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MAJOR ARTICLE

Associations Between the Gut Microbiome, Inflammation, and Cardiovascular Profiles in People With Human Immunodeficiency Virus

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Background. Inflammation and innate immune activation are associated with chronic human immunodeficiency virus (HIV) infection, despite effective treatment. Although gut microbiota alterations are linked to systemic inflammation, their relationship with HIV infection the relationships between the gut microbiome, inflammation, and HIV remains unclear.

Methods. The HIV UPBEAT Coronary Artery Disease sub-study evaluated cardiovascular disease (CVD) in people with and without HIV. Subclinical CVD was assessed using coronary computed tomography angiography (CCTA). Thirty-four biomarkers were measured using quantitative immunoassays. Stool samples underwent 16S rRNA sequencing. Differentially abundant species were identified by analysis of compositions of microbiomes with bias correction (ANCOM-BC) and correlated to biomarkers, diet, and CCTA outcomes using Spearman correlation.

Results. Among 81 participants (median age, 51 years; 73% male), people with HIV (n = 44) had higher rates of hypercholesterolemia ($P < .025$). Gut microbiome β-diversity differed significantly by HIV status. Enriched *Bifidobacterium pseudocatenulatum*, *Megamonas hypermegale*, and *Selenomonas ruminantium* correlated with lower plaque burden, while depleted *Ruminococcus bromii* correlated with higher plaque burden and fat intake. Depleted *Bacteroides* spp and *Alistepes* spp correlated with elevated biomarkers (D-dimer, CD40 ligand, C-reactive protein, and interferon-γ).

Conclusions. Gut microbiota differences in people with HIV were linked to subclinical CVD, diet, and inflammation, highlighting the microbiome's role in cardiovascular risk in HIV infection.

Keywords. HIV; cardiovascular disease; inflammation; microbiome; ageing.

Since the introduction of antiretroviral therapy (ART), many people with human immunodeficiency virus (HIV) now achieve a life expectancy approaching that of the general population [1]. However, some experience an accentuated aging phenotype, with increased risk of developing age-related noncommunicable diseases, such as cardiovascular disease (CVD), at a younger age than those without HIV [2]. CVD is now a leading cause of death in those receiving ART [1], with traditional CVD risk factors, although prevalent in this group, not fully accounting for this excess risk [3]. Initially, prolonged ART exposure was suspected, but recent data suggest its impact on CVD is relatively small [4].

Unresolved chronic inflammation is a major contributor to CVD pathogenesis, involving endothelial activation, vascular inflammation, and type 1 T-helper (Th1) cell activation [5], all implicated in coronary artery plaque (CAP) development [5]. Innate immune activation and systemic inflammation are also associated with subclinical coronary artery disease (CAD) in those with HIV, with markers like high-sensitivity C-reactive protein (hsCRP) and interleukin 6 predicting worse outcomes [6].

HIV infection has been associated with gut dysbiosis due to alteration of the gut epithelial barrier and depletion of $CD4^+$ T cells in the gut-associated lymphoid tissue [7]. This leads to changes in gut microbial diversity and community compositions. Moreover, commensal bacteria crucial for a healthy gut are replaced by taxa that may contribute to chronic inflammation and immune dysfunction [8]. Important commensal bacteria include short chain fatty acid (SCFA)–producing organisms, which produce butyrate, propionate, and acetate as metabolic

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products through the fermentation of dietary fibers and other indigestible carbohydrates [9]. *Firmicutes* and *Bacteroides* are the main butyrate-producing phyla [9]. Depletion of butyrate-producing bacteria has been associated with CVD, including atherosclerosis and dyslipidemia [10, 11].

In the context of HIV, decreased abundance of SCFA-producing bacteria has been associated with lower $CD4^+$ T-cell counts and immune activation [12]. A reduction of butyrate-producing bacteria in particular has been observed in people with HIV with metabolic disorders such as hyperglycemia, hypertension, and dyslipidemia [13]. HIV infection also increases microbial translocation (MT), with movement of bacteria (or bacterial products) from the gastrointestinal tract into the systemic circulation, driving inflammation [14, 15]. Markers used for MT include lipopolysaccharide, soluble C14 (sCD14), and intestinal fatty acid–binding protein (I-FABP), a marker of enterocyte damage. Reductions in butyrate-producing bacteria have been associated with MT as well as immune activation and vascular inflammation in people with HIV [15, 16].

A previous study of this cohort identified distinct biomarker-derived inflammatory patterns associated with both subclinical CAD and prevalent CVD events [17]. One particular inflammatory pattern displayed higher markers of gut epithelial dysfunction (I-FABP), T-cell stimulation, and systemic inflammation and was distinguished from the other clusters by being significantly associated with higher CAP and prevalent clinical CVD, independent of HIV status [17]. Similarly, in the Pharmacokinetic and Clinical Observations in People Over Fifty (POPPY) study, the systemically inflamed/gut epithelial dysfunction group showed higher estimated CVD risk [18]. These studies suggest a distinct inflammatory profile in persons with HIV that is associated with a high clinical CVD risk, with biomarkers linked to gut dysfunction implicated.

Despite these significant findings, the potential role of the gut microbiome on influencing this high-risk inflammatory phenotype remains unclear. Understanding the role of the gut microbiome in host inflammatory responses in HIV infection could provide valuable insights into the pathogenesis of premature aging and help identify individuals at higher risk of noncommunicable diseases like CVD, aiding earlier therapeutic or preventive strategies to reduce CVD risk. Our study aim was to investigate the interactions between host gut microbiome compositions and systemic inflammatory responses and to correlate these with subclinical CVD outcomes.

MATERIALS AND METHODS

Study Cohort

The Understanding the Pathology of Comorbid Disease in HIV-Infected Individuals Coronary Artery Disease (HIV UPBEAT CAD) study is a substudy of the HIV UPBEAT study, a single-center, prospective, observational cohort that enrolled people with and without HIV from similar demographic backgrounds. Participants with HIV were recruited from the infectious diseases clinics at the Mater Misericordiae University Hospital, Dublin, Ireland, whereas people without HIV were recruited from the local geographical area. Participants were included if they were >40 years old, with no known history of CVD. Those with and without HIV were propensity score matched for traditional CVD risk factors to controls, ensuring an even distribution of estimated CVD risk across both groups.

Participants underwent coronary computed tomography angiography (CCTA) to assess subclinical CAD, as described previously [17]. Participants also provided clinical/medical history, in addition to stool samples and fasting bloods, from which serum and plasma were derived and stored at −80°C. The study was approved by the Mater Misericordiae University Hospital and Mater Private Hospital Institutional Review Board and all participants provided written informed consent.

Nucleic Acid Purification and 16S rRNA Amplification

DNA was extracted from thawed stool samples using the repeated bead-beating Qiagen DNA extraction method as previously described [19]. A part of the 16S rRNA gene was amplified from isolated DNA using primers for the V3–V4 region according to the Illumina 16S Metagenomic Sequencing Protocol. Library DNA concentration was quantified using a Qubit fluorometer (Invitrogen) using the high-sensitivity assay, and samples were pooled at a standardized concentration. The uniquely barcoded amplicons were sequenced on an Illumina MiSeq platform utilizing 2 × 300 bp chemistry.

Processing and Quality Control

Resulting sequence data were quality-filtered using TrimGalore v0.6.7 [20]. Read de-replication, learning of the error rates, and sample sequence variant inference with pooled samples followed by the construction of amplicon sequence variant (ASV) table and removal of chimaeras were performed using Lotus2 pipeline (with dada2 clustering) at the ASV level for α-diversity analysis [21]. To ensure fair comparison across samples, we applied rarefaction, randomly subsampling reads to a uniform depth across all samples for α-diversity estimation, thereby controlling for differences in sequencing depth. Annotate of the 16S sequences at the species level was constructed using SPINGO [22]. In addition, we also applied a quality filtering step removing rare species from the analysis, keeping those present in 10% or more of samples. Absolute counts were transformed to relative abundance to normalize the data and focus on the proportional representation of taxa across samples.

Measurement of Inflammatory Biomarkers

The biomarkers analyzed in this study have been previously assessed using quantitative immunoassays as previously described (Supplementary Table 1) [17].

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Collection of Dietary Data

Dietary data were collected using a semi-quantitative food frequency questionnaire (FFQ) weighted by 10 consumptive frequencies according to the method used by Claesson et al [23]. Participants were asked to recall dietary intakes, including nutritional supplements, over the previous 4 weeks to provide an average daily nutritional intake. FFQ coding, data cleaning, and data checks were conducted by a single, trained individual to ensure consistency of data. The Healthy Food Diversity (HFD) index is based on dietary proportions derived from FFQ data and associated health values from nutritional guidelines [23]. It considers diversity of food in conjunction with using a weighted health value to circumnavigate many of the traditional problems of measures of dietary diversity. The HFD provides a score between 0 and 1, where a higher value indicates a more diverse diet.

Statistical Analysis

For clinical factors, continuous and categorical variables were summarized using median with interquartile range (IQR) and frequency (%), respectively. Categorical and continuous variables were compared using χ^2 and Kruskal-Wallis or Wilcoxon rank-sum tests, respectively.

Kruskal-Wallis and Mann-Whitney tests were used to explore significant differences in clinical and biochemical measures and α-diversity (Chao1 and Shannon indices). The permutational multivariate analysis of variance (PERMANOVA) was performed with Bray-Curtis dissimilarity, Jaccard dissimilarity, and binary Jaccard dissimilarity at the species and genus level, respectively, through vegan adonis2 function. The covariates of sex, age, comorbidities, and medications (antihypertensives, statins, and ART) were included in the model, and the marginal sums of squares was applied to avoid issues related to the order of factors in adonis2. To identify differentially abundant species and genera between people with and those without HIV, we used analysis of compositions of microbiomes with bias correction (ANCOM-BC; version 2.2.0) [24]. ANCOM-BC incorporates a centered log-ratio transformation to address the compositional nature of microbiome data and reports bias-corrected log fold changes in abundance between groups, adjusting for covariates (ie, sex, age, comorbidities, and the use of statins and ART). The *P* values were adjusted using the Benjamini-Hochberg procedure for multiple hypothesis testing [25]. We used nonparametric Spearman correlation to test associations between biomarkers, the CCTA outcome data, and the dietary data with the differential abundance of the identified microbiome species and genera. Prior to formation of the correlation matrix, the biomarkers were log-transformed (for approximate normality) and scaled to ensure that biomarkers with larger intrinsic variation did not dominate the correlation matrix.

Table 1. Baseline Demographics and Coronary Artery Disease (CAD) Risk Factors of Participants of the HIV UPBEAT CAD Study, Stratified by Human Immunodeficiency Virus Status

Characteristic	People Without HIV (n = 37)	People With HIV (n = 44)	*P* Value[a]
Age, y, median (IQR)	50 (46–56)	50 (45–57)	.67
Sex			.63
Female	11 (30)	11 (25)	
Male	26 (70)	33 (75)	
Race/ethnicity			>.99
African	8 (22)	10 (23)	
Asian	1 (2.7)	2 (4.5)	
Caucasian	28 (76)	32 (73)	
Smoking history			.98
Current smoker	7 (19)	9 (20)	
Former smoker	11 (30)	13 (30)	
Never smoker	19 (51)	22 (50)	
Statin use	4 (11)	22 (50)	<.001
History of hypertension	7 (19)	16 (36)	.083
Diabetes	1 (2.7)	3 (6.8)	.62
High cholesterol (TC level in mmol/L)	11 (30)	24 (55)	.025
BMI, kg/m^2, median (IQR)	27.2 (25.0–29.3)	29.4 (24.5–32.2)	.38
ART duration, y, median (IQR)	…	9.5 (7.2–13.9)	

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: ART, antiretroviral therapy; BMI, body mass index; HIV, human immunodeficiency virus; IQR, interquartile range; TC, total cholesterol.

[a]Wilcoxon rank-sum test, Pearson χ^2 test, Fisher exact test, or Wilcoxon rank-sum exact test.

RESULTS

Clinical Characteristics of the Study Population

Eighty-one participants with available stool samples were included in this analysis, of whom 44 were people with HIV and 37 were people without HIV (Table 1). The median age was 51 (IQR, 46–56) years, 73% were male, and 74% were Caucasian. Although estimated CVD risk was similar between the 2 groups (as a result of the propensity score matching), those with HIV had a higher prevalence of hypercholesterolemia ($P < .025$) and were more likely to be on statins ($P < .001$).

Microbiome Diversity in People With and Without HIV

While there was no significant difference in α-diversity at the species and genus level (using Chao1 and Shannon indices) between people with and without HIV (Figure 1), a significant separation in β-diversity was observed between the 2 groups, using the Bray-Curtis dissimilarity (PERMANOVA, $P < .001$, $R^2 = 0.034$) and Jaccard dissimilarity (PERMANOVA, $P < .001$, $R^2 = 0.027$). ANCOM-BC differential abundance analysis identified 42 species (Figure 2 and Supplementary Figure 1) and 10 genera (Supplementary Figure 2) that were significantly enriched or depleted in people with HIV compared to people without HIV. In this group, *Olsenella uli* was the most significantly enriched of these species, followed by *Anaerovibrio*

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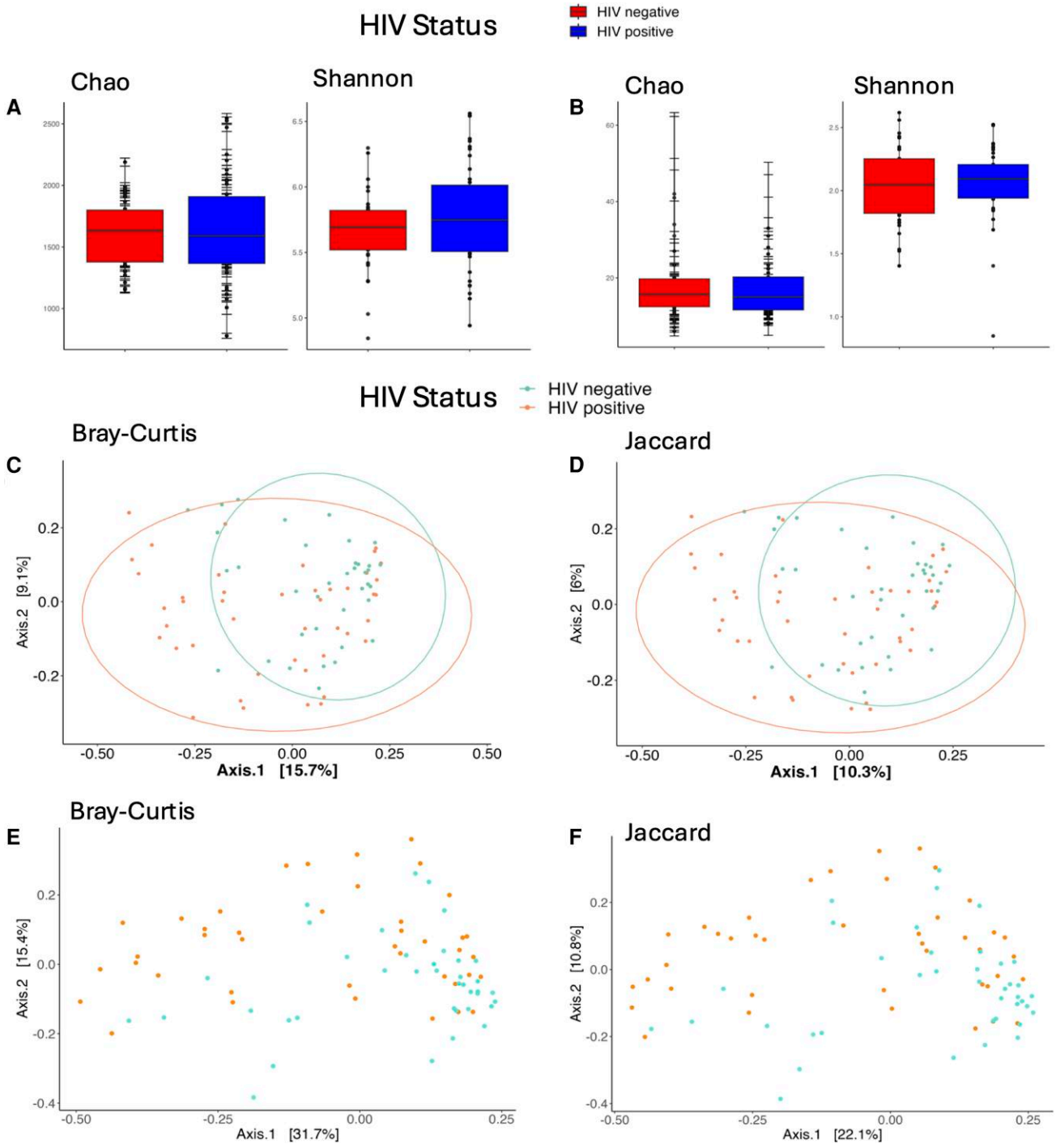


Figure 1. Alpha-diversity at the amplicon sequence variant level for species (*A*) and genus (*B*) for people with and without human immunodeficiency virus (HIV). Beta-diversity with Bray-Curtis dissimilarity and Jaccard similarity at the species level (*C* and *D*) and genus level (*E* and *F*), respectively. Permutational multivariate analysis of variance was performed, and the effect size for Axis 1 in the β-diversity results was interpreted using the 95% confidence interval.

lipolyticus and *Slackia exigua*, respectively. The most significantly depleted species, primarily SCFA producers, were *Odoribacter splanchnicus*, followed by *Alistipes shahii*, *Bacteroides uniformis*, and *Barnesiella intestinihominis*. *Barnesiella intestinihominis* is a taxa that has been positively associated with healthy aging, and its reduced abundance has been linked to an increased burden of cerebral small vessel disease [26]. *Olsenella* was the most significantly enriched genus, followed by *Megasphaera*, *Mitsuokella*, and *Flavonifractor*, respectively. The most significantly depleted genera were *Bacteroides*, followed by *Faecalibacterium* and *Fusicatenibacter*.

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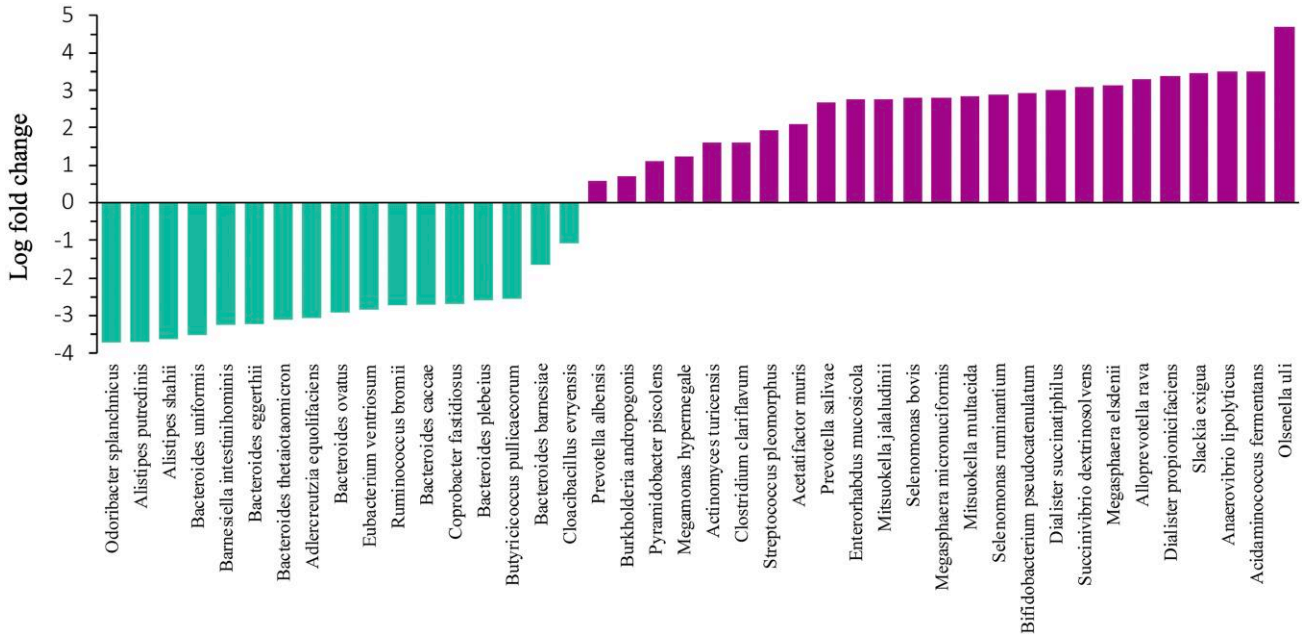


Figure 2. Differential abundance (DA) analysis of 42 species by in people with human immunodeficiency virus (HIV) compared to those without HIV. DA was assessed by analysis of compositions of microbiomes with bias correction while adjusting for the covariates based on filtered species abundance (present in at least 10% of samples and with maximum reads ≥10). False discovery rate <0.1 was considered as DA species.

Comparison of Microbiome Taxa With Subclinical Cardiovascular Disease

Overall, 30 (37%) participants had evidence of subclinical CAD on CCTA. Focusing on Agatston score and CAP burden as measures of subclinical CAD, CAP burden was similar in both groups, but those without HIV were more likely to be on a statin (n = 22 vs n = 4, $P < .001$) and have an Agatston score >100 (n = 9 vs n = 3, $P < .05$) (Table 2). In people with HIV, an increased abundance of the bacterium *Bifidobacterium pseudocatenulatum*, a potentially probiotic microbe that is associated with SCFA production and bile acid metabolism, correlated with a lower Agatston score and overall CAP burden (Supplementary Figure 3) [27]. Similarly, an increased abundance of *Megamonas hypermegale* and *Selenomonas ruminantium*, both SCFA-producing bacteria involved in carbohydrate fermentation, was linked to a lower CAP burden in people with HIV. These findings suggest a role of SCFA-producing bacteria in modulating inflammation and maintaining cardiovascular health. No significant associations at the genus level were associated with Agatston score or CAP burden (Supplementary Figure 4).

Dietary Data

Dietary data were collected from 81 participants (Table 3). There was no significant difference in daily nutrient intake or the HFD index between the groups with and without HIV. However, dietary patterns correlated with several microbiome changes relevant to CVD risk. For example, higher intake of saturated and trans-unsaturated fatty acids was associated with an enrichment of *Ruminococcus bromii*, a SCFA producer, and depletion of key gut commensals such as *Pyramidobacter piscolens* and *Bacteroides* spp (Figure 3). Additionally, higher intake of sugars and calories correlated with depletion of SCFA-producing *Bacteroides* spp and *Alistipes* spp (Supplementary Figure 5). These important gut commensals are involved in the breakdown of complex dietary fibers and polysaccharides, contributing to digestion. Importantly, the observed depletion of SCFA producers is consistent with findings from the CCTA analysis, further emphasizing their potential role in modulating cardiovascular outcomes.

Associations Between Microbial and Inflammatory Biomarkers

Building on the links between diet, microbiota, and cardiovascular outcomes, we explored associations between microbial taxa and 38 inflammatory biomarkers. Previous work within this cohort identified a distinct inflammatory phenotype defined by elevated circulating levels of I-FABP, T-cell stimulation, and systemic inflammation markers that were associated with subclinical CAD and prevalent CVD [17]. In this study, we found an increased abundance of several SCFA bacteria, including *Prevotella salivae*, *Megasphaera elsdenii*, *Enterorhabdus mucosicola*, and *Succinivibrio dextrinosolvens*, that correlated with elevated circulating I-FABP levels (Figure 4). Similarly, levels of interleukin 18 (IL-18), a potent proinflammatory cytokine that facilitates type 1 inflammatory responses [28], were positively associated with increased abundance of several gram-negative microbes (*P salivae*, *Prevotella albensis*, *M hypermegale*, *Burkholderia andropogonis*) and lipid-metabolizing bacteria, such as *A lipolyticus*. An overabundance of lipid-metabolizing bacteria may contribute

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Table 2. Subclinical Coronary Artery Disease (CAD) as Measured by Coronary Computed Tomography Angiography in HIV UPBEAT CAD Substudy Participants

Characteristic	HIV Negative (n = 37)	People With HIV (n = 44)	*P* Value[a]
History of statin use	4 (11)	22 (50)	<.001
Any coronary plaque	16 (43)	14 (32)	.29
Calcified plaque	15 (41)	13 (30)	.30
Noncalcified plaque	3 (8.1)	6 (14)	.50
Partially calcified plaque	9 (24)	8 (18)	.50
Worst stenosis			.30
<25% stenosis	4 (11)	7 (16)	
26%–50% stenosis	9 (24)	4 (9.1)	
>50% stenosis	3 (8.1)	3 (6.8)	
No significant stenosis	21 (57)	30 (68)	
Agatston score, median (IQR)	0.00 (0.00–4.20)	0.00 (0.00–2.10)	.12
Agatston score >100	9 (24)	3 (6.8)	.027
Calcium volume score, median (IQR)	0.00 (0.00–3.73)	0.00 (0.00–1.87)	.15
Calcium volume score >100	6 (16)	2 (4.5)	.13

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: HIV, human immunodeficiency virus; IQR, interquartile range.

[a]Pearson χ^2 test, Fisher exact test, or Wilcoxon rank-sum test.

Table 3. Median Daily Dietary Nutritional Intake and Healthy Food Diversity Index in People With and Without Human Immunodeficiency Virus

Characteristic	People Without HIV (n = 35)	People With HIV (n = 41)	*P* Value[a]
Energy, kcal	7.75 (7.42–7.98)	7.71 (7.52–8.05)	.66
Carbohydrates, g	5.28 (5.05–5.82)	5.51 (5.11–5.94)	.24
Sugars, g	4.63 (4.15–4.88)	4.71 (4.31–5.14)	.14
Fiber, g	3.34 (2.90–3.63)	3.33 (3.07–3.66)	.51
Saturated fatty acids, g	3.37 (3.09–3.52)	3.48 (3.15–3.80)	.36
Polyunsaturated fatty acids, g	2.53 (2.18–2.81)	2.51 (2.38–3.01)	.32
Trans-saturated fatty acids, g	0.28 (0.09–0.39)	0.36 (0.01–0.65)	.43
Cholesterol, mg	5.80 (5.40–6.05)	5.75 (5.54–6.08)	>.99
Protein, g	4.85 (4.53–5.18)	4.58 (4.45–4.95)	.15
Alcohol, g	2.18 (1.38–2.55)	1.38 (0.00–2.60)	.18
Caffeine, mg	7.16 (4.79–9.42)	6.12 (5.47–7.09)	.17
HFD index	0.49 (0.46–0.51)	0.49 (0.47–0.52)	.58

Data are presented as median (interquartile range) unless otherwise indicated.

Abbreviations: HFD, Healthy Food Diversity; HIV, human immunodeficiency virus.

[a]Wilcoxon rank-sum exact test, Wilcoxon rank-sum test, Pearson χ^2 test, or Fisher exact test.

to an imbalance in lipid metabolism, potentially leading to adverse metabolic outcomes [29].

In people with HIV, a depletion of SCFA-producing bacterial species, including *Bacteroides* spp, *Alistipes* spp, and *R bromii* (Figure 4), as well as the genus *Succinivibrio* (Supplementary Figure 6), correlated with upregulated inflammatory markers (hsCRP, D-dimer, CD40 ligand, interferon-γ, and lipopolysaccharide-binding protein [LBP]) and endothelial dysfunction markers (Vascular cell adhesion protein 1 [VCAM-1], E-selectin). These inflammatory patterns align with the microbiota associations seen in the CCTA and dietary findings, further connecting changes in the gut microbiome with cardiovascular risk.

DISCUSSION

This study describes the differences in fecal microbiota compositions associated with CVD risk in people with HIV. We identified decreased abundance of SCFA-producing bacteria, linked to subclinical CVD findings on CCTA, elevated inflammatory biomarkers, and higher dietary intake of fatty acids and sugars. Additionally, an increased abundance of proinflammatory bacteria correlated with elevated levels of I-FABP and IL-18, markers previously associated with CVD in this cohort [17]. While the cross-sectional design precludes causal inference, these findings suggest that the gut microbiome may influence inflammation and CVD risk in HIV.

Compared to people without HIV, an enrichment of *B pseudocatenulatum* was associated with a lower CAP burden and Agatston score in people with HIV, indicating a more favorable cardiovascular profile. Conversely, in this group, we found an increased abundance of bacteria known to be associated with CVD, including *Megasphaera micronuciformis*, *Streptococcus* spp, and *Prevotella* spp [15]. Additionally, in people with HIV, a depletion of *Bacteroides plebeius*, *Bacteroides barnesiae*, *Alistipes* spp, the butyrate-producing *Eubacterium ventriosum*, and the keystone species *R bromii* was associated with greater CAP burden and elevated Agatston scores. Although these are not the typical major butyrate producers, these other SCFA-producing microbes may have a role to play in CVD outcomes. Previous studies have shown that SCFA-producing microbes may influence immune regulation and can exert positive effects on blood pressure, CAD, and atherosclerosis [30]. The depletion of SCFA-producing organisms has been associated with atherosclerosis [9] and heart failure [31] in the general population.

A reduction of SCFA microbes may lead to increased systemic inflammation through multiple interconnected mechanisms. SCFAs play a key role in maintaining intestinal barrier integrity by serving as an energy source for colonocytes and promoting tight junction function [32]. Reduced SCFA production compromises this barrier, leading to increased gut permeability and MT [33]. Furthermore, SCFAs modulate inflammation through anti-inflammatory pathways, including the activation of regulatory T cells and inhibition of histone deacetylases, both of which help suppress excessive immune responses [32].

While studies investigating the role of SCFA-producing microbes in relation to CVD outcomes in people with HIV are limited, a reduction in production of a key SCFA, butyrate, has been found to be associated with diabetes in women with HIV [34]. Similarly, reductions in butyrate-producing

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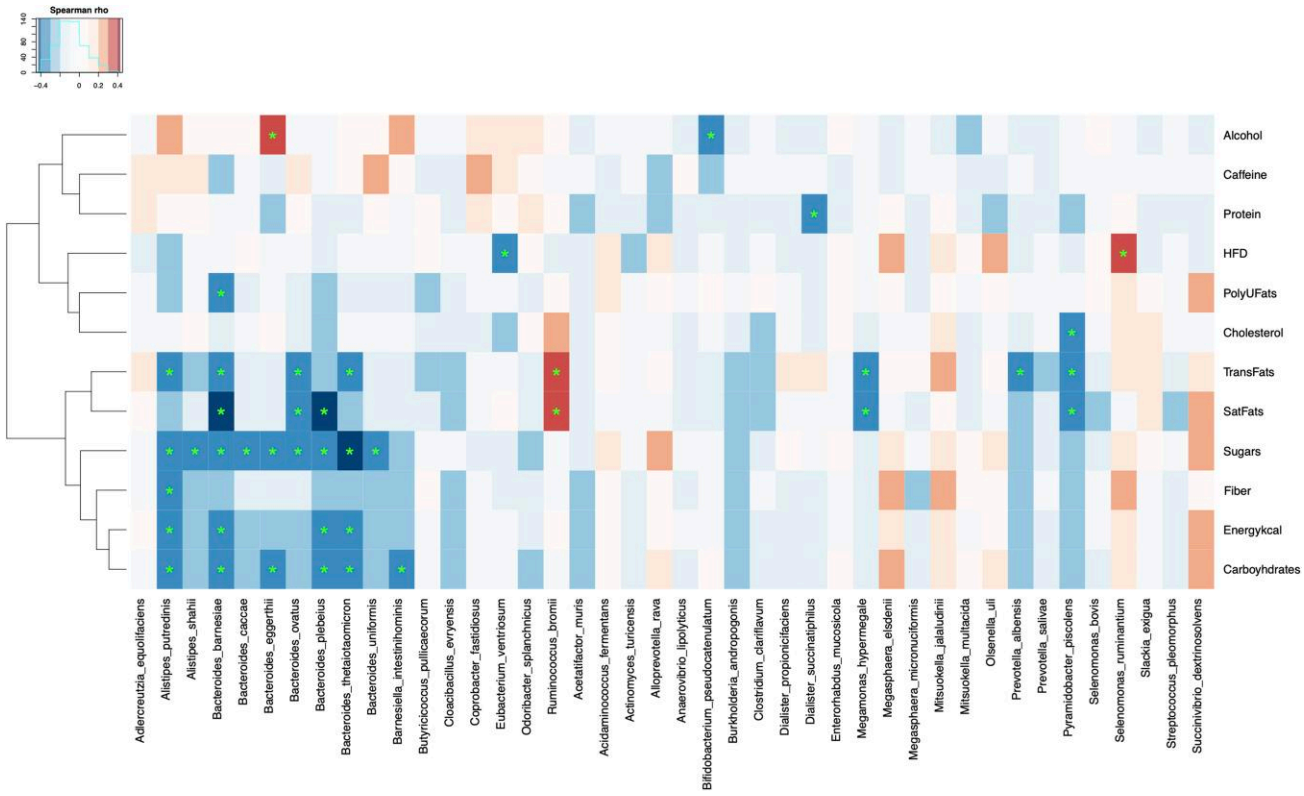


Figure 3. Heatmap showing the correlation of the differentially abundant (DA) species with mean nutrient intake and the Healthy Food Diversity (HFD) index. DA was assessed by analysis of compositions of microbiomes with bias correction while adjusting for the covariates based on filtered species abundance (present in at least 10% of samples and with maximum reads ≥10). False discovery rate <0.1 was considered as DA species. Green stars indicate $^{*}P < .05$; $^{**}P < .01$; $^{***}P < .001$.

microbes have been observed in people with HIV with hyperglycemia, hypertension, and dyslipidemia [13]. Our findings are in line with these previous studies and suggest that alterations in gut microbiota, particularly a reduction in SCFA-producing bacteria like butyrate producers, may be linked to cardiovascular and metabolic conditions in people with HIV.

In keeping with previous studies, we found no significant differences in nutrient intake or HFD index between people with and without HIV [35]. However, a depletion of SCFA-producing species was linked to higher intake of fats and sugars in both groups, affecting gut microbiota and CVD risk. Several studies have shown that a high-fat diet has a negative impact on gut microbiota, with a reduction in SCFA-producing bacteria affecting host metabolism and linked with obesity, insulin sensitivity, and lipid metabolism in the general population [9]. In the context of HIV, studies in simian immunodeficiency virus–infected macaques have shown that a high-fat diet exacerbates inflammation, increasing levels of proinflammatory cytokines such as IL-18 [36]. Consistent with these findings, we observed elevated circulating IL-18, alongside other proinflammatory biomarkers, which were associated with the depletion of SCFA-producing species. IL-18 has been previously reported to be elevated in obesity and linked with lipodystrophy in people with HIV, underscoring its relevance to metabolic and inflammatory dysregulation in this population [37, 38].

An increased abundance of *Megasphaera* and *Prevotella* spp, along with *A lipolyticus*, was associated with elevated I-FABP and IL-18 levels in people with HIV. I-FABP, a marker of endothelial damage, has been associated with CVD [39] and hypertension [40] in the general population. In HIV groups, I-FABP has been associated with increased adiposity, suggesting that intestinal damage is linked with nutrient malabsorption and inflammation [41]. The elevation of I-FABP likely reflects increased enterocyte turnover due to systemic metabolic derangements rather than, in the absence of suggestive markers (sCD14, LBP), gut MT. Together, these findings suggest an inflammatory state driven by reduced SCFA-producing microbes and elevated proinflammatory biomarkers, contributing to CVD risk in HIV infection.

Of note, a number of oral microbes were identified in people with HIV in this study, including the species *O uli* and *S exigua*. The oral microbiome plays a significant role not only in maintaining oral health but also in normal homeostasis. The translocation of oral microbes to the gut is particularly evident in inflammatory bowel diseases, sporadic colorectal cancer, and

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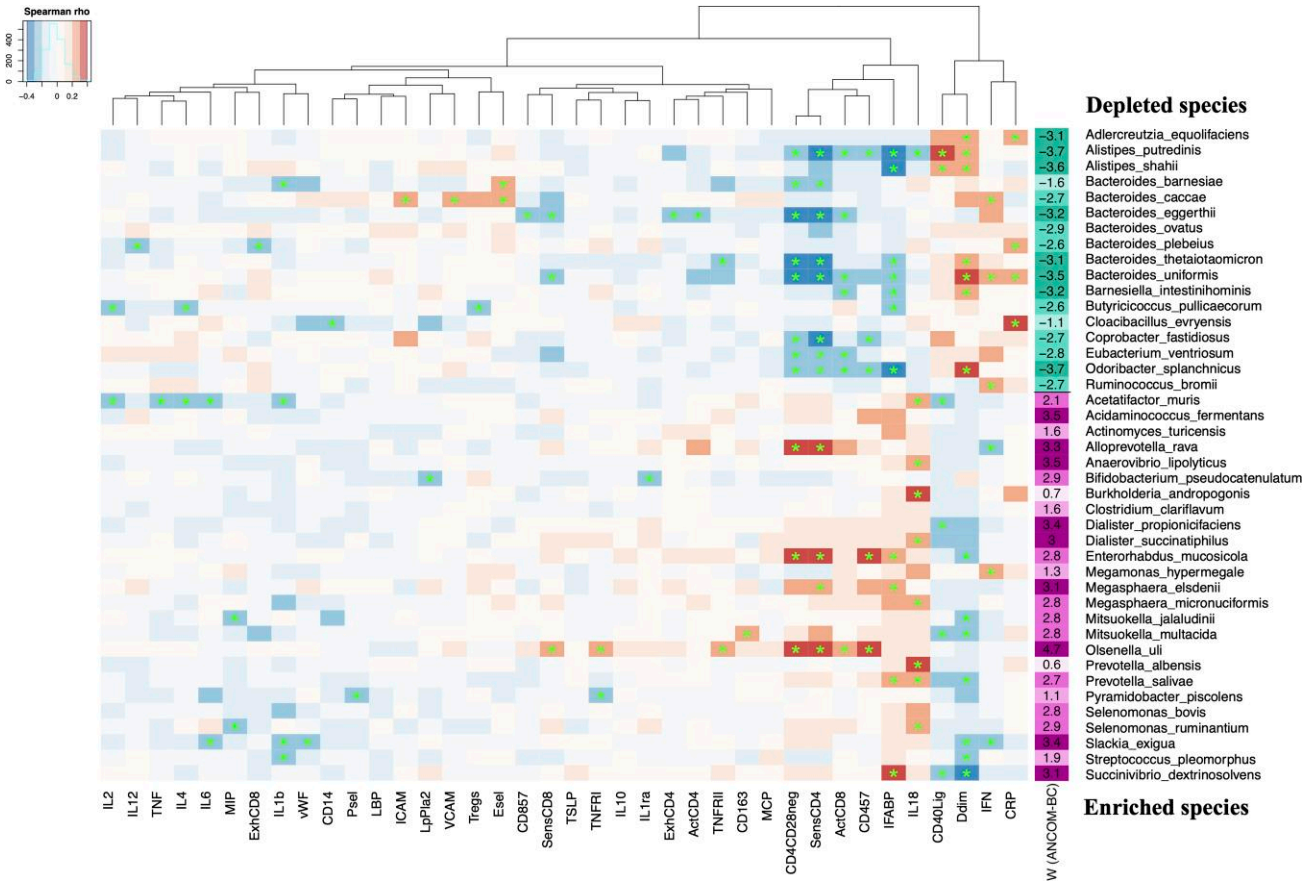


Figure 4. Heatmap showing the correlation of the differentially abundant (DA) species with the inflammatory cytokines and T-cell surface markers for people with human immunodeficiency virus (HIV) and those without HIV. DA was assessed by analysis of compositions of microbiomes with bias correction (ANCOM-BC) while adjusting for the covariates based on filtered species abundance (present in at least 10% of samples and with maximum reads ≥10). False discovery rate <0.1 was considered as DA species. Green stars indicate $*P < .05$; $**P < .01$; $***P < .001$. actCD8, activated CD8 T cells; CD14, cluster of differentiation 14; ExhCD4, exhausted CD4 T cells; ExhCD8, exhausted CD8 T cells; hsCRP, high sensitivity C Reactive Protein; I-FABP, intestinal fatty acid binding protein; IFN, interferon; IL, interleukin; IL1b, interleukin 1 beta; IL1RA, IL-1 receptor antagonist; LBP, LPS binding protein; Lp-PLA2, lipoprotein-associated phospholipase A2; MCP-1, monocyte chemoattractant protein; MIP-1, macrophage inflammatory protein; sCD163, soluble cluster of differentiation 163; sCD40L, soluble CD40 ligand; s-ICAM, soluble intercellular adhesion molecule; sensCD8, sensitised CD8 T cells; TNFR, tumor necrosis factor receptor; Tregs, regulatory T cells; TSLP, thymic stromal lymphopoietin; VCAM1, vascular cell adhesion molecule; vwf, von Willebrand factor.

liver disease [42–44]. Furthermore, HIV infection can significantly alter the composition of the oral microbiome, potentially contributing to dysbiosis and increasing susceptibility to systemic diseases [45]. Meta-analysis also shows that increased abundance of oral microbes is associated with unhealthy aging, suggesting that microbial shifts in the oral cavity can contribute to inflammation and chronic diseases associated with aging [46]. Additionally, a number of rumen microbes were identified in our results, including *M elsdenii*, *S dextrinosolvens*, and *S ruminantium*. The presence of these microbes could be attributed to diet or animal exposure, but these species have also been identified in studies of the human gut [47] and in human disease states [47–50].

The strengths of this study include (1) a well-matched CVD risk cohort of participants representing a diverse population of both men and women from different ethnicities; (2) a comprehensive comparison of diet, inflammatory phenotypes, and gut microbiome composition in the context of both subclinical and clinical CVD risk; and (3) a direct comparison of people with and without HIV, allowing for the identification of unique differences attributable to HIV status.

Limitations of this study include its cross-sectional design and relatively small sample size. This highlights the need for replication in larger, longitudinal cohorts with diverse demographics. Although SPINGO provides the highest accuracy at the species level compared to other tools, the annotation of 16S amplicon sequences at the species level is still limited. We included genus-level classification to aid a more reliable microbial community analysis. While the FFQ offers easy administration, low burden on participants, and cost-efficiency, it is subject to recall and social desirability bias. Inclusion of other dietary assessment tools, such as dietary records and 24-hour food recall, may enhance dietary data accuracy. Finally, while propensity scores were used to match groups, unmeasured factors, including sexual orientation, which

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was not assessed in this cohort but is known to influence microbiome changes, may still affect outcomes. However, as both study groups were recruited from similar socioeconomic backgrounds, this likely helps mitigate some of these unmeasured behavioral and socioeconomic factors. Future research should explore the functional capacities of specific microbial species and examine SCFA associations through targeted metabolomics to assess how gut microbiome interventions, such as probiotic use or dietary interventions, may impact inflammatory phenotypes in people with HIV.

CONCLUSIONS

In conclusion, we describe how gut microbiome alterations, particularly the depletion of SCFA-producing microbes, is associated with diet and systemic inflammation, contributing to clinical and subclinical CVD in people with HIV. These findings underscore the importance of dietary counseling and targeted interventions to address gut dysbiosis, as well as the potential for monitoring inflammatory markers to enhance CVD risk prediction and prevention. This evidence supports a precision medicine approach to managing HIV-associated comorbidities and improving CVD outcomes.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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