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Combined Effects of HIV and Obesity on the Gastrointestinal Microbiome of Young Men who have Sex with Men

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Abstract

Objectives.—The prevalence of obesity is rising among people living with HIV, which may synergistically increase inflammation and the risk of associated diseases. Disruption of gut bacterial communities may be one of the key drivers of this inflammation; however, the combined effects of HIV and obesity on the microbiome have not been explored.

Methods.—This study included 381 men who have sex with men. Thirty-nine were HIV+ and obese (H+O+), 143 were HIV+ and non-obese, 64 were HIV- and obese, and 135 were HIV- and non-obese. Microbiome composition was assessed by targeted sequencing of the V4 region of the 16S rRNA gene using rectal swab samples. Inverse probability of treatment-weighted marginal

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structural models were used to investigate differences in microbial composition between groups while controlling for numerous clinical and behavioral confounders.

Results.—Significant variability in microbial composition was explained by the combination of HIV and obesity, over and above each condition alone (R^2 for the marginal contribution of the H+/O+ group = .008, $p = .001$). H+O+ participants had the highest ratios of *Prevotella* to *Bacteroides,* a pro-inflammatory enterotype that has been described in HIV and obesity independently. H+O+ participants had lower levels of *Bacteroides* and *Veillonella* than all other groups, suggesting a synergistic effect of HIV and obesity on these genera.

Conclusions.—Our findings support the hypothesis that HIV and obesity act together to disrupt gut microbial communities, which may help explain higher levels of generalized inflammation among people living with both HIV and obesity.

Keywords

HIV; Microbiome; Obesity; Inverse probability of treatment weighting; Men who have sex with men

Introduction

Surveillance data from 2015–2016 shows that 40 percent of United States adults are obese and at high risk of heart disease, stroke, diabetes, and cancer(1). The obesity epidemic is a worsening public health crisis, projected to affect 50% of U.S. adults by the year 2030(2). The prevalence of obesity among people living with HIV (PLWH) is similar to the general population and rates are rising rapidly(3, 4), partially because of improved life expectancy due to antiretroviral therapy (ART) and partially as a side effect of the drugs themselves(5). HIV and obesity disproportionately affect racial and ethnic minorities in the United States; the highest age-adjusted prevalences of obesity are found among Hispanics (47.0%) and non-Hispanic blacks (46.8%)(1), and the highest rates of HIV are among non-Hispanic Blacks (43.6 per 100,000) and Hispanics (17 per 100,000)(6).

Studies suggest that obesity plays a role in HIV pathogenesis and the development of comorbid illnesses(4). Higher levels of generalized inflammation and monocyte activation have been observed in PLWH who are obese as compared to non-obese(7), and these immune parameters have been correlated with weight gain following ART initiation(8). The consequences of combined inflammatory effects may be severe; one study found the prevalence of multimorbidity was nearly 80% in PLWH and obesity(9), and inflammatory biomarkers are associated with mortality in PLWH(10).

Numerous studies have shown that HIV disrupts the composition and function of the gastrointestinal (GI) microbiome(11–15). This disruption, termed “dysbiosis,” is generally characterized by a shift from commensals to pro-inflammatory and potentially pathogenic bacteria. Dysbiosis has also been found in obese individuals(16–19) and a number of commonalities between HIV-associated and obesity-associated dysbiosis have been observed. Reduced overall bacterial diversity has been described in both HIV and obesity(11, 12, 16). Animal models and some human studies have found an elevated *Firmicutes* to *Bacteroidetes* ratio associated with obesity(17, 18, 20), but several re-analyses

and meta-analyses have failed to establish this as a robust phenomenon(16, 21, 22). However, as an elevated ratio has also been observed in cohorts of PLWH(23), it is plausible that there may be a synergistic effect of HIV and obesity together. Within *Bacteroidetes*, increased relative abundance of *Prevotella* and decreased *Bacteroides* is one of the most common enterotypes associated with HIV infection(12), and similar findings have been described in obese individuals(19). Receptive anal intercourse has been strongly linked with *Prevotella* enrichment(24, 25) and inflammation of the rectal mucosa(26), which may increase susceptibility to HIV infection. HIV and obesity have independently been shown to disrupt barrier function of the mucosal epithelium, allowing translocation of microbes and microbial products into the GI tract(27, 28). This process is highly inflammatory and may exacerbate dysbiosis, significantly contributing to chronic inflammation observed in both HIV and obesity(11, 13, 29–31).

Despite the role of the microbiome in mediating inflammation and data showing that HIV and obesity may synergistically contribute to immune dysfunction and ultimately increase the risk of non-AIDS related morbidity and mortality(7, 32, 33), the joint effect of HIV and obesity on microbial dysbiosis has not been described. Therefore, we examined the combined effects of HIV and obesity on dysbiosis in a cohort of young, mostly racial/ethnic minority men who have sex with men (MSM). We hypothesized that HIV and obesity would act synergistically to decrease overall bacterial diversity and increase the abundance of pro-inflammatory bacterial taxa while simultaneously decreasing those with anti-inflammatory properties.

Methods

Study population.

Specimens were obtained from an ongoing cohort, the Minority Men who have Sex with Men Cohort at UCLA Linking Infections Noting Effects (MASCULINE, or mSTUDY, NIDA U01 DA036267). Both the current study and the mSTUDY were approved by a UCLA Institutional Review Board and all study procedures were done in accordance with ethical standards for research involving human subjects. Sample selection procedures have been previously described(34).

Specimen collection and DNA preparation.

Included specimens were rectal swabs (FLOQSwabs, Copan Diagnostics, Murrieta, CA), the majority (76%) of which were collected via anoscopy under direct mucosal visualization and without preparatory enema at approximately 8 cm from the anal verge. Due to an mSTUDY protocol change, others (24%) were participant self-collected at approximately 4–5 cm from the anal verge. Collection method was taken into account in the analysis (Tables 1 and S1). Swabs were immediately frozen neat at −80°C until processing in bulk. For DNA processing the samples were transferred to Lysing Matrix E tubes (MP Biomedicals, Burlingame, CA) containing RLT lysis buffer (Qiagen, Hilden, Germany) and bead-beated on a TissueLyser (Qiagen). DNA was then extracted using the AllPrep DNA/RNA/Protein kit (Qiagen) per manufacturer's protocol.

16S rRNA gene sequencing and data processing.

Microbiome profiling was performed by sequencing of the V4 region of the 16S rRNA gene as previously described(34–36). Briefly, the V4 region was amplified in triplicate reactions using Golay-barcoded primers 515F/806R. PCR products were then pooled and sequenced on the Illumina MiSeq platform using 2×150bp v2 chemistry. The sequences were demultiplexed with Golay error correction using QIIME v1.9.1(37), and Divisive Amplicon Denoising Algorithm (DADA2) version 1.8 was used for error correction, exact sequence inference, read merging, and chimera removal(38). Likely due to the use of rectal swabs(39), several amplicon sequence variants (ASVs) commonly associated with skin bacteria were removed prior to analyses. Skin contaminants were identified in an aggregated ASV table by the authors, guided by previously published literature on the skin metagenome (e.g., 40, 41). The ASV table utilized in the analyses comprised 17,968,147 total merged read pairs (mean per sample = 47,160; range 5,429 to 122,693). Taxonomic assignment was performed using RDP trainset 16(https://doi.org/10.5281/zenodo.810827). Rarefaction was performed at a depth of 10,906 reads for alpha diversity analyses. To normalize all other analyses, estimates of relative library sizes (“size factors”) were obtained by calculating geometric means of pairwise read count ratios(42). All sequencing data has been deposited into BioProject with the accession number PRJNA422134.

HIV serostatus, obesity, and covariates.

HIV testing was conducted using the OraQuick Advance® HIV 1/2 (OraSure Technologies, Bethlehem, PA) and plasma HIV RNA was quantified using a standard clinical laboratory assay (Cobas® AmpliPrep/Cobas® TaqMan® HIV-1 Test, Version 2.0). Anthropometrics including height, weight, and waist circumference were gathered by trained clinical staff, and participants were classified as obese if they had BMI > 30 or waist circumference > 40 inches. Measurement of waist circumference is recommended by the National Heart, Lung, and Blood Institute as part of an obesity-related risk assessment(43); for men, a waist circumference > 40 inches indicates high risk for the development of obesity-related health conditions.

Demographic and behavioral covariates included in the analyses were age, race/ethnicity, country of origin, a dichotomous variable for homelessness in past six months, number of receptive anal intercourse (RAI) acts in past month, number of sex partners in the past 6 months, positive PCR test for STI (rectal gonorrhea, rectal chlamydia, or syphilis), positive test for hepatitis C, frequency of methamphetamine and marijuana use in the past 6 months, tobacco smoking, and binge drinking. All demographic and behavioral data were self-reported by participants using a computer-aided self-interview; measures have been previously described(34). Antibiotic use in the past month as well as drugs currently used for ART were also controlled in the analyses. These data were collected by trained clinic staff.

Statistical analyses.

To explore the combined effects of HIV and obesity on the microbiome, most analyses in this study compare the “index” group of HIV+ obese (H+O+) participants with three reference groups of HIV+ non-obese (H+O-), HIV- obese (H-O+), and HIV- non-obese (H-

O-) participants. We also compare obese to non-obese participants within strata of HIV status. As we examined the effects of HIV on the microbiome in detail in a previous study(34), we do not make this comparison here. Analyses utilize inverse probability of treatment weighting (IPTW) to control for confounding. In an IPTW analysis, the study sample is re-weighted to balance treatment/exposure groups with respect to covariates used to calculate the weights, creating a "pseudo-population" where these covariates no longer act as confounders. See Tables 1 and S1 for a list of covariates included in the IPTW models. IPTW were estimated using generalized boosted models (R package 'twang') and robust standard errors for IPTW-adjusted analyses were obtained using the sandwich estimator (R package 'sandwich'). See Supplemental Content for a description of the IPTW calculation process.

Prior to analysis, differences in clinical and behavioral covariates between the four HIV and obesity groups were described using standardized mean differences and tested for significance using Chi-square, Kruskal-Wallis, or multinomial logistic regression models. The R package 'phyloseq' (version 1.24.2) was used to calculate alpha diversity statistics, distance matrices, and to create ordination plots. Permutational multivariate ANOVA (PERMANOVA) was used to test for overall differences in microbial composition between groups (R package 'vegan'). IPTW-adjusted linear regression analyses were utilized to test for mean differences in alpha diversity, *Firmicutes/Bacteroidetes* and *Prevotella/Bacteroides* ratios between HIV and obesity groups. These analyses utilized a threshold of $p < .05$ to determine statistical significance and Wald-type 95% confidence intervals are displayed where appropriate.

Zero-inflated negative binomial models (ZINB) with IPTW adjustment were used to test for differential abundance of specific genera; see Supplemental Content for an overview of the ZINB model selection and analytic procedures. A pre-filtering step excluded genera appearing in less than 10% of samples as well as those with less than 100 total reads across all samples, resulting in 73 genera included in ZINB analyses. Because ZINB models may be sensitive to the effects of outliers, differential abundance testing was repeated using nonparametric alternatives (Wilcoxon and Kruskal-Wallis tests). In order to account for the large amount of tests, p values obtained from differential abundance analyses were corrected using Benjamini & Hochberg's False Discovery Rate (FDR) method(44). FDR-adjusted p values are labelled as q values, and $q < .1$ was used as a threshold to determine statistical significance. Accordingly, we display 90% false coverage rate (FCR)-adjusted confidence intervals(45) to accompany these analyses. All statistical analyses were performed using R v.3.5.1.

Results

Demographics and clinical characteristics.

Three hundred eighty-one participants were included; 39 were H+O+, 143 were H+O-, 64 were H-O+, and 135 were H-O-. All participants were MSM, their average age was 31, and most were Hispanic (49%) or non-Hispanic Black (39%). Among the obese participants, the mean BMI was 34.8 and waist circumference was 43.7 inches. Obese participants had less frequent RAI, fewer sex partners, and were less likely to test positive for a rectal STI than

their non-obese peers. Ninety percent of HIV+ participants reported current ART, their mean (log 10) plasma RNA level was 2.0 and CD4 cell count was 626 cells/mm^3. As compared to HIV- participants, HIV+ men were older, more likely to have hepatitis C infection, and more likely to report using methamphetamine and binge drinking in the past 6 months (Tables 1 and S1).

Effects of HIV and obesity on overall microbial composition.

Figure 1A displays the average microbial composition within each group defined by HIV and obesity status after adjustment with IPTW; individual-level compositions are shown in Figures S1 and S2. We calculated Bray-Curtis, Jaccard, and Jensen-Shannon dissimilarity statistics to quantitatively examine differences in overall composition between the HIV and obesity groups. Figure 1B displays ordination of the Bray-Curtis distance by principal coordinates analysis, which suggests similarity of H+O+ subjects. PERMANOVA models suggest that HIV and obesity combined explain a significant amount of between-subject variation in the microbiome, over and above each factor alone (Using Bray-Curtis distance, R^2 for the marginal contribution of H+/O+ = .008, p = .001; additional results in Table S2 and Figure S3). In addition, we estimated the effects of obesity stratified by HIV status. The effect of obesity on overall microbiome composition was larger in the HIV+ stratum as compared to the HIV- stratum [Bray-Curtis R^2 for the effect of obesity in HIV+ = .02 (p = .001), in HIV- = .01 (p = .01); additional results in Table S2].

We also calculated and compared measures of alpha diversity between groups. Figure 1C displays boxplots of Chao1 index values, split by HIV and obesity status. HIV+ individuals generally showed higher diversity than HIV-, with little difference by obesity. Results of a linear regression analysis provide support for these observations. Mean Chao1 diversity among the H+O+ group was higher than the H-O+ group (mean difference = 17.7, p = .036) and the H-O- group (mean difference = 15.8, p = .054), while the difference between H+O+ and H+O- participants was smaller (mean difference = 7.9, p = .3). In metrics that account for evenness (e.g. Shannon index), H+O+ remained significantly different from H-O-, but were not statistically distinguishable from H-O+ or H+O-; additional results are presented in Table S3 and Figure S4.

Differences in Firmicutes/Bacteroidetes and Prevotella/Bacteroides ratios.

Figure 2A displays boxplots of the (natural log) *Firmicutes* to *Bacteroidetes* ratios within each HIV and obesity group. No significant differences were seen on a regression analysis between the H+O+ group and any other group (Figure 2B; Table S4). Boxplots of *Prevotella* to *Bacteroides* ratios are shown in Figure 2C, which show H+O+ participants with the highest values of this ratio. H-O+ and H+O- groups appear to have similar values, and the *Prevotella* to *Bacteroides* ratio is lowest among H-O- participants. A regression analysis confirms that the *Prevotella* to *Bacteroides* ratio was significantly higher among H+O+ participants compared to H-O- (mean difference in log ratio = 1.94, $p < .001$); however, the H+O+ group was not different than the H-O+ or H+O- groups (Figure 2D; Table S4).

Differences in specific genera associated with obesity, stratified by HIV status.

In the absence of HIV, there were few significant differences in relative abundance due to obesity (Figure 3). Obese participants showed enrichment in *Allisonella* and *Succinivibrio* and depletion in *Arcanobacterium* and *Mannheimia* relative to non-obese participants (all $q < .1$). However, within the HIV+ stratum, the microbial signature of obese participants was more distinct, showing enrichment in *Bifidobacterium, Butyricicoccus,* and *Faecalibacterium* and depletion in *Bacteroides, Escherichia/Shigella* and *Gardnerella,* among others, relative to those without obesity ($q < .1$; Figure 3; Table S5). Analyses were repeated using nonparametric tests; generally, this strategy identified additional genera altered by obesity in both the HIV+ and HIV- strata (Figure S5; Table S6).

Effects of HIV and obesity together on microbial abundance.

Finally, we compared the abundance of each taxa between the H+O+ group and H+O-, H-O+, and H-O- groups. We first conducted a joint test of the three comparisons, which indicated that there was at least one significant difference ($q <.1$) between H+O+ and the others in 18 genera including *Bacteroides, Bifidobacterium, Brachyspira, Escherichia/Shigella, Faecalibacterium, Porphyromonas,* and *Prevotella,* among others (Table S7). We then examined individual comparisons for those genera, and as can be seen in Figure 4, different interactions are evident. H+O+ participants had lower levels of *Bacteroides* and *Veillonella* and higher levels of *Bifidobacterium* than all other groups, suggesting a synergistic effect of HIV and obesity on these genera. *Dietzia* was reduced and *Faecalibacterium* was enriched in H+O+ compared to H+O- and H-O+, but not compared to H-O- controls. This suggests that HIV and obesity may have antagonistic effects on these genera, e.g., perhaps both conditions alter the relative abundance of other taxa enough to "normalize" levels of *Dietzia* and *Faecalibacterium*. Finally, *Barnesiella, was* altered by obesity only in the HIV+ stratum*, Porphyromonas* was altered by HIV only in the obese stratum, and *Prevotella* was only enriched in H+O+ compared to H-O- participants. Analyses were repeated using nonparametric tests, which identified many of the same alterations when comparing H+O+ to H+O- and H-O-, but no differences between H+O+ and H-O+ (Figure S5; Table S8)

Discussion

In this study, we explored the combined effects of HIV and obesity on the gastrointestinal microbiome of young, mostly racial/ethnic minority MSM. Analyses of overall microbial composition revealed significant differences between H+O+ participants and those without HIV and/or obesity. Findings were supported by PERMANOVA models showing significant variability in microbial composition explained by the combination of HIV and obesity, over and above the contribution of each condition alone. The effect size we observed ($R^2 = .008$) is small on an absolute scale, likely because of the huge between-person variability in gut microbiome composition, but larger than or comparable to previously published estimates of the effects of BMI(46), diet(47), and HIV itself(25, 34). HIV and obesity did not jointly alter the *Firmicutes*/*Bacteroidetes* ratio, but H+O+ subjects did have the highest *Prevotella/Bacteroides* ratios. We found that obesity was associated with altered abundance of several genera only in the presence of HIV (i.e., only in the HIV+ stratum). Finally, we also noted

that HIV and obesity were associated with synergistically decreased levels of *Bacteroides* and *Veillonella* and increased levels of *Bifidobacterium*. In general, these findings support the hypothesis that microbial composition may be synergistically altered by HIV and obesity.

Contrary to our hypothesis, neither HIV nor obesity was associated with reduced alpha diversity; in fact, PLWH appeared to have greater diversity than HIV-uninfected individuals. Although many studies have previously reported a decrease in richness and diversity due to HIV(48), others have shown that ART may normalize this difference(49, 50) and 90% of PLWH in our study were taking ART and had high CD4 counts. Generally, a healthy rectal microbiome should be highly diverse(51), and our findings may reflect a return to health following successful treatment. In addition, although a recent meta-analysis found reduced diversity in PLWH overall, the difference was no longer evident when the analysis was restricted to MSM(48). With regards to obesity, our results are consistent with most of the studies included in a pooled analysis by Sze and Schloss(16). Although a significant difference in diversity was noted in only two of ten individual studies, Sze and Schloss showed that obesity did in fact reduce richness and evenness when pooling data from multiple cohorts. They concluded that obesity likely has a significant, but small effect size that is unlikely to be detected in moderately-sized studies.

We also hypothesized that HIV and obesity together may be associated with an increased ratio of *Firmicutes* to *Bacteroidetes*, which was not supported by our data. As previously discussed, several re-analyses and meta-analyses have found that this ratio is not elevated by obesity(16, 21, 22), and our findings suggest that the addition of HIV does not change these results. Furthermore, we were able to control for a number of clinical and behavioral confounders such as sexual behavior, which may have exaggerated or spuriously generated previously described differences. On the other hand, we found a significantly higher *Prevotella* to *Bacteroides* ratio among H+O+ individuals compared to H-O- controls. *Bacteroides* species have immune-regulatory properties(52) and, in the context of MSM and HIV, *Prevotella* are considered pro-inflammatory(24). Therefore, this finding is consistent with the theory that HIV and obesity may act synergistically to increase inflammation. Dominance of either *Prevotella* or *Bacteroides* constitutes a microbial enterotype that may be useful in predicting susceptibility to HIV infection(53) as well as response to diabetes medications(54) and weight-loss diets(55), making this ratio highly important to individuals living with HIV and obesity. Although some studies have linked *Prevotella* dominance to sexual behavior(24, 25), all participants in our study were MSM and we controlled for multiple measures of sexual behavior, making it unlikely to explain the increases in *Prevotella* we observed in the H+O+ group. Although our data are observational and it is not possible to rule out residual confounding, our study provides substantial evidence for alterations to the *Prevotella*/*Bacteroides* ratio by HIV and obesity independent of sexual behavior.

We found that *Bifidobacterium* was increased in H+O+ individuals relative to all other groups, a surprising finding, as *Bifidobacterium* is thought to be protective against obesity based on observed associations with weight loss, better glycemic control, reduced adiposity and ability to counteract leptin resistance(56). Interestingly, there may be some interaction

between the shift from *Bacteroides* to *Prevotella* associated with HIV and obesity and the protective effects of *Bifidobacterium*: One study showed that increasing *Bifidobacterium* resulted in improved metabolic parameters in *Bacteroides* but not *Prevotella*-rich subjects(54) and another found a negative association between obesity and *Bifidobacterium,* but only among study subjects of the *Bacteroides*-dominant microbial enterotype(57). Therefore, it is possible that the joint effects of HIV and obesity on the *Prevotella*/*Bacteroides* ratio inhibit the potential metabolic benefits of increased *Bifidobacterium*. We also found that *Faecalibacterium* was increased in H+O+ relative to H+O- and H-O+, but unchanged relative to H-O- controls, a pattern suggesting antagonism between HIV and obesity on the relative abundance of *Faecalibacterium.* A randomized study of 6 weeks of prebiotic therapy among PLWH found a compositional shift in favor of *Faecalibacterium,* which correlated strongly with butyrate production and reduction in inflammatory biomarkers(58). However, if HIV and obesity are truly antagonistic with respect to increasing *Faecalibacterium,* PLWH who are also obese may fail to benefit from such therapy. Validating these suppositions would require an experimental study, but these interactions are examples of insights that may be gained by studying the simultaneous effects of multiple diseases on the microbiome.

Our study is subject to a number of limitations. Primarily, diet information was not available for this cohort. Diet has been shown to have little impact on the microbiome relative to HIV and other confounding factors (e.g. sexual behavior)(25); however, diet is undoubtedly a major determinant of obesity status. We adjusted our analyses for race/ethnicity, country of origin, and homelessness in order to mitigate this limitation; however, diet remains an important omitted confounder. Although most of our behavioral data were self-reports of sensitive topics (e.g. substance use, sexual behavior), we utilized a computer-aided self-interview to reduce social desirability bias. Additionally, IPTW adjustment does not achieve perfect covariate balance between exposure groups in most real-world research applications, and residual confounding is possible. However, our IPTW achieved excellent balance on many of the most important covariates (e.g. antibiotic use, alcohol drinking, sexual behavior), increasing the likelihood that our findings are truly attributable to HIV and obesity. The ability to integrate a large amount of clinical and behavioral data into our analyses using IPTW is a significant strength of this study. Because they rely on assumptions that generally fit microbiome data(59), we used ZINB models to carry out our primary differential abundance analyses. However, because these models have been shown to be sensitive to outliers(60), we repeated our analyses with nonparametric statistics. As expected, results were not fully consistent between strategies. In addition to reducing the influence of unusual observations, a likely reason for the disagreement is that standard nonparametric analyses are not able to adjust for covariates and may be confounded by the variables that we were able to include in our ZINB models. Finally, our study was conducted exclusively in young MSM. Restricting to this group increases internal validity by preventing the influence of some confounders (e.g. gender differences, sexual preferences) but may limit the generalizability of our findings to women or other HIV risk groups. Despite this, our large cohort included adequate numbers of PLWH and obese participants to examine the joint effects of both exposures. Although it is widely accepted that many factors simultaneously impact microbial composition, there have been few studies examining the

combined effects of multiple exposures or behaviors on the microbiome. Finally, our sampling strategy (rectal swabs) likely resulted in stochastic sampling of skin communities(39). Although we removed these bacteria to the best of our ability, we cannot fully rule out the inclusion of skin containments in our samples.

In conclusion, this study of a diverse group of young MSM identified numerous alterations to the gastrointestinal microbiome among H+O+ individuals relative to those with only one or neither conditions. Although this study was observational, research linking dysbiosis with coronary heart disease in obese individuals(61) and PLWH(62) provides an example of the potential clinical implications of dysbosis in this population. We found that the alterations could be synergistic, antagonistic or have no effect, demonstrating the importance of taking bacteria-specific interactions into account when evaluating interventions to address dysbiosis and ameliorate its inflammatory consequences. Finally, HIV and obesity disproportionally affect racial and ethnic minorities(1, 6, 63) and their joint inflammatory effects may partially explain why minorities who are living with HIV experience higher rates of non-AIDS related chronic diseases than Whites(64). Therefore, interventions to reduce microbial dysbiosis in this vulnerable population could have added benefit.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

1. Centers for Disease Control and Prevention. Overweight & Obesity [Available from: https://www.cdc.gov/obesity/data/adult.html.
2. Wang YC, McPherson K, Marsh T, Gortmaker SL, Brown M. Health and economic burden of the projected obesity trends in the USA and the UK. Lancet (London, England). 2011;378(9793):815–25.
3. Crum-Cianflone N, Roediger MP, Eberly L, Headd M, Marconi V, Ganesan A, et al. Increasing rates of obesity among HIV-infected persons during the HIV epidemic. PloS one. 2010;5(4):e10106.
4. Erlandson KM, Lake JE. Fat Matters: Understanding the Role of Adipose Tissue in Health in HIV Infection. Current HIV/AIDS reports. 2016;13(1):20–30. [PubMed: 26830284]
5. Taramasso L, Ricci E, Menzaghi B, Orofino G, Passerini S, Madeddu G, et al. Weight Gain: A Possible Side Effect of All Antiretrovirals. Open forum infectious diseases. 2017;4(4):ofx239.
6. Centers for Disease Control and Prevention. HIV Surveillance Report 2016 [Available from: https://www.cdc.gov/hiv/pdf/library/reports/surveillance/cdc-hiv-surveillance-report-2016-vol-28.pdf.

7. Conley LJ, Bush TJ, Rupert AW, Sereti I, Patel P, Brooks JT, et al. Obesity is associated with greater inflammation and monocyte activation among HIV-infected adults receiving antiretroviral therapy. AIDS (London, England). 2015;29(16):2201–7.
8. Laidlaw E, Gouel-Cheron A, Kuriakose S, Pau A, Rupert A, Nason M, et al., editors. Weight Gain After ARV Initiation Correlates with Increased Inflammatory Biomarkers. 22nd International AIDS Conference; 2018 July 23–27; Amsterdam, Netherlands.
9. Kim DJ, Westfall AO, Chamot E, Willig AL, Mugavero MJ, Ritchie C, et al. Multimorbidity patterns in HIV-infected patients: the role of obesity in chronic disease clustering. Journal of acquired immune deficiency syndromes (1999). 2012;61(5):600–5. [PubMed: 23023101]
10. Kuller LH, Tracy R, Belloso W, De Wit S, Drummond F, Lane HC, et al. Inflammatory and coagulation biomarkers and mortality in patients with HIV infection. PLoS medicine. 2008;5(10):e203. [PubMed: 18942885]
11. Dillon SM, Frank DN, Wilson CC. The gut microbiome and HIV-1 pathogenesis: a two-way street. AIDS (London, England). 2016;30(18):2737–51.
12. Gootenberg DB, Paer JM, Luevano JM, Kwon DS. HIV-associated changes in the enteric microbial community: potential role in loss of homeostasis and development of systemic inflammation. Current opinion in infectious diseases. 2017;30(1):31–43. [PubMed: 27922852]
13. Zevin AS, McKinnon L, Burgener A, Klatt NR. Microbial translocation and microbiome dysbiosis in HIV-associated immune activation. Current opinion in HIV and AIDS. 2016;11(2):182–90. [PubMed: 26679414]
14. Zilberman-Schapira G, Zmora N, Itav S, Bashiardes S, Elinav H, Elinav E. The gut microbiome in human immunodeficiency virus infection. BMC medicine. 2016;14(1):83. [PubMed: 27256449]
15. Liu J, Williams B, Frank D, Dillon SM, Wilson CC, Landay AL. Inside Out: HIV, the Gut Microbiome, and the Mucosal Immune System. Journal of immunology (Baltimore, Md : 1950). 2017;198(2):605–14.
16. Sze MA, Schloss PD. Looking for a Signal in the Noise: Revisiting Obesity and the Microbiome. mBio. 2016;7(4).
17. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Human gut microbes associated with obesity. Nature. 2006;444:1022. [PubMed: 17183309]
18. Armougom F, Henry M, Vialettes B, Raccah D, Raoult D. Monitoring Bacterial Community of Human Gut Microbiota Reveals an Increase in Lactobacillus in Obese Patients and Methanogens in Anorexic Patients. PloS one. 2009;4(9):e7125. [PubMed: 19774074]
19. Zhang H, DiBaise JK, Zuccolo A, Kudrna D, Braidotti M, Yu Y, et al. Human gut microbiota in obesity and after gastric bypass. Proceedings of the National Academy of Sciences of the United States of America. 2009;106(7):2365–70. [PubMed: 19164560]
20. Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. Proc Natl Acad Sci U S A. 2005;102(31):11070–5.
21. Walters WA, Xu Z, Knight R. Meta-analyses of human gut microbes associated with obesity and IBD. FEBS letters. 2014;588(22):4223–33. [PubMed: 25307765]
22. Finucane MM, Sharpton TJ, Laurent TJ, Pollard KS. A taxonomic signature of obesity in the microbiome? Getting to the guts of the matter. PloS one. 2014;9(1):e84689.
23. Ling Z, Jin C, Xie T, Cheng Y, Li L, Wu N. Alterations in the Fecal Microbiota of Patients with HIV-1 Infection: An Observational Study in A Chinese Population. Scientific reports. 2016;6:30673.
24. Neff CP, Krueger O, Xiong K, Arif S, Nusbacher N, Schneider JM, et al. Fecal Microbiota Composition Drives Immune Activation in HIV-infected Individuals. EBioMedicine. 2018;30:192–202. [PubMed: 29650491]
25. Noguera-Julian M, Rocafort M, Guillen Y, Rivera J, Casadella M, Nowak P, et al. Gut Microbiota Linked to Sexual Preference and HIV Infection. EBioMedicine. 2016;5:135–46. [PubMed: 27077120]
26. Kelley CF, Kraft CS, de Man TJ, Duphare C, Lee HW, Yang J, et al. The rectal mucosa and condomless receptive anal intercourse in HIV-negative MSM: implications for HIV transmission and prevention. Mucosal immunology. 2017;10(4):996–1007. [PubMed: 27848950]

27. Marchetti G, Tincati C, Silvestri G. Microbial translocation in the pathogenesis of HIV infection and AIDS. Clinical microbiology reviews. 2013;26(1):2–18. [PubMed: 23297256]
28. Cox AJ, West NP, Cripps AW. Obesity, inflammation, and the gut microbiota. The lancet Diabetes & endocrinology. 2015;3(3):207–15. [PubMed: 25066177]
29. Cavalcante-Silva LH, Galvao JG, da Silva JS, de Sales-Neto JM, Rodrigues-Mascarenhas S. Obesity-Driven Gut Microbiota Inflammatory Pathways to Metabolic Syndrome. Frontiers in physiology. 2015;6:341. [PubMed: 26635627]
30. Sanz Y, Moya-Perez A. Microbiota, inflammation and obesity. Advances in experimental medicine and biology. 2014;817:291–317. [PubMed: 24997040]
31. d'Ettorre G, Ceccarelli G, Giustini N, Serafino S, Calantone N, De Girolamo G, et al. Probiotics Reduce Inflammation in Antiretroviral Treated, HIV-Infected Individuals: Results of the "Probio-HIV" Clinical Trial. PloS one. 2015;10(9):e0137200.
32. Lake JE. The Fat of the Matter: Obesity and Visceral Adiposity in Treated HIV Infection. Current HIV/AIDS reports. 2017;14(6):211–9. [PubMed: 29043609]
33. Koethe JR, Heimburger DC, PrayGod G, Filteau S. From Wasting to Obesity: The Contribution of Nutritional Status to Immune Activation in HIV Infection. The Journal of infectious diseases. 2016;214 Suppl 2:S75–82. [PubMed: 27625434]
34. Cook RR, Fulcher JA, Tobin NH, Li F, Lee D, Javanbakht M, et al. Effects of HIV viremia on the gastrointestinal microbiome of young MSM. AIDS (London, England). 2019;33(5):793–804.
35. Bender JM, Li F, Martelly S, Byrt E, Rouzier V, Leo M, et al. Maternal HIV infection influences the microbiome of HIV-uninfected infants. Science translational medicine. 2016;8(349):349ra100.
36. Bender JM, Li F, Adisetiyo H, Lee D, Zabih S, Hung L, et al. Quantification of variation and the impact of biomass in targeted 16S rRNA gene sequencing studies. Microbiome. 2018;6(1):155. [PubMed: 30201048]
37. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK, et al. QIIME allows analysis of high-throughput community sequencing data. Nature methods. 2010;7(5):335–6. [PubMed: 20383131]
38. Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP. DADA2: High-resolution sample inference from Illumina amplicon data. Nature methods. 2016;13(7):581–3. [PubMed: 27214047]
39. Biehl LM, Garzetti D, Farowski F, Ring D, Koeppel MB, Rohde H, et al. Usability of rectal swabs for microbiome sampling in a cohort study of hematological and oncological patients. PloS one. 2019;14(4):e0215428.
40. Grice EA. The intersection of microbiome and host at the skin interface: genomic- and metagenomic-based insights. Genome Research. 2015;25(10):1514–20. [PubMed: 26430162]
41. Oh J, Byrd AL, Deming C, Conlan S, Program NCS, Barnabas B, et al. Biogeography and individuality shape function in the human skin metagenome. Nature. 2014;514:59. [PubMed: 25279917]
42. Chen L, Reeve J, Zhang L, Huang S, Wang X, Chen J. GMPR: A robust normalization method for zero-inflated count data with application to microbiome sequencing data. PeerJ. 2018;6:e4600. [PubMed: 29629248]
43. National Heart Lung and Blood Institute. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: The evidence report 1998 [Available from: https://www.nhlbi.nih.gov/files/docs/guidelines/ob_gdlns.pdf.
44. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. Journal of the royal statistical society Series B (Methodological). 1995:289–300.
45. Benjamini Y, Yekutieli D. False Discovery Rate–Adjusted Multiple Confidence Intervals for Selected Parameters. Journal of the American Statistical Association. 2005;100(469):71–81.
46. Zhernakova A, Kurilshikov A, Bonder MJ, Tigchelaar EF, Schirmer M, Vatanen T, et al. Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. Science (New York, NY). 2016;352(6285):565–9.
47. Falony G, Joossens M, Vieira-Silva S, Wang J, Darzi Y, Faust K, et al. Population-level analysis of gut microbiome variation. Science (New York, NY). 2016;352(6285):560–4.

48. Tuddenham SA, Koay WLA, Zhao N, White JR, Ghanem KG, Sears CL. The Impact of HIV Infection on Gut Microbiota Alpha-Diversity: An Individual Level Meta-analysis. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America. 2019.
49. Lozupone CA, Rhodes ME, Neff CP, Fontenot AP, Campbell TB, Palmer BE. HIV-induced alteration in gut microbiota: driving factors, consequences, and effects of antiretroviral therapy. Gut microbes. 2014;5(4):562–70. [PubMed: 25078714]
50. Monaco CL, Gootenberg DB, Zhao G, Handley SA, Ghebremichael MS, Lim ES, et al. Altered Virome and Bacterial Microbiome in Human Immunodeficiency Virus-Associated Acquired Immunodeficiency Syndrome. Cell host & microbe. 2016;19(3):311–22. [PubMed: 26962942]
51. Human Microbiome Project Consortum. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486(7402):207–14. [PubMed: 22699609]
52. Troy EB, Kasper DL. Beneficial effects of Bacteroides fragilis polysaccharides on the immune system. Frontiers in bioscience (Landmark edition). 2010;15:25–34. [PubMed: 20036803]
53. Sui Y, Dzutsev A, Venzon D, Frey B, Thovarai V, Trinchieri G, et al. Influence of gut microbiome on mucosal immune activation and SHIV viral transmission in naive macaques. Mucosal immunology. 2018;11(4):1219–29. [PubMed: 29858581]
54. Gu Y, Wang X, Li J, Zhang Y, Zhong H, Liu R, et al. Analyses of gut microbiota and plasma bile acids enable stratification of patients for antidiabetic treatment. Nature communications. 2017;8(1):1785.
55. Hjorth MF, Roager HM, Larsen TM, Poulsen SK, Licht TR, Bahl MI, et al. Pre-treatment microbial Prevotella-to-Bacteroides ratio, determines body fat loss success during a 6-month randomized controlled diet intervention. International journal of obesity (2005). 2018;42(2):284. [PubMed: 29406520]
56. Mulders RJ, de Git KCG, Schele E, Dickson SL, Sanz Y, Adan RAH. Microbiota in obesity: interactions with enteroendocrine, immune and central nervous systems. Obesity reviews : an official journal of the International Association for the Study of Obesity. 2018;19(4):435–51. [PubMed: 29363272]
57. Nakayama J, Watanabe K, Jiang J, Matsuda K, Chao SH, Haryono P, et al. Diversity in gut bacterial community of school-age children in Asia. Scientific reports. 2015;5:8397. [PubMed: 25703686]
58. Serrano-Villar S, Vazquez-Castellanos JF, Vallejo A, Latorre A, Sainz T, Ferrando-Martinez S, et al. The effects of prebiotics on microbial dysbiosis, butyrate production and immunity in HIV-infected subjects. Mucosal immunology. 2017;10(5):1279–93. [PubMed: 28000678]
59. Xu L, Paterson AD, Turpin W, Xu W. Assessment and Selection of Competing Models for Zero-Inflated Microbiome Data. PloS one. 2015;10(7):e0129606.
60. Chen J, King E, Deek R, Wei Z, Yu Y, Grill D, et al. An omnibus test for differential distribution analysis of microbiome sequencing data. Bioinformatics (Oxford, England). 2018;34(4):643–51.
61. Kasselman LJ, Vernice NA, DeLeon J, Reiss AB. The gut microbiome and elevated cardiovascular risk in obesity and autoimmunity. Atherosclerosis. 2018;271:203–13. [PubMed: 29524863]
62. Kehrmann J, Menzel J, Saeedghalati M, Obeid R, Schulze C, Holzendorf V, et al. Gut Microbiota in Human Immunodeficiency Virus-Infected Individuals Linked to Coronary Heart Disease. The Journal of infectious diseases. 2019;219(3):497–508. [PubMed: 30202890]
63. Ogden CL, Lamb MM, Carroll MD, Flegal KM. Obesity and socioeconomic status in adults: United States, 2005–2008. NCHS data brief. 2010(50):1–8.
64. Buchacz K, Baker RK, Palella FJ Jr., Shaw L, Patel P, Lichtenstein KA, et al. Disparities in prevalence of key chronic diseases by gender and race/ethnicity among antiretroviral-treated HIV-infected adults in the US. Antiviral therapy. 2013;18(1):65–75. [PubMed: 23111762]

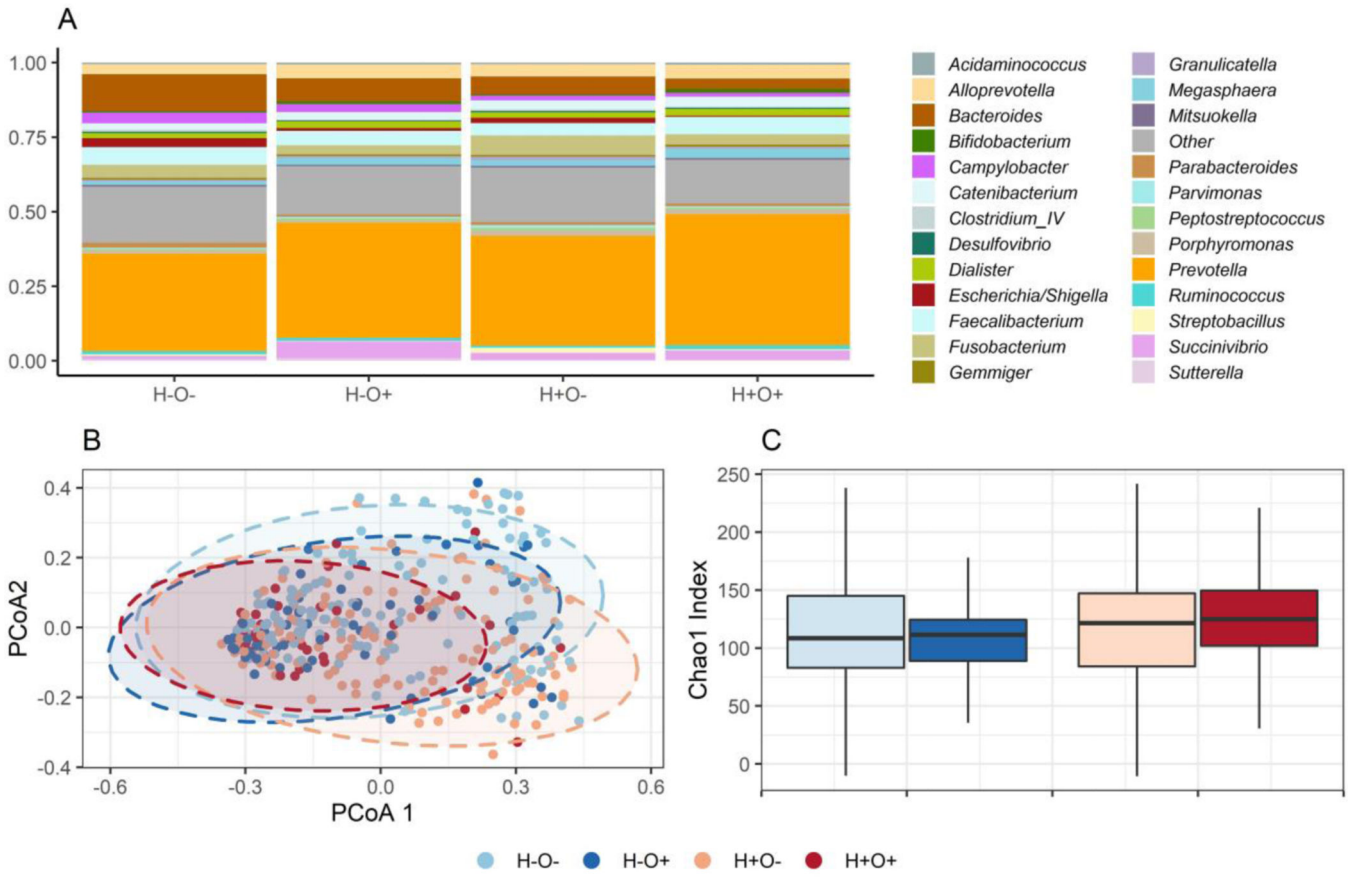

Figure 1. Rectal microbial composition, ordination of Bray-Curtis distances, and Chao1 diversity of study participants, N = 381.

A) Average microbial composition within each HIV and obesity category, adjusted for behavioral and clinical confounders using inverse probability of treatment weighting. Groups are HIV-/Non-obese (H-O-), HIV-/Obese (H-O+), HIV+/Non-obese (H+O-), and HIV+/Obese (H+O+). Bacterial genera representing less than 1% of the overall relative composition or present in less than 20% of the samples were grouped into "Other." (B) Ordination of Bray-Curtis distances between samples using principal coordinates analysis. PCoA = Principal coordinate axis. Ellipses are 95% confidence regions for each group assuming points follow a multivariate *t* distribution. (C) Boxplots of Chao1 index vales. Boxes represent the lower, median, and upper quartile of the data and whiskers are 1.5*interquartile range.

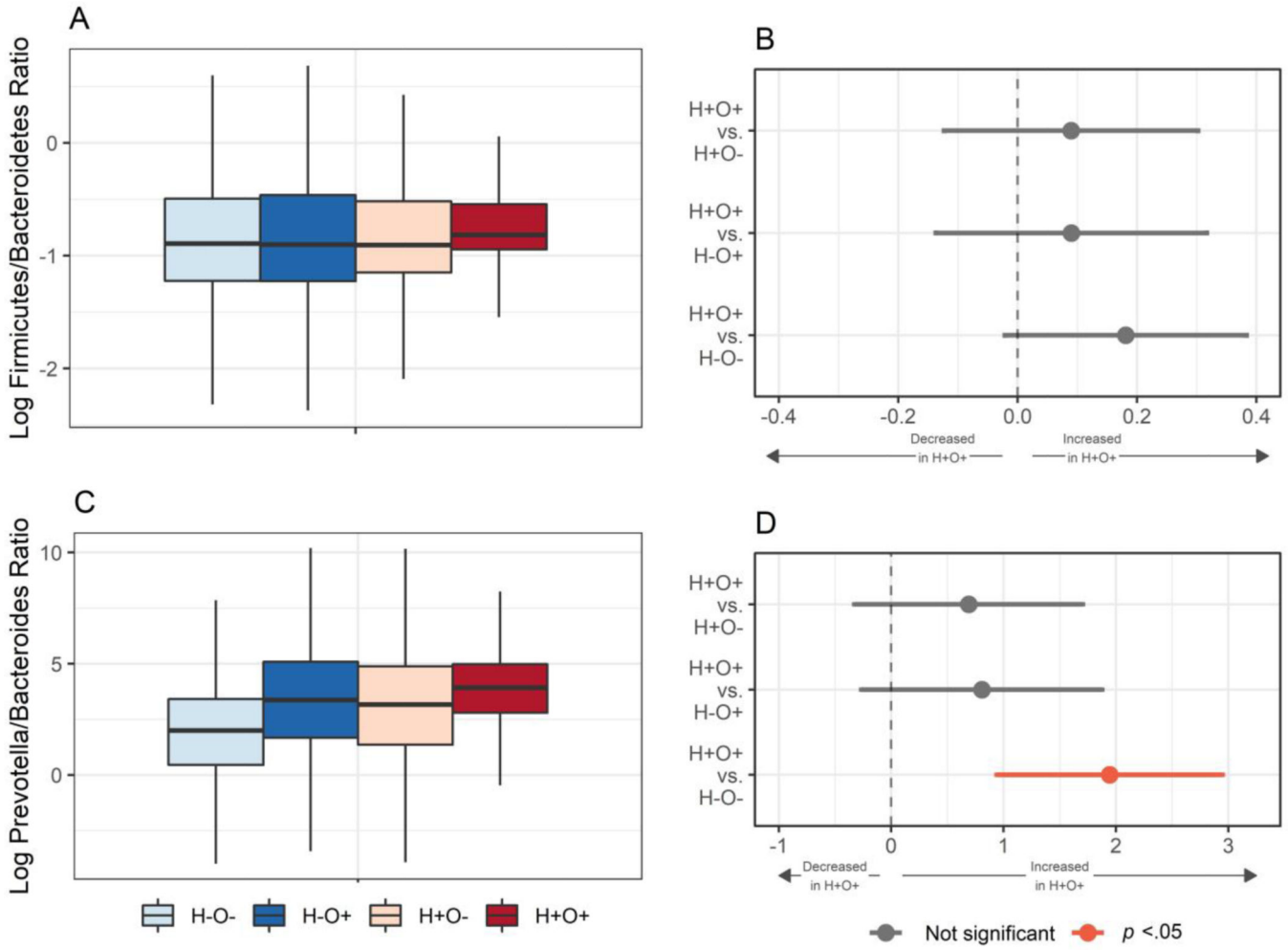

Figure 2. Ratios of *Firmicutes* to *Bacteroidetes* and *Prevotella* to *Bacteroides*.

(A) Boxplots of log *Firmicutes* to *Bacteroidetes* ratios. Boxes represent the lower, median, and upper quartile of the data and whiskers are 1.5*interquartile range. (B) Mean differences in ratios and Wald 95% confidence intervals, adjusted for behavioral and clinical confounders using inverse probability of treatment weighting (IPTW). The HIV+/Obese (H+O+) group is compared to the HIV-/Non-obese (H-O-), HIV-/Obese (H-O+), and HIV+/Non-obese groups (H+O-). (C) Boxplots of log *Prevotella* to *Bacteroides* ratios. (D) IPTW-adjusted mean differences in ratios and 95% confidence intervals. *p* values for comparisons are presented in table S4.

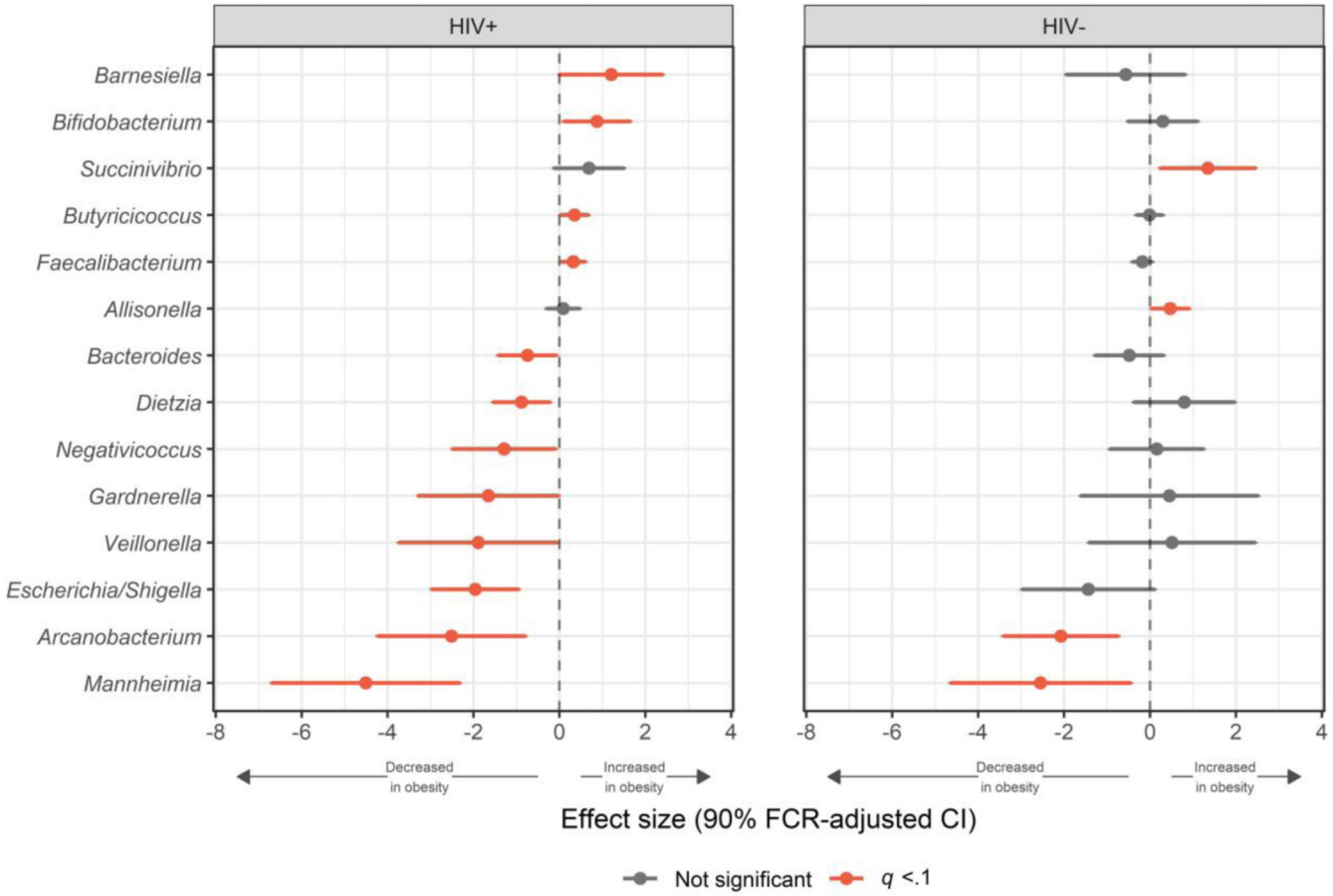

Figure 3. Effects of obesity on individual bacterial genera, stratified by HIV status.

Forest plots of results of zero-inflated negative binomial models comparing genus-level bacterial counts between obese and non-obese participants, stratified by HIV status. Inverse probability of treatment-weighted effect sizes and false coverage rate (FCR)-adjusted 90% confidence intervals (truncated at −6, 6) are plotted, with statistical significance ($q < 0.1$) indicated in red. Effect sizes are log ratios of normalized genera counts. Genera without significant differences in either strata are not shown; p and q values for all genera are presented in table S5.

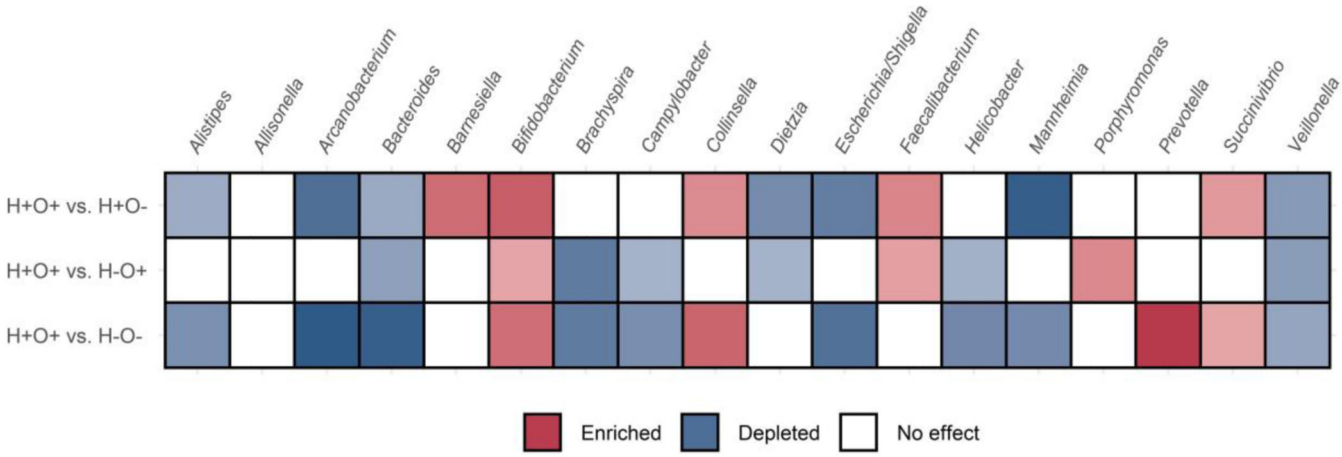

Figure 4. Combined effects of HIV and obesity on individual bacterial genera.

Heatmap of results of zero-inflated negative binomial models comparing genus-level bacterial counts between HIV+/Obese (H+O+) and HIV-/Non-obese (H-O-), HIV-/Obese (H-O+), and HIV+/Non-obese (H+O-) participants. Statistically significant results ($q < .1$) are colored with intensity proportional to effect size; "no effect" indicates $q > .1$. Genera with joint test $q > .1$ are not shown; p and q values for all comparisons are presented in table S7.

Table 1.

Participant characteristics, N = 381 men who have sex with men in Los Angeles, CA

	HIV-/ Non-obese mean (sd)/ n (%)	HIV-/Obese	HIV+/ Non-obese	HIV+/ Obese	*p*¶
n	135	64	143	39	
Age	28.1 (6.3)	30.5 (6.6)	33.1 (6.7)	35.6 (5.4)	<.001
Race/Ethnicity					.263
Black Non- Hispanic	53 (39.3)	29 (45.3)	54 (37.8)	14 (35.9)	
Hispanic	63 (46.7)	33 (51.6)	70 (49.0)	20 (51.3)	
Other Non- Hispanic	19 (14.1)	2 (3.1)	19 (13.3)	5 (12.8)	
Country of origin					.150
United States	113 (83.7)	58 (90.6)	115 (80.4)	29 (74.4)	
Other	22 (16.3)	6 (9.4)	28 (19.6)	10 (25.6)	
Homeless in past 6 months	50 (37.0)	16 (25.0)	52 (36.4)	11 (28.2)	
Number of RAI acts in past month	2.3 (4.7)	1.3 (2.6)	3.2 (6.5)	2.0 (3.1)	.175
Number of anal sex partners in past 6 months	7.3 (8.1)	6.2 (7.2)	8.0 (9.2)	5.9 (8.2)	.182
Positive for STI by PCR test †	18 (13.3)	3 (4.7)	23 (16.1)	3 (7.7)	.101
Positive for HCV	3 (2.2)	0	10 (7.0)	4 (10.3)	.02
Methamphetamine use in past 6 months					<.001
Daily/Weekly	16 (11.9)	6 (9.4)	46 (32.2)	7 (17.9)	
Monthly/less	35 (11.1)	17 (26.6)	40 (28.0)	9 (23.1)	
Never	104 (77.0)	41 (64.1)	57 (39.9)	23 (59.0)	
Marijuana use					.289
Daily/Weekly	49 (36.3)	21 (32.8)	48 (33.6)	11 (28.2)	
Monthly/less	38 (28.1)	19 (29.7)	29 (20.3)	7 (17.9)	
Never	48 (35.6)	24 (37.5)	66 (46.2)	21 (53.8)	
Tobacco smoker	53 (39.3)	25 (39.1)	74 (51.7)	16 (41.0)	.140
Binge drinking in past 6 months ‡					.002
Weekly	18 (13.3)	6 (9.4)	26 (18.2)	5 (12.8)	
Monthly/less	70 (51.9)	41 (64.1)	50 (35.0)	13 (33.3)	
Never	48 (34.8)	17 (26.6)	67 (46.9)	21 (53.8)	
Antibiotic use	9 (6.7)	2 (3.1)	15 (10.5)	5 (12.8)	.191
Sample collection strategy					.463
Anoscopy	107 (79.3)	46 (71.9)	111 (77.6)	27 (69.2)	
Self-collected	28 (20.7)	18 (28.1)	32 (22.4)	12 (30.8)	
HIV RNA $\log_{10}$ copies/mL (median, IQR) §			1.6 (1.9)	1.5 (1.5)	N/A
CD4 cells/mm^3 (median, IQR) §			603 (341)	632 (419)	N/A
CD4 cells/mm^3 < 200			11 (0.08)	3 (0.08)	N/A
ART regimen					N/A

	HIV-/ Non-obese mean (sd)/ n (%)	HIV-/Obese	HIV+/ Non-obese	HIV+/ Obese	*p*¶
NRTI + INSTI			51 (35.7)	17 (43.6)	
NRTI + NNRTI			43 (30.1)	5 (12.8)	
NRTI + PI			22 (15.4)	8 (20.5)	
Other			11 (7.7)	5 (12.8)	
Missing/Not reported/NA			16 (11.2)	4 (10.3)	
Tenofovir disoproxil fumarate/emtricitabine for pre-exposure prophylaxis (PrEP)	24 (17.8)	12 (18.8)			N/A
BMI §	24.3 (2.9)	34.8 (5.7)	24.4 (3.0)	35.0 (7.3)	N/A
Waist circumference (inches) §	33.2 (3.1)	43.6 (6.0)	34.5 (3.1)	43.8 (5.9)	N/A

RAI = Receptive anal intercourse; STI = Sexually transmitted infection; HCV = hepatitis C virus; ART = Antiretroviral therapy; INSTI = Integrase strand transfer inhibitor; NRTI = Nucleoside reverse transcriptase inhibitor; NNRTI = Non-nucleoside reverse transcriptase inhibitor; PI = Protease inhibitor

† Sexually transmitted infections include rectal gonorrhea and chlamydia as well as syphilis.

‡ Binge drinking defined as 6 or more drinks on one occasion.

§ HIV RNA, CD4 cell count, waist circumference, and BMI were not included in the inverse probability of treatment weight model, all other variables in the table were included.

¶ *p* values are from Kruskal-Wallis tests, Chi-square tests, or multinomial logistic regression models depending on variable distributions. If the latter, the p value represents a likelihood ratio test of all model coefficients vs. an intercepts-only model.