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PATHOGENESIS

Circulating Short-Chain Fatty Acids: Association with Vaginal Microbiota, Genital Inflammation, and HIV Acquisition

Rupak Shivakoti,[1] Marothi Letsoalo,[2] Lara Lewis,[2,3] Lyle R. Mckinnon,[2,4] Jo-Ann S. Passmore,[2,5,6]
Salim S. Abdool Karim,[1,2] and Lenine J.P. Liebenberg[2,7]

Abstract

Little is known about the relationships between circulating short-chain fatty acids (SCFAs) and genital microbiota, inflammation, and the risk for HIV infection in women. As circulating SCFAs are potentially modifiable, for example, through dietary fiber or probiotics, we investigated association of circulating SCFA levels with these outcomes. We carried out a nested matched case–control study within a randomized trial of an antiretroviral microbicide to prevent HIV infection to study the association between circulating SCFAs and HIV acquisition (primary outcome for case definition), vaginal microbiota, and genital inflammation. Levels of the SCFAs butyrate, acetate, and propionate were quantified in plasma using mass spectrometry. Vaginal microbiota was assessed using metaproteomics and characterized as *Lactobacillus* dominant (LD) or low *Lactobacillus* (LL). Genital inflammation was measured using multiplex immunoassays. Logistic regression models were used to study the association of SCFAs with each outcome. Study population ($N = 99$) characteristics were similar between cases (33 who acquired HIV) and controls (66 who did not acquire HIV). We did not observe any associations between any of the circulating SCFAs with HIV acquisition or with LL vaginal microbiota status. However, there was an inverse association between circulating SCFAs and several pro-inflammatory genital cytokines, including interleukin-6 (IL-6), IL-1α, and IL-8. In our study of women with high risk of HIV infection, higher levels of circulating SCFAs were associated with lower levels of various genital inflammatory markers, but not with HIV acquisition or a LL microbiota profile. Future larger studies, including genital SCFA assessment, are needed to confirm these findings.

Keywords: cervicovaginal, molecular bacterial vaginosis, butyrate, acetate, propionate

Introduction

Studies have characterized the vaginal microbiota of reproductive-age women, grouping women into multiple categories of vaginal microbial communities called community state types (CSTs).[1,2] A low-*Lactobacillus* (LL) vaginal microbiota characterizes CST IV, whereas the other CSTs (e.g., CST I, II, III, V) are characterized by the dominance of various *Lactobacillus* species.[1] Notably, the LL vaginal microbiota is positively associated with a variety of adverse health outcomes, including bacterial vaginosis,[3] preterm birth,[2,4,5] and increased risk of sexually transmitted infections (STIs).[2,6,7] Related to STIs, women with LL vaginal microbiota were at a higher risk of acquiring HIV infection compared with those with *Lactobacillus*-dominant (LD) profiles.[6] Furthermore, studies such as the CAPRISA 004 show that women with LL microbiota also had higher levels of cervicovaginal inflammation[8,9] and lower efficacy for preventive vaginal microbicides.[10]

These results suggest that modulating the vaginal microbiota from a LL profile toward a more optimal LD profile may reduce the risk of STI acquisition, especially HIV acquisition. Topical *Lactobacillus*-based probiotics show promise in improving *Lactobacillus* levels,[11–13] but have generally had limited success with conditions like recurrent bacterial vaginosis.[11] Interestingly, in a small study of healthy U.S. women without HIV, we observed that women with higher intakes of dietary fiber were less likely to have LL microbiota.[14] While

[1]Department of Epidemiology, Columbia University, New York, New York, USA.
[2]Centre for the AIDS Programme of Research in South Africa (CAPRISA), Durban, South Africa.
[3]School of Nursing and Public Health, Discipline of Public Health Medicine, University of KwaZulu-Natal, Durban, South Africa.
[4]Department of Medical Microbiology and Infectious Diseases, University of Manitoba, Winnipeg, Canada.
[5]Institute of Infectious Diseases and Molecular Medicine (IDM), University of Cape Town, Cape Town, South Africa.
[6]National Health Laboratory Service, Cape Town, South Africa.
[7]School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal, Durban, South Africa.

dietary fiber is known to increase levels of *Lactobacillus* in the gut microbiota,[15] there is a lack of other data to show that dietary fiber intake might also affect the vaginal microbiota. Interestingly, some studies using specific oral *Lactobacillus* probiotics have shown to increase the levels of vaginal *Lactobacillus*.[16,17] These preliminary results would suggest that prebiotics (i.e., fiber) or oral probiotics could be a potential intervention to modulate vaginal microbiota and inflammation and, ultimately, reduce the risk of HIV and other STIs.

In this nested analysis, we leverage the data and samples from CAPRISA 004[18] to study the association of circulating short-chain fatty acids (SCFAs) with cervicovaginal microbiota, cervicovaginal inflammation, and HIV acquisition. As CAPRISA 004 did not collect data on dietary fiber intake or have participants on probiotics, we use circulating SCFAs as a downstream marker[19,20] which, if associated with cervicovaginal microbiota, could potentially be modified (e.g., through prebiotics/probiotics) to reduce HIV risk. Considering the established relationships among HIV risk, cervicovaginal microbial dysbiosis, and inflammation in women participating in the CAPRISA 004 trial cohort,[9,10,21] and the observed relationship between dietary fiber/probiotics and increased observations of LL microbiota,[14,16,17] we test the hypothesis that women with higher levels of SCFAs would be less likely to acquire HIV infection, presumably due to a profile of *Lactobacillus* dominance and reduced genital inflammation. More specifically, we hypothesize that higher levels of circulating SCFAs, as a gut-microbiota mediated effect of dietary fiber/probiotics, can also affect the cervicovaginal microbiota profile and inflammation and, ultimately, HIV acquisition risk.

Materials and Methods

Study design and population

This study was nested within the CAPRISA 004 trial conducted between 2007 and 2010.[18] In the parent CAPRISA 004 study, 889 sexually active women without HIV were randomized to either tenofovir (1% vaginal gel formulation of this nucleotide reverse transcriptase inhibitor) or placebo gel and followed for 30 months to compare HIV incidence between the 2 study arms.[18] Study participants were women from rural Vulindlela and the urban eThekwini clinics in KwaZulu-Natal, South Africa. Eligibility criteria are detailed elsewhere[18] but include sexually active, nonpregnant women 18–40 years of age.

The primary objective of this study was to determine the relationship of circulating SCFAs with HIV acquisition. We nested a 1:2 case–control study ($N = 99$) within CAPRISA 004 to address this objective. Of the 889 participants of the CAPRISA 004 trial conducted in South Africa, 99 (11.14%) acquired HIV (cases). A total of 33 cases who acquired HIV infection during the 2.8 years of follow-up had available ethylenediaminetetraacetic acid (EDTA) plasma specimens (for SCFA measurements) along with paired genital cytokine and microbiota data from cervicovaginal lavage (CVL) samples at the last visit before seroconversion. We selected 66 controls (2 controls per case) who remained uninfected during the study. Controls were matched to cases by treatment group (tenofovir or placebo arms) and sample storage duration (mean ± standard deviation difference in storage duration was 5.6 ± 10.7 days). HIV testing was conducted monthly using two rapid HIV antibody tests and confirmed with two independent RNA-polymerase chain reaction assays at least 1 week apart.[18] All case samples were analyzed for cytokine levels before HIV infection [median 4.5 months preinfection (interquartile range or IQR 2.4–6.9 months)].

A secondary objective of this study was to determine the relationship between circulating SCFA and vaginal microbiota (LL vs. LD based on proteome). Another secondary objective was to study the association between circulating SCFA and genital inflammation.

Ethics statement

Research Ethics Committees at Columbia University and the University of KwaZulu-Natal approved this study, and all participants provided written informed consent to related ancillary analyses before participation in the CAPRISA 004 trial. We followed U.S. Department of Health and Human Services guidelines for human studies.

Specimen collection

CVL samples were collected as previously described[21] by bathing the cervix with 3 mL sterile saline and retrieving the liquid from the posterior fornix by aspiration with a plastic bulb pipette. Specimens were transported on ice to CAPRISA laboratories within 4 hours of collection, where they were centrifuged and the supernatant stored at −80°C. CVL samples were not collected from menstruating participants, so sampling was postponed to the following week. At the same visits, blood was collected by venipuncture into EDTA vacutainer tubes, and isolated blood plasma was stored at −80°C. The samples included in this study had not been previously thawed.

SCFA quantification

The following three relevant SCFAs were quantified in plasma specimens by Metabolon using liquid chromatography with tandem mass spectrometry (LC-MS/MS; Agilent 1290/AB Sciex 5500 system): acetic acid, propionic acid, and butyric acid. Plasma specimens were stored at Metabolon in a freezer at approximately −80°C until analysis. Briefly, plasma samples were spiked with stable labeled internal standards, homogenized, and subjected to protein precipitation before assessment by LC-MS/MS. Quantitation was performed using a weighted linear least squares regression analysis with fortified calibration standards. Analyte concentrations that fell below the limit of quantitation are extrapolated, and the reported values, referred to as below the lower limit of quantification (BLOQ), are below the level of quantitation. The raw data for the LC-MS/MS were collected and processed using SCIEX software and SCIEX OS-MQ software, with data reduction carried out using Microsoft Excel.

Microbial proteome to characterize LD and LL

Mass spectrometry was used as previously described in this population[10] to characterize the microbial and host metaproteomes. Briefly, CVLs were prepared by determining protein content, denaturing proteins, and digesting them with trypsin. Peptides were eluted, dried, and isolated using

chromatography. Mass spectrometry analysis was conducted using a Q-Exactive Plus instrument, with peptides loaded onto a C18-reversed phase column and eluted using a gradient. Protein identification was performed using the Mascot search engine against a curated bacterial protein database. The presence and abundance of microbial proteins were determined based on peptide identification and spectral counts. The methodology included database construction, taxonomic search, and sample quality assessment.[10] Women were classified as LD if the proportion of *Lactobacillus* species detected in CVL was greater than 0.5 and non-*Lactobacillus* dominant (nonLD, i.e., LL) otherwise.[10]

Cytokine quantification

Cytokine concentrations were determined in CVL fluid secretions by multiplex enzyme-linked immunosorbent assays. Samples were filtered by centrifugation using 0.2 μm cellulose acetate filters (Sigma, USA) before the quantification of the following 48 cytokines involved in inflammation, chemotaxis, hematopoiesis, regulation, and growth (Bio-Plex Pro Human Cytokine Kits, Bio-Rad)[22,23]: interleukin (IL)-1β, IL-1RA, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p70, IL-12p40, IL-16, IL-18, IL-1A, IL-2Rα, IL-3, IL-13, IL-15, IL-17, basic fibroblast growth factor, cutaneous T cell attracting chemokine, eotaxin, granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor, growth regulated-alpha, hepatocyte growth factor, interferon-γ, IFN-α2, interferon gamma-induced protein (IP)-10, leukemia inhibitory factor, monocyte chemotactic protein (MCP)-1, MCP-3, macrophage colony-stimulating factor, monokine induced by gamma-interferon, macrophage migration inhibitory factor, macrophage inflammatory protein (MIP)-1α, MIP-1β, nerve growth factor-β, platelet-derived growth factor-$\beta\beta$, regulated upon activation normal T cell expressed and presumably secreted, stem cell factor, stem cell growth factor-β, stromal-derived factor-1α, tumor necrosis factor (TNF)-α, TNF-β, TNF-related apoptosis inducing ligand, and vascular endothelial growth factor. Assays were conducted according to the manufacturer's instructions. The sensitivity of the kits ranged between 0.2 and 45.2 pg/mL for each cytokine measured and between 2 and 200 pg/mL for each matrix metalloproteinase. A Bio-Plex 200 array reader with Bio-Plex Manager software was used to collect data, and the sample concentrations were calculated using five-parametric curve fitting. Genital inflammation was defined based on previous studies[9,21,24] as not inflamed or inflamed, with the latter being defined by having any five or more of nine pro-inflammatory/chemotactic cytokines (IL-1α, IL-1β, MIP-1α, MIP-1β, IL-6, IL-8, TNF-α, IP-10, and MCP-1) above the 75th percentile (calculated using the entire dataset).

Statistical considerations

Box plots and Wilcoxon rank-sum tests were used to explore the relationship between each SCFA and outcomes of HIV acquisition, *Lactobacillus* status, and inflammation status. For the primary analysis of the relationship between SCFA and HIV acquisition, as matching was used for the creation of the case–control sample, conditional logistic

Table 1. Demographic and Clinical Characteristics of the Study Population by HIV Status

		HIV acquisition		
Variable	*Group/measurement*	*Did not acquire HIV* N = *66*	*Acquired HIV* N = *33*	*Total* N = *99*
Treatment	Placebo (%)	48 (72.7)	24 (72.7)	72 (72.7)
	Tenofovir (%)	18 (27.3)	9 (27.3)	27 (27.3)
Study site	eThekwini (%)	18 (27.3)	9 (27.3)	27 (27.3)
	Vulindlela (%)	48 (72.7)	24 (72.7)	72 (72.7)
Contraceptive	Injectables (%)	56 (84.8)	30 (90.9)	86 (86.9)
	Other (%)	10 (15.2)	3 (9.1)	13 (13.1)
Cohabitation	No (%)	60 (90.9)	32 (97.0)	92 (92.9)
	No Regular Partner (%)	1 (1.5)	—	1 (1.0)
	Yes (%)	5 (7.6)	1 (3.0)	6 (6.1)
Condom use	Always (%)	16 (24.2)	11 (33.3)	27 (27.3)
	Most Times (%)	20 (30.3)	8 (24.2)	28 (28.3)
	Never (%)	9 (13.6)	5 (15.2)	14 (14.1)
	Occasionally (%)	21 (31.8)	9 (27.3)	30 (30.3)
Marital status	Casual (%)	1 (1.5)	1 (3.0)	2 (2.0)
	Married (%)	3 (4.5)	1 (3.0)	4 (4.0)
	Stable (%)	58 (87.9)	29 (87.9)	87 (87.9)
	Stable casual (%)	4 (6.1)	2 (6.1)	6 (6.1)
Baseline HSV serology	Negative (%)	38 (57.6)	9 (27.3)	47 (47.5)
	Positive (%)	28 (42.4)	24 (72.7)	52 (52.5)
Age	Median (IQR)	22 (20–25)	22 (20–24)	22 (20–25)
Age of sexual debut	Median (IQR)	18 (16–19)	17 (16–18)	18 (16–18)
Frequency of sex in past 30 days	Median (IQR)	6 (3–10)	4 (2–8)	4 (3–9)
Number of sexual partners	Median (IQR)	2 (1–3)	2 (1–3)	2 (1–3)

Study population characteristics shown by case (i.e., HIV acquisition) and control (no HIV acquisition) status and for total women in the study. Median and interquartile range are shown for continuous variables and the number and percent are shown for categorical variables.

HSV, Herpes simplex virus; IQR, interquartile range.

regression was used to model the probability of not acquiring HIV. The exposure of interest was SCFAs that included separate models for each SCFA as follows: acetic, butyric, and propionic acids. The values for acetic, propionic, and butyric acids were divided by 100, 10, and 10, respectively, to aid the interpretation of odds ratios. A piece-wise regression procedure was implemented to model values BLOQ separately from quantifiable (LOQ) values.[25] The model also adjusts for measured variables potentially associated with both SCFA and HIV acquisition [i.e., age, study site, contraceptive use, and Herpes simplex virus (HSV) serology].

For the secondary analyses, unconditional weighted logistic regression models were used to fit the association of each SCFA with separate outcomes of *Lactobacillus* dominance and inflammation status. As the sample for this analysis was selected based on case and control status for the primary analysis, applying the weighted logistic regression technique involves the calculation of inverse probability weights that rely on the probability of selecting the cases and controls. The weights used in this study were calculated as per design variables, HIV status, treatment, and site. The analytical approach, such as how to deal with SCFA values (e.g., BLOQ and LOQ) and the variables adjusted for in the model, was the same as for the primary objective. For the inflammation analysis, we also studied the relationship between SCFA and individual cytokines (primary analysis assessed the 9 cytokines detailed above to define inflamed status; secondary analysis assessed correlation with all of the cytokines tested) using Pearson correlation.[26] In exploratory analyses, relationship of SCFAs with *Lactobacillus* was also assessed using beta regression models. For all statistical tests, a *p*-value <.05 was considered significant, and a false discovery rate adjustment was applied to account for multiple comparisons. All statistical analyses were performed in R version 4.5.0.

Results

Clinical characteristics of the study population

A total of 99 participants were included in this study: 33 cases who acquired HIV during the CAPRISA 004 trial and 66 controls who did not seroconvert during the trial. Most of the study population characteristics at study enrolment, including age, study site, contraceptive use, marital status, and number of sexual partners, were similar between cases and controls (Table 1). However, a higher proportion of cases were HSV seropositive at baseline compared with

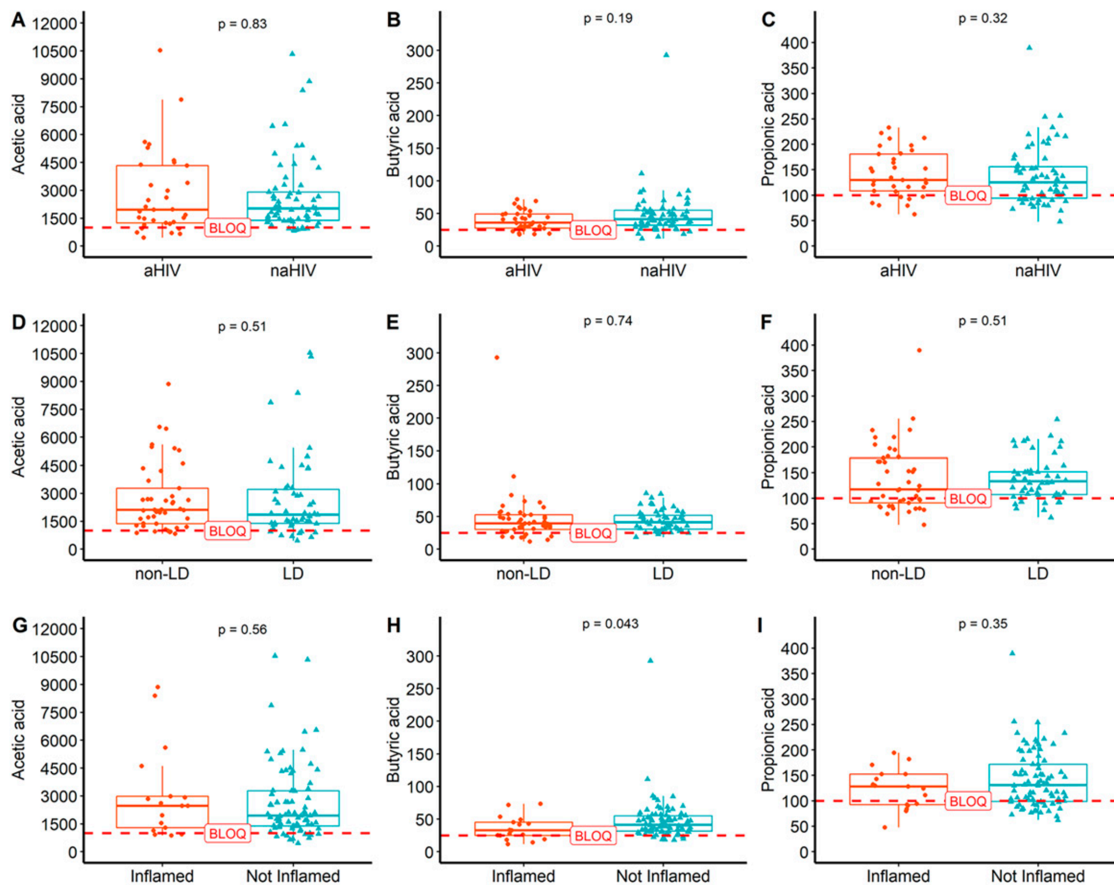

FIG. 1. Comparison of SCFA concentrations by HIV outcome, microbial profile, and inflammation status. Concentrations (ng/mL) of acetic **(A, D,** and **G)**, butyric **(B, E,** and **H)**, and propionic acid **(C, F,** and **I)** are compared between each end point. Box plots and Wilcoxon rank-sum tests were used to assess the relationship between SCFAs and HIV acquisition **(A, B,** and **C)**, *Lactobacillus* status **(D, E,** and **F)**, and inflammation status **(G, H,** and **I)**. The detection limit per analyte is represented by a *dashed line*. aHIV, acquired HIV; LD, *Lactobacillus* dominant; naHIV, did not acquire HIV; SCFA, short-chain fatty acid.

controls (72% vs. 42%). Due to matching, cases and controls were well-balanced in terms of their assignment to the randomization arm of the CAPRISA 004 study (Table 1).

Associations between SCFA and HIV acquisition

Median plasma concentrations of SCFAs ranged from 40.8 ng/mL (IQR 30.4–52.3 ng/mL; butyric acid) to 1980.4 ng/mL (1369.1–3268.1 ng/mL; acetic acid) (Supplementary Table S1). Within-range detection of SCFAs varied across analytes, with propionic acid detectable in 73% of specimens, whereas butyric acid was detectable in 91% of plasma specimens (Supplementary Table S1).

Wilcoxon rank-sum tests identified no differences in the magnitudes of SCFA by HIV outcome (Fig. 1). In addition, conditional logistic regression models in the primary analysis identified no evidence of associations between acetic, butyric, or propionic acid and the odds of not acquiring HIV after adjusting for baseline age, study site, contraceptive use, and HSV infection (Table 2).

Associations between SCFA, microbiota, and inflammation status

Of the 99 participants, 54 (54.5%) were classified as having a LD genital microbiota. Study population characteristics were similar between LD and LL participants, except for a higher proportion of LL with HSV infection at baseline (Supplementary Table S2). No differences in the magnitudes of SCFA by LD status were noted (Fig. 1), and the weighted unconditional multivariable logistic regressions demonstrated that acetic, butyric, or propionic acids were not associated with the odds of *Lactobacillus* dominance (Table 2).

Seventeen percent of the participants were classified as having genital inflammation [acquired HIV: 9 (27%) and not acquired HIV: 8 (12%)]. Wilcoxon rank-sum tests identified lower levels of butyric acid ($p = .04$) in the inflamed group than in the not-inflamed group (Fig. 1). In multivariable models, none of the SCFAs was significantly associated with inflamed status (Table 2). When we assessed the correlations between each SCFA and each of the 9 individual immune markers used to define inflamed status (Fig. 2), butyric acid was significantly and inversely correlated with pro-inflammatory cytokines IL-6 ($\rho = -0.24$) and IL-1α ($\rho = -0.22$) as well as the chemokines MIP-1α ($\rho = -0.17$) and IL-8 ($\rho = -0.30$). Propionic acid also demonstrated an anti-inflammatory effect, showing significant negative correlations with IL-6 ($\rho = -0.20$) and the chemokine MIP-1β ($\rho = -0.21$). In contrast, acetic acid showed no significant correlation with any of the markers included in the inflammation definition. Correlation between each SCFA and the wider panel of all tested cytokines is also presented in Supplementary Figure S1.

Discussion

In our study of South African women with high risk for HIV infection, we did not observe an association between circulating SCFA and non-LD vaginal microbiota or HIV acquisition. However, butyric acid and propionic acid had an inverse association with several genital pro-inflammatory markers, including IL-6 and IL-1α and IL-8. These results suggest that circulating SCFAs might have minimal impact on the vaginal microbiota or HIV acquisition risk, although they may influence genital inflammation to some extent. Future studies should confirm the potential relationship between circulating SCFAs and genital inflammation.

Our results did not find an association between circulating SCFAs and vaginal microbiota profile. Literature is abundant on the relationship between gut microbiota profiles, gut SCFAs, and circulating SCFAs.[27–30] For example, specific gut bacteria produce fecal SCFAs, a subset of which will be absorbed into circulation.[27–30] In the vaginal

Table 2. Associations Between Short-Chain Fatty Acid and HIV Acquisition, Microbial Profile, and Inflammation Status

	Conditional	*Weighted unconditional*	
	HIV acquisition	*Lactobacillus dominance*	*Inflamed*
SCFA	*OR (95% CI)*	*OR (95% CI)*	*OR (95% CI)*
Acetic acid			
Unadjusted	1.02 (0.99, 1.04) $p = .235$	1.00 (0.98, 1.02) $p = .824$	1.01 (0.98, 1.03) $p = .676$
Adjusted	1.02 (0.99, 1.05) $p = .279$	0.99 (0.97, 1.02) $p = .568$	1.01 (0.99, 1.04) $p = .298$
Butyric acid			
Unadjusted	0.78 (0.56, 1.09) $p = .145$	0.89 (0.65, 1.22) $p = .478$	0.93 (0.62, 1.34) $p = .716$
Adjusted	0.81 (0.58, 1.14) $p = .228$	0.89 (0.64, 1.22) $p = .463$	1.04 (0.67, 1.58) $p = .860$
Propionic acid			
Unadjusted	1.03 (0.93, 1.14) $p = .612$	0.93 (0.83, 1.05) $p = .266$	0.98 (0.85, 1.12) $p = .762$
Adjusted	1.01 (0.90, 1.14) $p = .812$	0.94 (0.83, 1.06) $p = .326$	1.00 (0.86, 1.15) $p = .968$

The odds ratio and 95% confidence intervals are shown for the association of acetic acid, butyric acid, and propionic acid with the following outcomes: (1) HIV acquisition (conditional logistic regression), (2) vaginal microbiota (unconditional), and (3) genital inflammation (unconditional). For HIV acquisition, the odds of not acquiring HIV infection is shown; for vaginal microbiota, the odds of *Lactobacillus* dominance is shown; and for genital inflammation, the odds of being inflamed is shown. The definitions of these outcomes are detailed in the methods section. Results are shown for unadjusted and adjusted model. The limit of quantification (LOQ) parameter reflects the association between SCFA values that are above the lowest LOQ (LLOQ) and the outcome variable. Unadjusted model measures the unadjusted association between SCFA and outcome, whereas adjusted model further adjusts for age, contraceptive use, study site, and HSV positivity. The values for acetic, butyric, and propionic were divided by 100, 10, and 10, respectively, to aid the interpretation of odds ratios.

CI, confidence interval; OR, odds ratio.

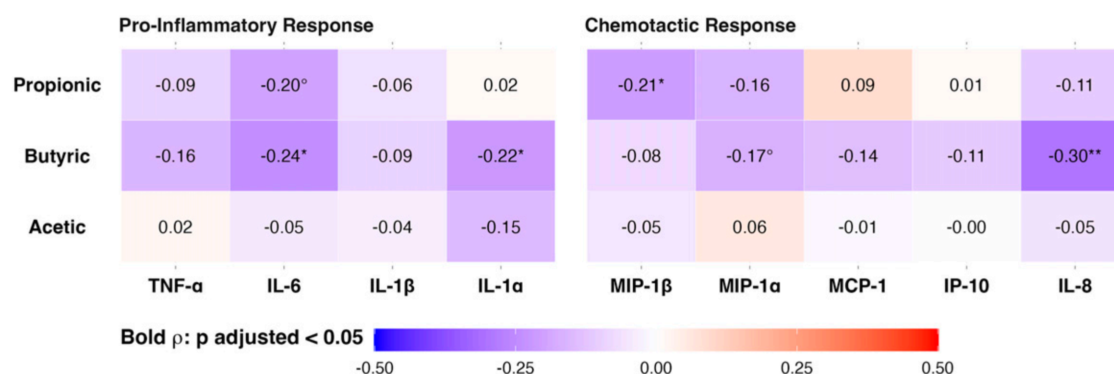

FIG. 2. Heat map of Pearson correlations between SCFAs and immune markers. Pearson's correlation coefficients (ρ) were calculated for the relationship between plasma propionic, butyric, and acetic acid levels and various pro-inflammatory and chemotactic markers. The color scale represents the correlation strength and direction, where *blue* is negative and *red* is positive. While some correlations showed nominal significance (°$p < .1$; *$p < .05$; **$p < .01$; ***$p < .001$), none remained statistically significant after adjustment for multiple comparisons (p-adjusted <.05), as indicated by the absence of bolded values.

microenvironment, there is also literature to suggest a relationship between vaginal microbiota and vaginal SCFA concentrations.[31–33] However, some studies have noted different relationships compared with the gut.[31] While the potential for cross talk between gut and genital tract microbiota and immunity has been proposed,[31] other studies have not yet looked at the relationship between circulating SCFAs and vaginal microbiota. Our current study was motivated by prior findings suggesting that dietary fiber and oral *Lactobacillus* probiotics might have an inverse association with LL microbiota.[14,16,17] However, we did not observe any association between circulating SCFA and LL microbiota. With the known links between LL microbiota and increased HIV risk in women,[6] this study also assessed the relationship between circulating SCFAs with HIV acquisition. We did not observe an association between circulating SCFAs and HIV acquisition. Given our findings with vaginal microbiota, this result was not surprising.

Our findings on the relationship between circulating SCFA and genital inflammation were more complex. Using the "inflamed" definition based on having at least five cytokines above the 75th percentile,[9,21,24] we did not observe significant relationships between SCFAs and genital inflammation. Given the minimal changes in effect estimates and specific definition used (i.e., ≥5 cytokines) with "inflamed," we further explored the relationship between circulating SCFAs with individual genital cytokines to better understand potential associations. We noted that butyric acid and propionic acid were inversely associated with IL-6, IL-1α, and IL-8. It is known that SCFAs can reduce inflammation, owing to their histone deacetylase properties to modulate inflammatory gene expression.[27–30]

As previous studies had shown that increased genital inflammation was linked to LL profile and increased HIV acquisition,[6,8,9] it was interesting to note the apparent discrepancy with an association between circulating SCFA with specific cytokines but not with vaginal microbiota or HIV acquisition. It is possible that SCFAs, which are circulating, can also have anti-inflammatory effects in the local vaginal microenvironment, but they are not sufficient to modify vaginal microbiota profile or HIV risk.

Our study has some limitations that should be noted. The sample size for this study was somewhat limited, with only 33 women acquiring HIV. Related to sample size, while the primary approach was to not adjust for multiple comparisons in the cytokine analysis due to the exploratory nature, we should note that there were no significant results observed after correction for multiple comparisons. Furthermore, dietary fiber intake data were not collected in the parent study. Another limitation of this study was that genital SCFA data were not available. Due to our hypothesis around dietary fiber and oral probiotics, this study did not aim to measure genital SCFAs. Still, it could be informative to understand the relationship between systemic and local SCFA and its relationship with vaginal microbiota and HIV acquisition. Despite these limitations, our study also has strengths. We utilize a 1:2 case–control design with matching on key characteristics and nesting within a longitudinal randomized trial. Furthermore, we have well-characterized paired data related to important outcomes of HIV acquisition over time, vaginal microbiota profile, and genital inflammation yielding novel information on their relationship with circulating SCFAs.

In conclusion, we observed that higher levels of SCFAs were associated with lower levels of various genital pro-inflammatory cytokines. However, circulating SCFAs were not predictive of HIV acquisition risk in our nested case–control study. Future studies are needed to confirm these findings and to better understand the relationship between circulating SCFAs and genital inflammation.

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Authors' Contributions

R.S.: Conceived the research question, obtained funding, led this study's implementation, helped guide the analysis,

and wrote the primary version of the article. M.L. and L.L.: Contributed to the study design and led the data analysis for this project. L.R.M. and J-A.S.P.: Contributed to the parent study and provided cytokine and microbiome data utilized in this study and helped with critical review of article. S.S.A.K.: Helped obtain funding, contributed to the study's design, interpretation, and article review, and was a leading investigator of the parent CAPRISA 004 study. L.J.P.L.: Helped conceive the research question and obtain funding, led this study's implementation, guided the analysis, and co-wrote the primary version of the article. All authors read and approved the final version of the article. All authors met the criteria for authorship as recommended by the International Committee of Medical Journal Editors and were fully responsible for all aspects of article development.

Author Disclosure Statement

The authors have no relevant conflicts of interest to declare.

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Supplementary Material

Supplementary Data

References

1. Ravel J, Gajer P, Abdo Z, et al. Vaginal microbiome of reproductive-age women. Proceedings of the National Academy of Sciences of the United States of America 2011; 108(Suppl 1):4680–4687; doi: 10.1073/pnas.1002611107
2. Kroon SJ, Ravel J, Huston WM. Cervicovaginal microbiota, women's health, and reproductive outcomes. Fertil Steril 2018;110(3):327–336; doi: 10.1016/j.fertnstert.2018.06.036
3. McKinnon LR, Achilles SL, Bradshaw CS, et al. The evolving facets of bacterial vaginosis: Implications for HIV transmission. AIDS Res Hum Retroviruses 2019;35(3):219–228; doi: 10.1089/AID.2018.0304
4. Elovitz MA, Gajer P, Riis V, et al. Cervicovaginal microbiota and local immune response modulate the risk of spontaneous preterm delivery. Nat Commun 2019;10(1):1305; doi: 10.1038/s41467-019-09285-9
5. Fettweis JM, Serrano MG, Brooks JP, et al. The vaginal microbiome and preterm birth. Nat Med 2019;25(6):1012–1021; doi: 10.1038/s41591-019-0450-2
6. Gosmann C, Anahtar MN, Handley SA, et al. Lactobacillus-deficient cervicovaginal bacterial communities are associated with increased HIV acquisition in young South African women. Immunity 2017;46(1):29–37; doi: 10.1016/j.immuni.2016.12.013
7. Taha TE, Hoover DR, Dallabetta GA, et al. Bacterial vaginosis and disturbances of vaginal flora: Association with increased acquisition of HIV. AIDS 1998;12(13):1699–1706; doi: 10.1097/00002030-199813000-00019
8. Masson L, Arnold KB, Little F, et al. Inflammatory cytokine biomarkers to identify women with asymptomatic sexually transmitted infections and bacterial vaginosis who are at high risk of HIV infection. Sex Transm Infect 2016;92(3): 186–193; doi: 10.1136/sextrans-2015-052072
9. Masson L, Passmore JA, Liebenberg LJ, et al. Genital inflammation and the risk of HIV acquisition in women. Clin Infect Dis 2015;61(2):260–269; doi: 10.1093/cid/civ298
10. Klatt NR, Cheu R, Birse K, et al. Vaginal bacteria modify HIV tenofovir microbicide efficacy in African women. Science 2017;356(6341):938–945; doi: 10.1126/science.aai9383
11. Cohen CR, Wierzbicki MR, French AL, et al. Randomized trial of lactin-V to prevent recurrence of bacterial vaginosis. N Engl J Med 2020;382(20):1906–1915; doi: 10.1056/NEJMoa1915254
12. Hemmerling A, Govender V, Dong K, et al. Acceptability of the live biotherapeutic LACTIN-V (Lactobacillus crispatus CTV-05) among young women at high risk of HIV acquisition in South Africa: Data from the phase 2 placebo-controlled trial. Front Reprod Health 2025;7:1544458; doi: 10.3389/frph.2025.1544458
13. Ravel J Gje Gottfried S, Greene M, Kellogg-Spadt S, Gevers D, Harper D. A novel multi-strain vaginal synbiotic is effective in optimizing the vaginal microbiome: Results from a randomized, placebo-controlled clinical trial. medRxiv 2024; doi: 10.1101/2024.05.20.24307554
14. Shivakoti R, Tuddenham S, Caulfield LE, et al. Dietary macronutrient intake and molecular-bacterial vaginosis: Role of fiber. Clin Nutr 2020;39(10):3066–3071; doi: 10.1016/j.clnu.2020.01.011
15. Florowska A, Krygier K, Florowski T, et al. Prebiotics as functional food ingredients preventing diet-related diseases. Food Funct 2016;7(5):2147–2155; doi: 10.1039/c5fo01459j
16. Russo R, Edu A, De Seta F. Study on the effects of an oral lactobacilli and lactoferrin complex in women with intermediate vaginal microbiota. Arch Gynecol Obstet 2018;298(1): 139–145; doi: 10.1007/s00404-018-4771-z
17. Russo R, Karadja E, De Seta F. Evidence-based mixture containing Lactobacillus strains and lactoferrin to prevent recurrent bacterial vaginosis: A double blind, placebo controlled, randomised clinical trial. Benef Microbes 2019; 10(1):19–26; doi: 10.3920/BM2018.0075
18. Abdool Karim Q, Abdool Karim SS, Frohlich JA, et al.; CAPRISA 004 Trial Group. Effectiveness and safety of tenofovir gel, an antiretroviral microbicide, for the prevention of HIV infection in women. Science 2010;329(5996): 1168–1174; doi: 10.1126/science.1193748
19. Boets E, Deroover L, Houben E, et al. Quantification of in vivo colonic short chain fatty acid production from inulin. Nutrients 2015;7(11):8916–8929; doi: 10.3390/nu7115440

20. van der Beek CM, Canfora EE, Kip AM, et al. The prebiotic inulin improves substrate metabolism and promotes short-chain fatty acid production in overweight to obese men. Metabolism 2018;87:25–35; doi: 10.1016/j.metabol.2018.06.009
21. McKinnon LR, Liebenberg LJ, Yende-Zuma N, et al. Genital inflammation undermines the effectiveness of tenofovir gel in preventing HIV acquisition in women. Nat Med 2018;24(4):491–496; doi: 10.1038/nm.4506
22. Archary D, Liebenberg LJ, Werner L, et al. Randomized cross-sectional study to compare HIV-1 specific antibody and cytokine concentrations in female genital secretions obtained by menstrual cup and cervicovaginal lavage. PLoS One 2015; 10(7):e0131906; doi: 10.1371/journal.pone.0131906
23. Garrett N, Mtshali A, Osman F, et al. Impact of point-of-care testing and treatment of sexually transmitted infections and bacterial vaginosis on genital tract inflammatory cytokines in a cohort of young South African women. Sex Transm Infect 2021;97(8):555–565; doi: 10.1136/sextrans-2020-054740
24. Masson L, Mlisana K, Little F, et al. Defining genital tract cytokine signatures of sexually transmitted infections and bacterial vaginosis in women at high risk of HIV infection: A cross-sectional study. Sex Transm Infect 2014;90(8): 580–587; doi: 10.1136/sextrans-2014-051601
25. Q H. Hands-on Tutorial for Piecewise Linear Mixed-effects Models Using SAS® PROC MIXED. Pharma SUG China: Chapel Hill, NC; 2015.
26. Benesty J, Chen J, Huang Y, Cohen I. Noise Reduction in Speech Processing. Springer; 2009.
27. Tan J, McKenzie C, Potamitis M, et al. The role of short-chain fatty acids in health and disease. Adv Immunol 2014; 121:91–119; doi: 10.1016/B978-0-12-800100-4.00003-9
28. Fusco W, Lorenzo MB, Cintoni M, et al. Short-chain fatty-acid-producing bacteria: Key components of the human gut microbiota. Nutrients 2023;15(9):2211; doi: 10.3390/nu15092211
29. Birkeland E, Gharagozlian S, Valeur J, et al. Short-chain fatty acids as a link between diet and cardiometabolic risk: A narrative review. Lipids Health Dis 2023;22(1):40; doi: 10.1186/s12944-023-01803-5
30. Muller M, Hernandez MAG, Goossens GH, et al. Circulating but not faecal short-chain fatty acids are related to insulin sensitivity, lipolysis and GLP-1 concentrations in humans. Sci Rep 2019;9(1):12515; doi: 10.1038/s41598-019-48775-0
31. Amabebe E, Anumba DOC. Female gut and genital tract microbiota-induced crosstalk and differential effects of short-chain fatty acids on immune sequelae. Front Immunol 2020;11:2184; doi: 10.3389/fimmu.2020.02184
32. Baldewijns S, Sillen M, Palmans I, et al. The role of fatty acid metabolites in vaginal health and disease: Application to candidiasis. Front Microbiol 2021;12:705779; doi: 10.3389/fmicb.2021.705779
33. Mirzaei R, Kavyani B, Nabizadeh E, et al. Microbiota metabolites in the female reproductive system: Focused on the short-chain fatty acids. Heliyon 2023;9(3):e14562; doi: 10.1016/j.heliyon.2023.e14562

Address correspondence to:
Rupak Shivakoti
Department of Epidemiology
Columbia University
722 W 168th Street
New York
NY 10032
USA

E-mail: rs3895@cumc.columbia.edu