source	Baseline (mean $\pm$ SD)	Follow-up (mean $\pm$ SD)	p
Coffee	161.26 $\pm$ 194.95	190.40 $\pm$ 205.03	0.206
Decaffeinated coffee	0.83 $\pm$ 9.21	0.03 $\pm$ 0.43	0.329
Espresso	14.40 $\pm$ 64.35	13.92 $\pm$ 57.82	0.942
Tea	34.70 $\pm$ 64.32	31.58 $\pm$ 59.36	0.640
Green tea	7.34 $\pm$ 62.93	4.20 $\pm$ 21.62	0.594
Energy drinks	46.32 $\pm$ 134.42	9.61 $\pm$ 65.32	0.005
Hot cocoa	1.03 $\pm$ 5.00	0.00 $\pm$ 0.00	0.020
Caffeinated soft drinks	63.23 $\pm$ 92.98	29.66 $\pm$ 83.78	< 0.001
Chocolate candy bars	3.69 $\pm$ 13.81	2.00 $\pm$ 17.93	0.402
Anacin	0.24 $\pm$ 2.80	0.00 $\pm$ 0.00	0.319
Appetite control pills	4.61 $\pm$ 39.10	0.00 $\pm$ 0.00	0.181
Total	337.63 $\pm$ 304.97	281.04 $\pm$ 260.85	0.890

TABLE 3. LINEAR REGRESSION ANALYSES OF THE EFFECT OF CAFFEINE CONSUMPTION ON IMMUNOLOGICAL AND VIROLOGICAL MARKERS OF DISEASE PROGRESSION (N=130)

Variables	Unadjusted			Adjusted ^a		
	Beta	Standard error	p	Beta	Standard error	p
CD4 square root	1.215	0.099	0.465	1.532	0.241	0.049
Log ₁₀ HIV viral load	-1.023	0.043	0.588	-1.067	0.081	0.048

^aModel adjusted for age, income, race/ethnicity, time from diagnosis of HIV, drug use, alcohol consumption, smoking status, BMI, and total body fat percentage.

BMI, body mass index.

Discussion

This study constitutes one of the few that investigated the association between caffeine intake and immunological and virological parameters of HIV disease progression. The demographic and substance use characteristics of our cohort were epidemiologically similar to those described by other studies among PLWH in Miami-Dade County (21,33). More than half of the participants were consuming alcohol and smoking cigarettes, and 48.2% used illicit drugs confirmed by urine toxicology. Therefore, we adjusted for these variables in the analyses to account for the powerful effects of substance use on disease progression variables.

Our study found that caffeine was associated with higher CD4 counts and lower HIV viral load, reflecting beneficial effects on HIV disease progression. The apparent beneficial effects of higher levels of caffeine consumption on HIV disease progression was unexpected, although there is some evidence from *in vitro* studies on caffeine decreasing the replication of the HIV viral strains in tissue cultures (8,23). In our study, only after adjusting for other relevant variables affecting HIV disease progression, caffeine consumption became significantly associated with both immunological and virological markers of disease progression. This suggests a complex and modest impact of caffeine on parameters of HIV disease progression. Our findings need to be confirmed by larger studies, as they could be incidental findings. Furthermore, there are very few studies that explore the relationship between caffeine and HIV disease progression, and there is lack of scientific consensus on this topic, probably due to the general acceptance of caffeine as a safe consumer product (30).

A study conducted by Raboud *et al.* among clinically stable HIV-infected patients showed significant reductions in CD4 counts among caffeine consumers compared to

nonconsumers, after adjusting for age, gender, medications, presence of opportunistic infections (oral candidiasis), and potential measurement errors (26). Thus the findings observed in this study were contrary to the results of our study. However, this study had several limitations and differences from our study. The study was conducted before the advent of combined ART, caffeine consumption was considered a categorical variable with subjects either reporting or not reporting caffeine consumption, and the study had a small sample size of 30 participants, thus limiting the validity of the results. Because of these shortcomings, this study could have missed the beneficial effects of caffeine on CD4 counts observed in our study. Furthermore, the findings in our study could be more valid because of the larger sample size and adjustments for several covariates that have significant confounding effects on immunological and virological parameters.

Bishop *et al.* conducted a study in clinically stable PLWH to establish the effects of caffeine consumption and exercise on CD4 and CD8 counts (3). The participants were asked to abstain from consumption of caffeine for 60 h before the experiment. Half of the subjects were administered 350 mg of caffeine, while the remaining subjects were administered placebo. There was 54% decrease in circulating CD4 counts and 55% decrease in circulating CD8 counts postexercise, in subjects administered caffeine compared to the placebo group, after adjusting for factors such as age, gender, BMI, LBM, and fluid input and output levels. This study also reports that postexercise plasma catecholamine levels were significantly higher in those administered caffeine when compared to placebo (3). This study hypothesized that caffeine produced alterations in lymphocyte subset trafficking as well as expression of CD69 molecules, which are important intermediate steps in generating and recruiting inflammatory cells. Through this process, caffeine decreased

TABLE 4. LINEAR MIXED MODEL ANALYSIS OF THE EFFECTS OF CAFFEINE CONSUMPTION ON IMMUNOLOGICAL AND VIROLOGICAL MARKERS OF DISEASE PROGRESSION (N=130)

Variables	Unadjusted			Adjusted ^a		
	Beta	Standard error	p	Beta	Standard error	p
CD4 square root ^b	0.900	0.301	0.402	1.720	0.901	0.042
Log ₁₀ HIV viral load ^c	-0.977	0.800	0.777	-1.389	0.786	0.033

^aModel adjusted for age, income, race/ethnicity, time from diagnosis of HIV, drug use, alcohol consumption, smoking status, BMI, and total body fat percentage.

^bModel 1: -2 Restricted Log Likelihood=1055.823; AIC=1059.823; Schwarz's Bayesian Information Criteria (BIC)=1065.910.

^cModel 2: -2 Restricted Log Likelihood=2597.274; AIC=2601.274; Schwarz's Bayesian Information Criteria (BIC)=2607.36.

AIC, Akaike's Information Criteria; BIC, Schwarz's Bayesian Information Criteria.

production and recruitment of inflammatory cells to the targeted areas of inflammation, which included decreased circulating CD4 and CD8 counts. Although the results of this study contradict our results, it is not clear whether they were produced by caffeine itself or the stress hormones such as catecholamines that were associated with exercise and caffeine consumption (17,19). Hence, the decrease in CD4 and CD8 counts observed in this study could be related to the increase in levels of stress hormones associated with caffeine intake and exercise rather than the biochemical effects of caffeine itself on immunological cells.

Our study found mostly beneficial effects of caffeine consumption. The beneficial findings in our study are supported by two *in vitro* studies. The first *in vitro* study showed that caffeine suppressed replication of infectious HIV-1 strains in cultures of human peripheral mononuclear cells (23). Caffeine in 100 mM concentrations exerted these effects by inhibiting the integration step of the HIV-1 viral replication cycle. This study also showed that other methylxanthines such as theophylline, theobromine, and paraxanthine in 100 mM doses act through the same processes to inhibit HIV-1 replication. They observed a ninefold reduction in HIV-1 p24 antigen values in samples treated with caffeine, theobromine, paraxanthine, or theophylline when compared to control solutions (23). The second *in vitro* study found that caffeine in concentrations of 10 µg/L significantly inhibited retroviral transduction of dividing human neuronal precursor (hNT-2) cells, thereby blocking postintegration repair of HIV-1 viral strains. These effects have been postulated to a cellular target, the ataxia telangiectasia mutated-Rad3-related (ATR) kinase (8). Caffeine inhibits viral DNA repair at the DNA damage-activated checkpoints, thereby inhibiting viral replication cycle (8). The CXCR4 and CCR5 receptors are utilized by HIV viral particles as entry coreceptors during their viral replication cycles and transmission of the virus to other CD4 cells (14). Stimulation of adenosine A2A receptor is associated with downregulation of chemokine receptors in HIV-infected CD4 cells (6). Caffeine is associated with upregulation in the expression of A2A receptors in a dose-dependent manner (7). Thus, caffeine's action on A2A receptors could be associated with decreased HIV replication due to its indirect action on CXCR4 and CCR5 receptors. Our study observed an inverse association between caffeine consumption and viral load, which could be related to the mechanisms postulated in these studies. Nevertheless, we should also note that tissue culture studies and *in vivo* studies on human beings are not comparable. Many confounders associated with the substance use and lifestyle factors could have influenced the findings in our study.

This study consisted of participants attending Borinquen Health Care Center (BHCC) for MASH cohort visits. They could be significantly different from the substance-using PLWH with limited access to healthcare resources, thereby compromising the external validity of our study. In addition, many unaccounted confounders beyond the ones identified and controlled in this study could have affected our results, thereby limiting the internal validity of our findings. Only experimental designs such as randomized controlled trials, which have complete control over the study variables due to a comparable control arm, can overcome such biases. In this study, there were two time points when the data were

gathered and carryover effects from the initial baseline visit could have affected the outcomes during the follow-up visit. The follow-up visit was at 3 months and only short-term effects of caffeine could be studied. Long-term studies regarding the effects of caffeine should be planned in the future. Furthermore, the data are self-reported, and participants were substance-using PLWH. This could have increased the chances of recall and social desirability biases, thereby affecting the findings of our study.

Large-scale experimental studies with controlled intake of nutrients, accurate assessment of health and substance use characteristics, and adequate sample size should be planned in the future to understand the biochemical mechanisms underlying the complex relationship between caffeine consumption and immunological and virological measures of HIV disease progression. This would be helpful in developing guidelines for safe levels of caffeine consumption for the PLWH population and thereby significantly contribute to their general well-being.

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Author Disclosure Statement

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References

1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders. 5th ed. Arlington, VA: American Psychiatric Association, 2013.
2. Balk L, Hoekstra T, Twisk J. Relationship between long-term coffee consumption and components of the metabolic syndrome: the Amsterdam Growth and Health Longitudinal Study. *Eur J Epidemiol* 2009;24:203–209.
3. Bishop NC, Fitzgerald C, Porter PJ, Scanlon GA, Smith AC. Effect of caffeine ingestion on lymphocyte counts and subset activation *in vivo* following strenuous cycling. *Eur J Appl Physiol* 2005;93:606–613.
4. Blashill AJ, Mayer KH, Crane HM, Grasso C, Safren SA. Body mass index, immune status, and virological control in HIV-infected men who have sex with men. *J Int Assoc Provid AIDS Care* 2013;12:319–324.
5. Brewer TH, Metsch LR, Zenilman JM. Use of a public sexually transmitted disease clinic by known HIV-positive adults: decreased self-reported risk behavior and increased disease incidence. *J Acquir Immune Defic Syndr* 2002; 29:289–294.
6. By Y, Durand-Gorde JM, Condo J, Lejeune P-J, Fenouillet E, Guieu R, *et al.* Monoclonal antibody-assisted stimulation of adenosine A 2A receptors induces simultaneous

- downregulation of CXCR4 and CCR5 on CD4+ T-cells. *Hum Immunol* 2010;71:1073–1076.
7. By Y, Durand-Gorde JM, Condo J, Lejeune PJ, Mallet B, Carayon P, *et al.* Production of an agonist-like monoclonal antibody to the human A 2A receptor of adenosine for clinical use. *Mol Immunol* 2009;46:400–405.
 8. Daniel R, Marusich E, Argyris E, Zhao RY, Skalka AM, Pomerantz RJ. Caffeine inhibits human immunodeficiency virus type 1 transduction of nondividing cells. *J Virol* 2005; 79:2058–2065.
 9. Department of Health and Human Services. Panel on clinical practices for treatment of HIV infection. Guidelines for the use of antiretroviral agents in HIV-infected adults and adolescents, January 28, 2000. *HIV Clin Trials* 2000;1: 60–110.
 10. Dreher HM. The effect of caffeine reduction on sleep quality and well-being in persons with HIV. *J Psychosom Res* 2003;54:191–198.
 11. Fernández MI, Bowen GS, Varga LM, *et al.* High rates of club drug use and risky sexual practices among Hispanic men who have sex with men in Miami, Florida. *Subst Use Misuse* 2005;40:1347–1362.
 12. Geleijnse JM. Habitual coffee consumption and blood pressure: an epidemiological perspective. *Vasc Health Risk Manag* 2008;4:963.
 13. Hatsu I, Huffman F, Johnson P, Baum M, Thomlison B, Campa A. Association of Supplemental Nutrition Assistance Program (SNAP) with food security and nutrition status among persons living with HIV. *Eur J Nutr Food Saf* 2015;5:229–241.
 14. He J, Chen Y, Farzan M, Choe H, Ohagen A, Gartner S, *et al.* CCR3 and CCR5 are co-receptors for HIV-1 infection of microglia. *Nature* 1997;385:645.
 15. Hedges DW, Woon FL, Hoopes SP. Caffeine-induced psychosis. *CNS Spectr* 2009;14:127–129.
 16. Kent SJ. Loss of control of HIV viremia associated with the fat malabsorption drug orlistat. *AIDS Res Hum Retroviruses* 2012;28:961–962.
 17. King SL, Hegadoren KM. Stress hormones: how do they measure up? *Biol Res Nurs* 2002;4:92–103.
 18. Langford SE, Ananworanich J, Cooper DA. Predictors of disease progression in HIV infection: a review. *AIDS Res Ther* 2007;4:11.
 19. Marketon JIW, Glaser R. Stress hormones and immune function. *Cell Immunol* 2008;252:16–26.
 20. May M, Boule A, Phiri S, *et al.* Prognosis of patients with HIV-1 infection starting antiretroviral therapy in sub-Saharan Africa: a collaborative analysis of scale-up programmes. *Lancet* 2010;376:449–457.
 21. Moss T, Martin CW, Klausner JD, Brown BJ. Integration of screening for syphilis, hepatitis C, and other sexually transmitted infections with HIV testing in a community-based HIV prevention program in Miami, Florida. *LGBT Health* 2014;1:82–85.
 22. Nardi AE, Lopes FL, Freire RC, *et al.* Panic disorder and social anxiety disorder subtypes in a caffeine challenge test. *Psychiatry Res* 2009;169:149–153.
 23. Nunnari G, Argyris E, Fang J, Mehlman KE, Pomerantz RJ, Daniel R. Inhibition of HIV-1 replication by caffeine and caffeine-related methylxanthines. *Virology* 2005;335: 177–184.
 24. Passaes CP, Sáez-Cirión A. HIV cure research: advances and prospects. *Virology* 2014;454:340–352.
 25. Preston JD, O'Neal JH, Talaga MC. *Consumer's Guide to Psychiatric Drugs*. 1998. Gretna, LA: New Harbinger Publications, Inc.
 26. Raboud J, Haley L, Montaner J, Murphy C, Januszewska M, Schechter M. Quantification of the variation due to laboratory and physiologic sources in CD4 lymphocyte counts of clinically stable HIV-infected individuals. *J Acquir Immune Defic Syndr Hum Retrovirol* 1995;10:S67.
 27. Rukmana D. Comparing the residential origins of homeless families and homeless individuals in Miami-Dade County, Florida. *AREA* 2011;43:96–109.
 28. Schneider M, Chersich M, Neuman M, Parry C. Alcohol consumption and HIV/AIDS: the neglected interface. *Addiction* 2012;107:1369–1371.
 29. Soliman ML, Geiger JD, Chen X. Caffeine blocks HIV-1 Tat-induced Amyloid beta production and tau phosphorylation. *J Neuroimmune Pharmacol* 2016:1–8.
 30. United States Food and Drug Administration. Database of Select Committee on GRAS Substances (SCOG) Reviews: Caffeine. 2006. Available at www.accessdata.fda.gov/scripts/cfn/fcnDetailNavigation.cfm?rpt=scogsListing&id=42 Accessed September 12, 2016.
 31. United States Food and Drug Administration. FDA to investigate added caffeine. Available at www.fda.gov/downloads/ForConsumers/ConsumerUpdates/UCM350740.pdf Accessed September 2, 2016.
 32. Wang EA, McGinnis KA, Fiellin DA, *et al.* Food insecurity is associated with poor virologic response among HIV-infected patients receiving antiretroviral medications. *J Gen Intern Med* 2011;26:1012–1018.
 33. Wawrzyniak AJ, Rodríguez AE, Falcon AE, *et al.* Association of individual and systemic barriers to optimal medical care in people living with HIV/AIDS in Miami-Dade County. *J Acquir Immune Defic Syndr* 2015;69:S63–S72.
 34. World Health Organization. Mental and behavioural disorders F15. In: *International Statistical Classification of Diseases and Related Health Problems*. 2010; Chapter V (Mental and behavioural disorders due to use of other stimulants, including caffeine). Geneva: World Health Organization.
 35. Zampelas A, Panagiotakos DB, Pitsavos C, Chrysoshoou C, Stefanadis C. Associations between coffee consumption and inflammatory markers in healthy persons: the ATTICA study. *Am J Clin Nutr* 2004;80:862–867.

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