

# Circulating $\beta$ -D-Glucan as a Marker of Subclinical Coronary Plaque in Antiretroviral Therapy-Treated People With Human Immunodeficiency Virus

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**Background.** Despite antiretroviral therapy (ART), people with human immunodeficiency virus (PWH) have increased risk of inflammatory comorbidities, including cardiovascular diseases. Gut epithelial damage, and translocation of bacterial lipopolysaccharide (LPS) or fungal  $\beta$ -D-glucan (BDG) drive inflammation in ART-treated PWH. In this study, we investigated whether markers of gut damage and microbial translocation were associated with cardiovascular risk in asymptomatic ART-treated PWH.

**Methods.** We cross-sectionally analyzed plasma from 93 ART-treated PWH and 52 uninfected controls older than 40 years of age from the Canadian HIV and Aging Cohort. Participants were cardiovascular disease free and underwent a cardiac computed tomography (CT) to measure total coronary atherosclerotic plaque volume (TPV). Levels of bacterial LPS and gut damage markers REG3 $\alpha$  and I-FABP were measured by enzyme-linked immunosorbent assay. Fungal BDG levels were analyzed using the Fungitell assay.

**Results.**  $\beta$ -D-glucan levels but not LPS were significantly elevated in ART-treated PWH with coronary artery plaque ( $P = .0007$ ). Moreover, BDG but not LPS levels correlated with TPV ( $r = 0.26$ ,  $P = .01$ ). Intestinal fatty acid binding protein (I-FABP) but not REG3 $\alpha$  levels correlated with TPV ( $r = 0.23$ ,  $P = .03$ ). However, BDG and LPS levels were not elevated in uninfected controls with plaque. In multivariable models, elevated BDG levels were independently associated with the presence of coronary atherosclerosis in PWH but not in uninfected controls.

**Conclusions.** Translocation of fungal BDG was associated with coronary atherosclerosis assessed by CT-scan imaging in ART-treated PWH, suggesting a human immunodeficiency virus-specific pathway leading to cardiovascular disease. Further investigation is needed to appraise causality of this association. Translocation of fungal products may represent a therapeutic target to prevent cardiovascular disease in ART-treated PWH.

**Keywords.** beta-d-glucan; coronary plaque; CT scan; HIV; microbial translocation.

Antiretroviral therapies (ARTs) have effectively transformed human immunodeficiency virus (HIV) infection into a treatable chronic condition. Antiretroviral therapy has halted the onset of acquired immunodeficiency syndrome (AIDS) in

people with HIV (PWH), reducing morbidity, prolonging survival, while preventing secondary transmission of the virus [1]. However, chronic inflammation and immune activation persist in PWH, leading to the development of non-AIDS comorbidities including cancers and bone, kidney, liver, neurological, and cardiovascular diseases (CVD) [2]. The risk of developing coronary heart disease is 1.5 to 2 times higher among PWH in comparison to age-matched uninfected individuals, with increased carotid artery wall stiffness, plaque, and cardiomyopathy prevalence [3, 4]. Aside from classic risk factors for CVD and coinfections with hepatitis viruses and cytomegalovirus (CMV), such non-AIDS comorbidities are associated with gut dysbiosis, epithelial gut damage, and subsequent microbial translocation [5, 6].

Gut dysbiosis has been linked to increased activation of both CD4<sup>+</sup> and CD8<sup>+</sup> T cells, combined with neutrophil infiltration

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in the sigmoid in association with damages to the gut-mucosa barrier [7, 8]. After HIV infection, enterocytes undergo apoptosis resulting in a loss of intestinal integrity, allowing microbial translocation of bacterial and fungal products into the bloodstream [9]. Damage of the gut epithelial barrier can be assessed in PWH by circulating markers including intestinal fatty acid binding protein (I-FABP), expressed by enterocytes, and regenerating islet-derived protein 3 $\alpha$  (REG3 $\alpha$ ), an antimicrobial peptide secreted by Paneth cells [10–12].

The translocation of microbial products into circulation further contributes to systemic immune activation and the onset of non-AIDS comorbidities in ART-treated PWH [13, 14]. Microbial translocation was first described by quantifying levels of the bacterial lipopolysaccharide (LPS) in blood circulation [15, 16]. We and others have shown that plasma levels of a fungal cell wall component (1 $\rightarrow$ 3)- $\beta$ -D-glucan (BDG) were elevated in PWH and associated with inflammation markers and CD4 and CD8 T-cell activation, independently of age, sex, and CD4 and CD8 T-cell counts [17–20]. Furthermore, BDG levels were elevated in PWH independently of the duration of the infection and persisted on ART [17]. In this study, we cross-sectionally quantified markers of gut damage and microbial translocation to determine whether they were associated with subclinical CVD in asymptomatic ART-treated PWH.

## METHODS

### Study Design and Population

A total of 145 participants, 93 ART-treated participants and 52 uninfected controls from the cardiovascular imaging substudy of the Canadian HIV and Aging Cohort Study (CHACS), were included in a transversal study. The study design and protocol of the CHACS study have been reported [21]. In brief, (1) PWH over the age of 40 or having lived with HIV for at least 15 years and (2) HIV-negative controls over the age of 40 were recruited in HIV and sexual transmitted disease clinics, the majority of their population being men who have sex with men. Participants from the control group have been recruited at the same medical centers as the PWH participants. The CHACS participants who were free of overt cardiovascular disease (had never suffered a myocardial infarction, coronary revascularisation, angina, stroke or peripheral vascular revascularization) and had a 10-year Framingham risk score ranging from 5% to 20% were invited to participate to the cardiovascular imaging substudy. Data on all classic cardiovascular risk factors were collected prospectively as part of CHACS study visits.

### Cardiovascular Imaging

In all participants, a 256-slice computed tomography (CT) scanner (Brilliance iCT; Philips Healthcare, Best, The Netherlands) was used to perform noncontrast cardiac CT and

coronary computed tomography angiography (CCTA). The following parameters were used for noncontrast CT: slice thickness 2.5 mm, matrix 512  $\times$  512, field-of-view 250 mm, scan voltage 120 kV, and prospective electrocardiographic gating. Patients were given (1) 50–75 mg of metoprolol orally 45–60 minutes before CCTA if heart rate was >60 beats per minute and (2) 0.4 mg of nitroglycerin sublingually, in absence of contraindications. For coronary CCTA, contrast agent was injected at a flow rate of 5 mL/second, using 370 mg/mL iopamidol (Bracco Imaging, Milan, Italy). All images were reconstructed using a hybrid iterative reconstruction algorithm (Philips iDose, Philips Healthcare, level 3).

Coronary artery calcium (CAC) measurement was performed using Agatston method [22] on noncontrast CT. Coronary plaque analysis was performed using CCTA images as previously described by Chen et al [23]. The coronary segments were defined as reported in the American College of Cardiology/American Heart Association guidelines for coronary angiography [24]. Plaque volumetric analysis was performed in multiplanar reformat (MPR), using the aforementioned semi-automated software. First, proximal and distal plaque boundaries were traced by manual segmentation. Then, the software allowed for semiautomatic delimitations between lumen, vessel wall, and plaque followed by, if required, manual adjustment. Care was taken to exclude surrounding epicardial fat from coronary plaque segmentation. Plaque composition was assessed using attenuation-stratified measurements in the plaque volume:  $\leq$ 30 Hounsfield units (HU), 31–50 HU, 51–100 HU, 101–150 HU, 151–350 HU, and >350 HU.

Total plaque volume (TPV) per participant was defined as the sum of aforementioned attenuation-stratified measurements. Total low-attenuation plaque volume (LAPV) was defined as the sum of all plaque components with  $\leq$ 30 HU. A TPV > 0 mm<sup>3</sup> was defined as presence of subclinical cardiovascular disease (TPV<sup>+</sup>), and a TPV of 0 was defined as absence of subclinical cardiovascular disease (TPV<sup>-</sup>).

All imaging studies were performed at the Centre Hospitalier de l'Université de Montréal, Quebec, Canada, and interpreted by a board-certified cardiothoracic radiologist (C.C.-L.). All radiology personnel performing image interpretation and postprocessing were blinded to HIV status.

### Laboratory Measurements

Human immunodeficiency virus infection was diagnosed by quantifying HIV-1 p24 antigen/antibody in plasma and confirmed by Western blot as previously reported [25]. Plasma viral load was quantified by the Abbott RealTime HIV-1 assay (Abbott Laboratories). Plasma samples of study participants were stored at  $-80^{\circ}\text{C}$  until used. CD4 and CD8 T-cell counts were measured using 4-color flow cytometry [26]. Peripheral blood mononuclear cell samples of study participants were stored in liquid nitrogen until used.

### Measurement of Markers of Epithelial Gut Damage and Microbial Translocation

Levels of I-FABP, REG3 $\alpha$ , and LPS were measured by enzyme-linked immunosorbent assay (ELISA) as previously described [11]. Plasma BDG was measured by the Fungitell assay in duplicate as per the manufacturer's instructions with lower extended range (as low as 3.9 pg/mL) (Associates of Cape Cod, Inc., East Falmouth, MA).

### Statistical Analyses

Medians with interquartile range were calculated for all variables. Unpaired comparisons were conducted using *t* tests or Mann-Whitney *U* tests. Spearman rank correlation test identified associations between 2 quantitative measures. The Kruskal-Wallis test was used to compare more than 2 study groups.  $P < .05$  were considered to be significant. To control for potential confounding by classic cardiovascular risk factors on the association between BDG and coronary atherosclerosis, we performed a multivariable logistic regression analysis. To assess for effect modification by HIV on the association between BDG and coronary atherosclerosis, we added an interaction term between HIV status and BDG. The significance of the interaction term was obtained by a likelihood ratio test. In case of a significant interaction ( $P < .05$ ), we presented stratum-specific results. Total plaque volume was dichotomized as absent (TPV = 0) or present (TPV = 1). Classic cardiovascular risk factors (age, smoking, statin use as a measure of dyslipidemia, hypertension, and body mass index [BMI]) were considered as potential confounders of the association and were entered into the model sequentially and kept into the model if they modified the association between BDG and TVP by more than 10%. Although considered as potential confounders, sex and diabetes were not included into the final model due to small cell issues and absence of association with the outcome in our data. GraphPad Prism 8.0 (GraphPad Software) and R-software were used to perform descriptive statistical analyses.

### Patient Consent Statement

This study was approved by the Centre Hospitalier de l'Université de Montréal and by McGill University Health Centre research ethics board as well as by community medical centers, all in Montréal. Study participants provided written informed consent for study enrollment and participation. All matters were conducted in accordance with the principals of the Declaration of Helsinki.

## RESULTS

### Study Participant Characteristics

The median ages of HIV<sup>+</sup> participants ( $n = 93$ ) and uninfected controls ( $n = 52$ ) were 55.6 (interquartile range [IQR], 52.2–60.8) and 55.0 (IQR, 49.1–62.2) years, respectively. Eighty-six percent of the total population were males. Clinical characteristics are depicted in Table 1. Diabetes and statin use were

significantly more frequent in HIV<sup>+</sup> participants. High waist circumferences (over 102 cm in men, and 88 cm in women) were observed in 29% of HIV<sup>+</sup> participants and 36.5% of uninfected controls, respectively. The HIV<sup>+</sup> participants had a median CD4 T-cell count (cells/ $\mu$ L), CD8 T-cell count (cells/ $\mu$ L), and CD4/CD8 ratio of 594 (IQR, 446–735), 710 (IQR, 511–1023), and 0.86 (IQR, 0.56–1.14), respectively. All HIV<sup>+</sup> participants who were taking ART for a median of 15 years (12–18) had a suppressed viremia below 50 copies/mL. Plasma levels of I-FABP and REG3 $\alpha$ , but not LPS nor BDG, were significantly elevated in PWH compared with uninfected controls.

### Plasma Levels of $\beta$ -D-Glucan, But not Lipopolysaccharide, Were Elevated in People With Human Immunodeficiency Virus With Subclinical Cardiovascular Disease

As aforementioned, subclinical CVD was defined by the presence of atherosclerotic plaque in the coronary arteries whereby TPV  $> 0 \text{ mm}^3$  (TPV<sup>+</sup>). Cross-sectional analysis demonstrated significantly elevated plasma levels of BDG (21 [IQR, 14–29.5] vs 14 [IQR, 9–19.5] pg/mL;  $P = .0007$ ) (Figure 1A), but not LPS (55.46 [IQR, 38.1–86.1] vs 60.8 [IQR, 45.1–89.4] pg/mL) (Figure 1C) in ART-treated PWH with TPV<sup>+</sup> in comparison to those without the presence of atherosclerotic coronary plaque (TPV<sup>-</sup>). In contrast, plasma levels of both BDG (15.0 [IQR, 13.0–32.25] vs 16.5 [IQR, 10–26.8] pg/mL) (Figure 1B) and LPS (48.2 [IQR, 33.6–77.4] vs 57.2 [IQR, 30.2–78.0] pg/mL) (Figure 1D) were not different in TPV<sup>+</sup> and TPV<sup>-</sup> groups of uninfected controls ( $P = .87$  and  $P = .64$ , respectively).

### Plasma Levels of $\beta$ -D-Glucan, But Not Lipopolysaccharide, Correlated With Total Coronary Plaque Volume, Low-Attenuation Plaque Volume, and Coronary Artery Calcium Levels in People With Human Immunodeficiency Virus

Plasma levels of BDG, but not LPS, correlated with TPV ( $r = 0.26$ ,  $P = .01$  and  $r = -0.08$ ,  $P = .44$ , respectively) (Figure 2A, C) and LAPV ( $r = 0.26$ ,  $P = .01$  and  $r = -0.28$ ,  $P = .47$ , respectively) (Supplemental Figure 1) in ART-treated PWH. Likewise, CAC levels correlated with plasma levels of BDG, but not LPS (data not shown;  $r = 0.34$ ,  $P = .001$  and  $r = -.05$ ,  $P = .59$ ). Elevated BDG levels among PWH were independent of age, sex, BMI, diabetes, hypertension, and waist circumference and did not correlate with plasma levels of glucose, total cholesterol, HDL, LDL, triglycerides, CMV seropositivity, CD4 T-cell count, CD8 T-cell count, or CD4:CD8 ratio. In contrast, plasma levels of both BDG and LPS were not associated with TPV nor LAPV (Figure 2B, D and Supplementary Figure 1) nor CAC (data not shown) in uninfected controls.

### Plasma Levels of Intestinal Fatty Acid Binding Protein Correlated With Total Coronary Plaque Volume and Low-Attenuation Plaque Volume in People With Human Immunodeficiency Virus

No significant difference in both I-FABP (1504 [IQR, 888–2065] vs 1155 [IQR, 749–1676] pg/mL) and REG3 $\alpha$  (2572 [IQR, 1978–3856] vs 2156 [IQR, 1555–3136] pg/mL) plasma levels were observed in between TPV<sup>+</sup> and TPV<sup>-</sup> groups of ART-treated PWH or uninfected controls (Supplementary Figure 2).

**Table 1. Characteristics of Study Participants (N = 145)**

Characteristics	PWH (N = 93)	UC (N = 52)	P Value
Age, years			
Median (IQR)	55.6 (52.2–60.8)	55.0 (49.1–62.2)	.30
Range	44.1–73.8	39.2–74.5	
Sex, no. (%)			
Male	84 (90%)	40 (77%)	.05
Female	9 (10%)	12 (23%)	
Diabetes	9 (10%)	0 (0%)	.03
Hypertension	33 (35%)	12 (23%)	.17
Statin use	32 (34%)	5 (10%)	.002
High waist circumference	27 (29%)	19 (36.5%)	.39
Smoking Status			
Current smoker	23 (25%)	5 (10%)	
Former smoker	43 (46%)	19 (36%)	
Never smoked	26 (28%)	28 (54%)	
N/A	1 (1%)		
CD4 T-cells/ $\mu$ L			
Median (IQR)	594 (446–735)	N/A	
Range	117–1840	N/A	
CD8 T Cells/ $\mu$ L			
Median (IQR)	710.4 (511–1003)	N/A	
Range	145–2146.2	N/A	
CD4/CD8			
Median (IQR)	0.86 (0.56–1.14)	N/A	
Range	0.20–2.30	N/A	
Viral load, log <sub>10</sub> copies/mL	<1.7	N/A	
Presence of coronary artery plaque	68 (68%)	30 (57%)	.09
Presence of calcified plaque	45 (67%)	24 (54%)	.93
Plasma BDG (pg/mL)			
Median (IQR)	18 (13–25)	15.5 (10.5–26.8)	.80
Plasma LPS (pg/mL)			
Median (IQR)	56.7 (39.2–86.3)	52.0 (33.0–73.8)	.33
Plasma REG3 $\alpha$ (pg/mL)			
Median (IQR)	2680 (2027–4306)	2059 (1504–2581)	.0003
Plasma I-FABP (pg/mL)			
Median (IQR)	1581 (967–2081)	1010 (735–1614)	.002

Abbreviations: BDG, (1 $\rightarrow$ 3)- $\beta$ -D-glucan; HIV<sup>+</sup>, people with human immunodeficiency virus; I-FABP, intestinal fatty acid binding protein; IQR, interquartile range; LPS, lipopolysaccharide; N/A, not applicable or not available; REG3 $\alpha$ , regenerating islet-derived protein 3 $\alpha$ ; UC, uninfected controls.

NOTE: Mann-Whitney's test.

Plasma levels of gut damage marker I-FABP correlated with TPV ( $r = 0.23$ ,  $P = .03$ ) (Figure 3C) and LAPV ( $r = 0.27$ ,  $P = .01$ ) (Supplementary Figure 2) in ART-treated PWH. Such correlations were not observed in uninfected controls (Figure 3D; Supplementary Figure 2). Plasma levels of I-FABP also correlated with BMI, total cholesterol, and REG3 $\alpha$  levels (data not shown). In contrast, REG3 $\alpha$  did not correlate with TPV nor LAPV in ART-treated PWH (Figure 3A). However, REG3 $\alpha$  correlated with TPV ( $r = 0.30$ ,  $P = .04$ ) (Figure 3B) and LAPV ( $r = 0.32$ ,  $P = .03$ ) (Supplementary Figure 2) in uninfected controls.

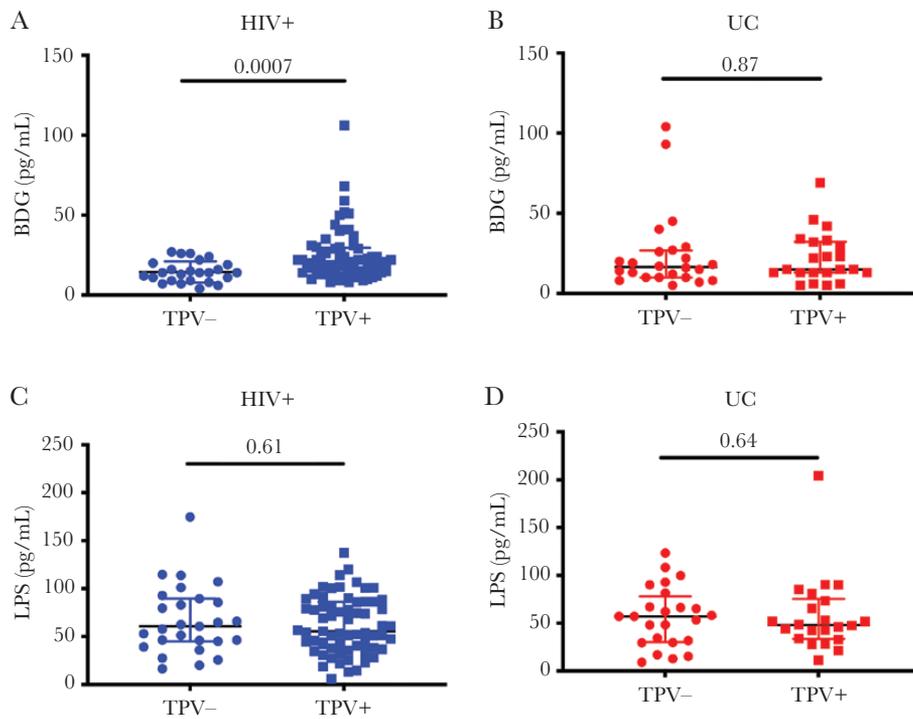
#### **$\beta$ -D-Glucan Is Associated With Total Coronary Plaque Volume Independently of Classic Cardiovascular Risk Factors in People With Human Immunodeficiency Virus**

In the complete population of HIV<sup>+</sup> and uninfected participants, after adjusting for classic cardiovascular risk factors,

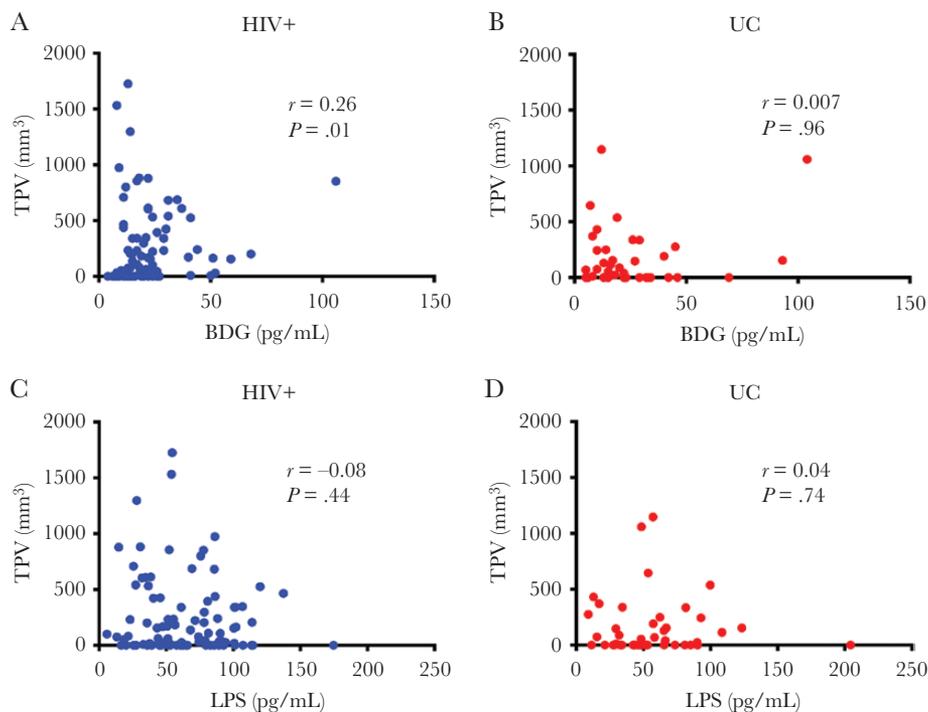
each 10-unit increase in plasma BDG level was associated to a nonsignificant odds ratio (OR) of 1.22 (95% confidence interval [CI], 0.93–1.69) for the presence of coronary artery atherosclerosis. However, there was evidence of effect modification by HIV status ( $P$  value for interaction 0.012). In PWH, each increase of 10 units in BDG was associated to an increased OR for coronary atherosclerosis of 1.11 (95% CI, 1.04–1.21;  $P = .01$ ). However, in uninfected controls, no evidence of an association between BDG and coronary atherosclerosis was observed (OR, 0.98; 95% CI, 0.94–1.02;  $P = .26$ ), but smoking was associated with coronary plaque (OR, 1.08; 95% CI, 1.02–1.017;  $P = .028$ ) (Table 2).

#### **DISCUSSION**

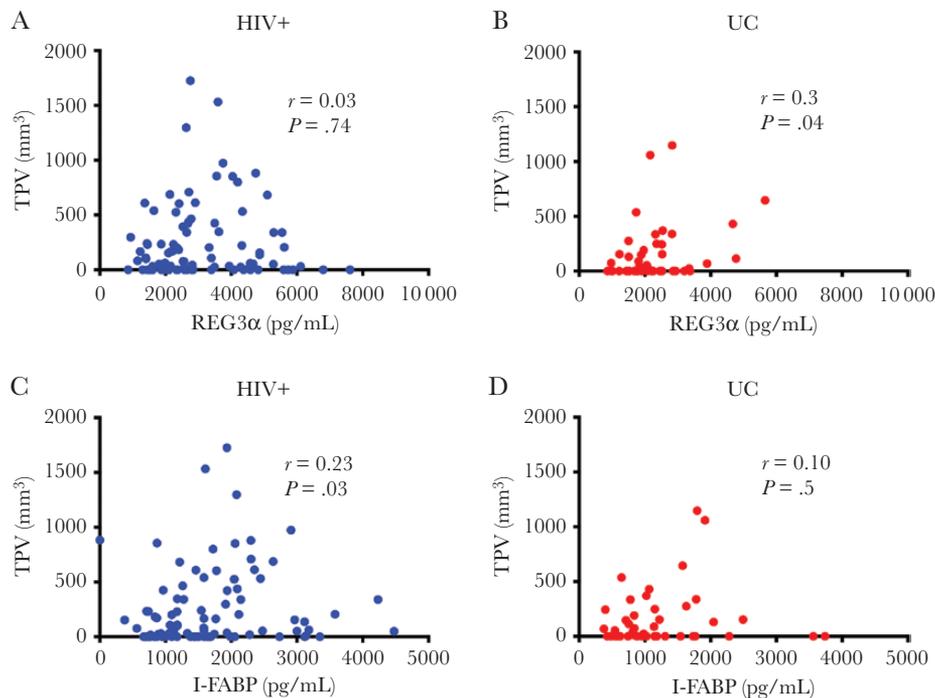
We showed that plasma levels of the fungal translocation marker BDG, but not levels of bacterial translocation marker LPS, were



**Figure 1.** Cross-sectional plasma levels of (1→3)-β-D-glucan (BDG) and the presence atherosclerotic plaque in the coronary arteries in people with human immunodeficiency virus (PWH) and uninfected controls (UC). Total plaque volume (TPV)<sup>+</sup> denotes the presence of any coronary plaque (>0). (A) Plasma BDG levels were significantly elevated in antiretroviral therapy-treated PWH with subclinical cardiovascular disease (TPV<sup>+</sup>) in comparison to those without atherosclerotic coronary plaque (n = 93). (B) Plasma BDG levels were not elevated among TPV<sup>+</sup> and TPV<sup>-</sup> groups of UCs (n = 52). (C) Plasma lipopolysaccharide (LPS) levels were not elevated among TPV<sup>+</sup> and TPV<sup>-</sup> groups of PWH (n = 93). (D) Plasma LPS levels were not elevated among TPV<sup>+</sup> and TPV<sup>-</sup> groups of UCs (n = 52). Mann-Whitney tests. HIV<sup>+</sup>, people with HIV.



**Figure 2.** Comparison of (1→3)-β-D-glucan (BDG) and lipopolysaccharide (LPS) plasma levels with total plaque volume (TPV) assessed by computed tomography scan. (A) Plasma BDG levels correlated with TPV in people with human immunodeficiency virus (PWH) (n = 93). (B) Plasma BDG levels did not correlate with TPV in uninfected controls (UCs) (n = 52). (C) Plasma LPS levels did not correlate with TPV in PWH (n = 93). (D) Plasma LPS levels did not correlate with TPV in UCs (n = 52). Spearman's tests. Abbreviations: HIV<sup>+</sup>, people with HIV.



**Figure 3.** Comparison of regenerating islet-derived protein 3 $\alpha$  (REG3 $\alpha$ ) and intestinal fatty acid binding protein (I-FABP) plasma levels with total plaque volume (TPV) assessed by computed tomography scan. (A) Plasma REG3 $\alpha$  levels did not correlate with TPV in people with human immunodeficiency virus (PWH) (n = 93). (B) Plasma REG3 $\alpha$  levels correlated with TPV in uninfected controls (UCs) (n = 52). (C) Plasma I-FABP levels correlated with TPV in PWH (n = 93). (D) Plasma I-FABP levels did not correlate with TPV in UCs (n = 52). Spearman's test. Abbreviations: HIV<sup>+</sup>, people with HIV.

elevated in ART-treated PWH with subclinical coronary atherosclerotic plaque compared with those without detectable plaque. We observed associations between plasma BDG levels and TPV in ART-treated PWH compared with uninfected controls. Classic cardiovascular risk factors such as statin use and smoking habit were not associated with TPV in this population. Gut damage marker I-FABP was also associated with TPV in ART-treated PWH, implying that the increased translocation of fungal products into systemic circulation may be due

to extensive damage at the gut epithelium related to subclinical CVD/coronary artery plaque.

Other markers of CVD such as carotid artery plaque have been previously found to be elevated in PWH and associated with increased mortality in those who do not present with symptoms of cardiovascular diseases [21, 27–29]. However, this is the first report on a relationship between subclinical coronary artery plaque and the translocation of BDG in PWH. It is interesting to note that the commonly used marker of microbial

**Table 2. Multivariable Logistic Regression Analysis for the Association of BDG With Coronary Plaque**

Parameter	All Participants N = 145		HIV <sup>+</sup> Participants N = 93		HIV <sup>-</sup> Participants N = 52	
	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
BDG <sup>a</sup>	1.22 (0.93–1.69)	.196	<b>1.11 (1.04–1.21)</b>	<b>.010</b>	0.98 (0.94–1.02)	.261
HIV	1.06 (0.45–2.47)	.894	-	-	-	-
Age	1.05 (0.99–1.11)	.128	1.08 (0.98–1.20)	.139	1.07 (0.99–1.17)	.090
Hypertension	1.73 (0.66–4.73)	.273	0.58 (0.15–2.04)	.400	-	-
Smoking <sup>b</sup>	<b>1.04 (1.01–1.07)</b>	<b>.007</b>	1.03 (1.00–1.06)	.075	<b>1.08 (1.02–1.17)</b>	<b>.028</b>
Statin use	2.48 (0.86–8.34)	.110	3.48 (0.98–5.08)	.068	-	-
BMI	0.98 (0.90–1.07)	.674	0.91 (0.80–1.04)	.168	1.16 (1.00–1.39)	.076

Abbreviations: BDG, (1→3)- $\beta$ -D-glucan; BMI, body mass index; CI, confidence interval; HIV<sup>+</sup>, people with human immunodeficiency virus; OR, odds ratio.

NOTE: Interaction term between HIV and BDG,  $P = .012$ . A parsimonious method was used to build the multivariable model. Age and smoking were kept into the model a priori. Classic risk factors (age, smoking, statin use as a measure of dyslipidemia, diabetes, hypertension and BMI >30) were entered into the model sequentially and kept into the model if they modified the association between BDG and TYP by more than 10%.  $P$  value was deemed significant when <5% as indicated in bold.

<sup>a</sup>OR per 10-unit increase in BDG.

<sup>b</sup>OR per 1 pack-year increase in smoking.

translocation LPS was not elevated in groups with coronary artery plaque compared with those without and was neither correlated with TPV, LAPV, nor CAC. The methodological challenges in measuring LPS levels, lack of precision, association with serum lipid levels, and cross-reactivity with BDG by limulus amebocyte lysate assays have questioned its role as a reliable marker of microbial translocation [18, 30]. We have showed that time of collection mitigates LPS plasma levels in PWH because those levels displayed a daily variation over 24 hours, whereas BDG did not significantly vary during the same period of time [31]. Furthermore, we have previously shown that glucan-rich food and time of food intake do not influence BDG plasma levels in HIV- and hepatitis C virus-infected patients, indicating a gut fungal origin [31, 32]. In contrast, circulating LPS has been shown to be rapidly detoxified in the liver and spleen, possibly explaining the absence of association between LPS and CVD [18, 33].

Epithelial gut damage has been shown to lead to subsequent microbial translocation and elevated systemic plasma levels of microbial products, such as LPS [16]. Furthermore, it has been demonstrated that microbial translocation further contributes to gut dysbiosis creating a vicious negative feedback loop. It has yet to be elucidated whether circulating plasma BDG originates from the gut; however, our team has demonstrated that plasma BDG correlated with plasma LPS, I-FABP, and REG3 $\alpha$  levels [11, 17]. These observations are consistent with demonstrated gut translocation of fungal BDG in an alcohol-induced model of liver disease [34]. Herein, low levels of gut damage and microbial translocation markers were observed in this long-term ART-treated population selected for low risk of chronic diseases, fitting previous observations [35–37]. In addition, we have further demonstrated an association with I-FABP and markers of CVD alongside plasma BDG levels speculating that the fungal product originates from the gut. More than 90% of healthy individuals have *Saccharomyces* and 60% *Candida* colonization in the gastrointestinal (GI) tract [38]. Considering the increased prevalence of gut dysbiosis in PWH and already prevalent GI-tract colonization of fungal species, increased frequency of *Saccharomyces* and *Candida* may account for the elevated plasma BDG levels noted in this aging population of PWH [18]. These findings are in line with previous works in which plasma BDG levels were shown to be associated with non-AIDS comorbidities [35]. Furthermore, elevated levels of microbial products have been associated with increased risk of CVD whereby elevated plasma BDG levels were associated with cardiopulmonary dysfunction, although elevation of BDG in PWH was mostly observed in untreated viremic participants [20].

We acknowledge the limitations of the cross-sectional nature of this study and the one-time CT scan assessing subclinical CVD. Despite accounting for a variety of confounding factors such as smoking, alcohol consumption, and drug use,

many participants presented with hypertension, statin use, and smoking habit, which could account for increased incidence of subclinical CVD. However, these characteristics are typical of this aging demographic and fit the aim of this study. Furthermore, multivariable analysis showed that BDG but not statin use nor smoking habits was associated with TPV in PWH. Influence of sex and diabetes will have to be assessed in larger studies. In addition, the study participants were free of cardiovascular/coronary artery disease at baseline and deemed to have low or intermediate cardiovascular risk. In addition, an ELISA was used to measure LPS plasma levels to avoid any cross-reactivity with plasma BDG. For BDG, the Fungitell kit has been extensively documented with both plasma and serum measurements making a bias selection of sample improbable.

Our finding of an effect modification by HIV status for the association between BDG and the presence of coronary atherosclerosis is of great interest. Increased BDG is associated with subclinical CVD only in PWH and not in uninfected controls, suggesting that mechanistic pathways leading to CVD in PWH are distinct from those in uninfected people and may be related to persistent microbial translocation-induced inflammation. The origin of the elevated BDG plasma levels noted in the subclinical CVD ART-treated group will have to be determined, and further studies assessing and characterizing gut damage and microbial translocation are needed to determine the influence of the translocation of such fungal products [39]. Such findings should be validated in larger cohorts of PWH and uninfected controls.

## CONCLUSIONS

The shift in ART and HIV care has allowed for sustained viral suppression and a better quality of life among PWH; however, chronic immune activation and inflammation contribute greatly to the onset of non-AIDS comorbidities including CVD [40]. Elevated BDG levels have been shown to be (1) associated with cardiopulmonary dysfunction despite being linked with increased viremia and low CD4 T-cell count and (2) a key independent predictor of non-AIDS comorbidities in PWH and not uninfected controls [20, 35]. Globally our study findings suggest a relationship between gut damage, subsequent translocation of BDG, and presence of coronary artery atherosclerosis in PWH. More research is needed to appraise the causality of the association; however, determining the implications of elevated BDG levels may represent novel diagnostic and therapeutic avenues to control the onset of cardiovascular disease in ART-treated PWH.

## Supplementary Data

Supplementary materials are available at Open Forum Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility

of the authors, so questions or comments should be addressed to the corresponding author.

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