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Alcohol use alters the colonic mucosa-associated gut microbiota in humans

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Abstract

Alcohol misuse is a risk factor for many adverse health outcomes. Alcohol misuse has been associated with an imbalance of gut microbiota in preclinical models and alcoholic diseases. We hypothesized that daily alcohol use would change the community composition and structure of the human colonic gut microbiota. Thirty-four polyp-free individuals donated 97 snap-frozen colonic biopsies. Microbial DNA was sequenced for the 16S rRNA gene V4 region. The SILVA database was used for operational taxonomic unit classification. Alcohol use was assessed using a food frequency questionnaire. We compared the biodiversity and relative abundance of the taxa among never drinkers (ND, $n = 9$), former drinkers (FD, $n = 10$), current light drinkers (LD, < 2 drinks daily, $n = 9$), and current heavy drinkers (HD, ≥ 2 drinks daily, $n = 6$). False discovery rate-

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Author's Contributions

Li Jiao: Conceptualization, Methodology, Data Curation and Analysis, Supervision, Funding Acquisition. **Shawn Gurwara, Annie Dai, Albert Jang:** Writing- Original Manuscript Preparation. **Nadim J. Ajami and Joseph H. Petrosino:** Software, Project Administration, Data Curation. **Liang Chen:** Investigation. **Donna L. White:** Investigation. Writing – Review and Editing. **David Y. Graham, Hashem B. El-Serag:** Resource. Writing – Review and Editing. **Ellie Chen:** Writing – Review and Editing

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Supplemental Materials

Supplemental materials were provided for this submission that include Table S1, Mean relative abundance (%) of less common bacterial genera (relative abundance between 0.05% and 1%) differed significantly based on alcohol use; Table S2, Mean relative abundance (%) of bacterial genera based on alcohol use in 23 sigmoid biopsies; Table S3, Bacterial functional pathways differed significantly by alcohol use ($q < 0.05$); and 3 supplemental figures.

adjusted P values (q values) < 0.05 indicated statistical significance. HD had the lowest alpha diversity (Shannon index q value < 0.001), and HD's microbial composition differed the most from the other groups (P value = 0.002). LD had the highest relative abundance of *Akkermansia* (q values < 0.001). HD had the lowest relative abundance of *Subdoligranulum*, *Roseburia*, and *Lachnospiraceaeunc91005*, but the highest relative abundance of *Lachnospiraceaeunc8895* (all q values < 0.05). The multivariable negative binomial regression model supported these observations. ND and FD had a similar microbial profile. Heavy alcohol use was associated with impaired gut microbiota that may partially mediate its effect on health outcomes.

Keywords

microbiome; ethanol; epidemiology; diet

1. Introduction

Heavy alcohol use, defined as at least one drink daily for women and two drinks daily for men, has been associated with a higher risk of cardiovascular diseases, cirrhosis, and malignancy of liver [1], colon-rectum [2], and pancreas [3]. Several mechanisms have been proposed for the pathogenesis of alcohol-induced complications, including direct toxicity of ethanol and its metabolites such as acetaldehyde [4–6], oxidative stress, and accumulation of fatty acid ethyl esters [7]. The accumulation of endogenous and exogenous ethanol promotes the production of carcinogenic and toxic acetaldehyde [8]. Acetaldehyde at high concentrations has been shown to disrupt the microbiota equilibrium and cause direct hepatocyte injury [9]. Preclinical animal models have shown that alcohol misuse can induce an imbalance of gut microbiota and alcoholic liver diseases [10, 11].

The association between alcohol use and gut microbiota has been examined in a few small-scale studies in patients with alcohol-induced diseases using the 16S ribosomal RNA (rRNA) gene sequencing analysis. Increased Proteobacteria and decreased Bacteroidetes in fecal samples or snap-frozen colonic biopsies have been found in patients with liver cirrhosis or alcoholic conditions [12, 13]. Another study in patients with excessive alcohol use showed increased Proteobacteria and decreased *Faecalibacterium* in feces, suggesting more inflammatory gut microbiota in long-term alcohol users [14]. A study in patients with alcohol dependence syndrome showed an increased abundance of *Bifidobacterium*, whose species possess the ability to accumulate acetaldehyde [15]. However, the association between alcohol use and the gut microbiota in the general human population is unknown.

We hypothesized that varying amounts of alcohol use would be associated with alterations in the gut microbial community composition and structure in the colonic mucosa. We performed the taxonomic analysis using the 16S rRNA gene sequencing in the colonic mucosal samples of 34 polyp-free participants in this cross-sectional study. We ascertained the alcohol use using the food frequency questionnaire (FFQ). We compared the biodiversity and the relative abundance of bacterial taxa in individuals who did not drink alcohol or who drank varying amounts of alcohol.

2. Methods and materials

2.1. Study setting

This research used convenience samples from two studies in which participants were prospectively recruited from veterans at the endoscopy suite of the Michael E. DeBakey VA Medical center (MEDVAMC) in Houston between July 2013 and April 2017. One was an individually matched case-control study investigating the associations between gut microbiota and the risk of developing colorectal adenoma. The other study was to examine the longitudinal distribution of gut microbiota along the large intestine in polyp-free individuals. We used the data and biospecimens collected from the controls, i.e., individuals with endoscopically confirmed normal colon, for the present analysis. The eligibility criteria, study cohort, and enrollment procedure have been described previously [16].

2.2. Data collection

After obtaining informed consent, the research coordinator administered a questionnaire to collect information on lifestyle, social history, and medical history. We assessed alcohol use (type: beer, wine or wine coolers, liquor or mixed drinks; frequency; and quantity) in the past 12 months using a self-administered validated Block FFQ (2005 version) [17]. The FFQ data were processed at the NutritionQuest, and daily intake of food and nutrients was calculated. Dietary intake data were energy-adjusted using the density method [18]. There was no missing information on the FFQ. Self-reported alcohol use information was used in conjunction with the FFQ to determine alcohol use status. Among current drinkers, we categorized the amount of alcohol use based on grams of alcohol consumed per day. One drink was defined based on the US Department of Agriculture's Food Guide Pyramid as 12 fluid ounces of regular beer (12.96 grams of alcohol), 5 fluid ounces of wine (13.72 grams of alcohol), or 1.5 ounces of 80 proof distilled spirits liquor (13.93 grams of alcohol) [19]. A healthy eating index (HEI) score was calculated to indicate each participant's dietary quality [20].

2.3. Colonoscopy and biopsy acquirement

Adequate endoscopic visualization was ensured. The endoscopists obtained biopsies from each colonic segment (cecum, ascending colon, transverse, descending, sigmoid colon, or rectum) when possible. The snap-frozen biopsy samples were stored at -80°C within 15 minutes after collection. Participants were advised to stop taking aspirin, anti-inflammatory drugs, blood thinners, iron, or vitamins with iron seven days before the procedure and stop diabetic medication one day before the procedure.

2.4. Study participants

We enrolled 612 eligible study participants. Of 174 study participants who were confirmed polyp-free, 135 consented to provide one or multiple colonic mucosal biopsies. We sent the biopsies of 69 participants for microbiota profiling in the parent studies. Among them, 46 were given the FFQ, and 40 returned the FFQ. Five participants who had self-reported energy consumption < 800 or $> 5,000$ kcal per day were excluded from the analysis. Therefore, we had the sequencing data of 99 mucosa samples from 35 study participants.

The flow chart of participant selection is shown in Fig 1. These 35 participants had the same distribution of age and BMI as 174 polyp-free participants in our study. However, the proportion of current smokers was higher in 35 participants than 174 polyp-free participants (20% vs. 14%). The proportion of never drinkers, former drinkers, and current drinkers was 27%, 29%, and 44% in 35 participants, compared to 22%, 21%, and 57% in 174 participants. The study protocol was approved by the Institutional Review Boards of BCM and MEDVAMC. An informed consent form was obtained from all participants.

2.5. 16S rRNA gene sequencing

2.5.1. Microbial DNA extraction and library preparation—The microbiota profiling was performed at the Alkek Center for Metagenomics and Microbiome Research (CMMR) at Baylor College of Medicine (BCM). Microbial genomic DNA was extracted from biopsies using the MO BIO PowerLyzer UltraClean Tissue & Cell DNA Isolation Kits (MO BIO Laboratories, Carlsbad, CA). All DNA samples were stored at -80°C until further analysis.

The 16S rRNA gene hypervariable region 4 (V4) was amplified by PCR using the barcoded Illumina adaptor-containing primers 515F and 806R and sequenced in the MiSeq platform (Illumina, San Diego, CA). The 2×250 bp paired-end protocol yields pair-end reads that overlap almost completely [21]. The samples were sequenced in two batches.

2.5.2. Bioinformatics and taxonomic assignment—We used the CMMR bioinformatics pipeline for data analysis. The reads were merged using USEARCH v7.0.1090. A quality filter was applied to the resulting merged reads, and those containing above 0.5% expected errors were discarded. The 16S rRNA gene sequences were clustered into the Operational Taxonomic Units (OTUs) at a similarity cutoff value of 97% using the UPARSE algorithm [22]. Chimeras were removed using the USEARCH v7.0.1090 and UCHIME. The OTUs were mapped to the SILVA database (v128) [23].

A rarefied OTU table was constructed for the downstream analyses of alpha and beta diversity, and phylogenetic trends using the Agile Toolkit for Incisive Microbial Analyses (ATIMA) [24]. The samples were rarefied to 1,648 reads. The rarefaction resulted in the loss of two mucosal samples with the poor sequence reads, including one sample from a participant who contributed one piece of biopsy. Therefore, we eventually included 97 mucosal samples from 34 participants in the final analysis (Fig. 1).

2.5.3. Tax4fun analysis—We used the Tax4fun in R package to predict the functional capabilities of the bacterial communities based on the 16S rRNA gene sequencing data linked to the prokaryotic KEGG Ortholog reference profile [25]. The metabolic functional pathway output (relative abundance $\geq 0.1\%$) inferred by Tax4Fun was analyzed using ATIMA.

2.6. Statistical analyses

Patients were categorized into never drinkers (ND, $n = 9$), former drinkers (FD, $n = 10$), current light drinkers (LD, < 2 drinks daily, $n = 9$), and current heavy drinkers (HD, ≥ 2 drinks daily, $n = 6$). All heavy drinkers consumed > 48 grams of alcohol daily in our study.

Sociodemographic and clinical characteristics of the study participants were compared based on alcohol use using ANOVA for continuous variables or Fisher's exact test for categorical variables. Alpha diversity was evaluated using the Shannon index and the Simpson index. The Simpson and Shannon diversity indices were compared using the Kruskal-Wallis test in ATIMA by default. The Shannon diversity index was also compared based on alcohol use using ANOVA because it followed the normal distribution. To evaluate beta-diversity, principal coordinate analysis (PCoA) plots were constructed to visualize the dissimilarity of the community composition based on alcohol use using the weighted UniFrac as the distance matrix [26]. The PERMANOVA was used to test if centroids of distance matrices differed by alcohol use [27].

The difference in the relative abundance of bacterial taxa (phylum and genera) based on alcohol use was compared using the Kruskal-Wallis test. For those major genera (relative abundance $\geq 1\%$) that differed significantly based on alcohol use, we used the multivariable negative binomial regression analysis [28] with panel data to estimate the incident rate ratio (IRR) and its 95% confidence interval (CI) of having a non-zero count of major bacterial genera among FD, LD, and HD, compared to ND, adjusting for age (continuous), race/ethnicity (Non-Hispanic Caucasian, African American, and Hispanic), body mass index (continuous), smoking status (never, former, and current), hypertension (yes vs. no), diabetes (yes vs. no), and biopsy segment. We further adjusted the HEI score (continuous) in the model to account for the confounding effect of diet. The rarefied bacterial count was the dependent variable. The panel data function was used because some participants contributed more than one biopsy to the analysis.

Because our previous study showed that the gut microbiota was associated with dietary quality [16], we examined the association between alcohol use and the gut microbiota stratifying by dietary quality. Lower dietary quality was defined as HEI < 60 , the median value in 34 participants. Additional stratified analyses were performed by smoking status (ever vs. never). Our previous study based on 27 individuals showed that the bacterial biodiversity did not differ by the colon segments (Supplemental Fig. S1). Nevertheless, we limited the analysis in 23 sigmoid samples as a sensitivity analysis.

2.7. Power calculation

We calculated the study power based on the ANOVA test of the difference in the Shannon index or relative abundance of a bacterial phylum between ND, FD, LD, and HD. The Shannon index followed the normal distribution in our samples. We assumed the distribution of the major phylum follows the normal distribution. The sample size weight was 4:4:2:1 in ND, FD, LD, and HD, the within-group variance was 0.1 for Shannon index and 0.024 for the relative abundance of bacterial phylum, and the alpha level was 0.05. We would have 99% power to detect the difference when the Shannon index was 2.80, 2.80, 2.80, and 2.10

in ND, FD, LD, and HD, respectively, with 97 mucosal biopsies. The power to detect such a difference in 23 sigmoid samples was 65%. Similarly, we would have 80% power to detect the difference when the relative abundance of the bacterial phylum was 20%, 20%, 10%, and 5% in ND, FD, LD, and HD, respectively, with 97 mucosal biopsies. The power to detect such a difference in 23 sigmoid samples was 32%.

We used STATA 9.3 (Stata Corp LLC, College Station, TX, USA) and the latest R program for data analysis and power calculation. All tests were two-sided. In the general analysis, P value < 0.05 indicated statistical significance. In the microbiota analysis, all P -values were adjusted for multiple comparisons using the false discovery rate (FDR) algorithm [29]. FDR-adjusted P -values (q values) < 0.05 indicated statistical significance [30].

3. Results

3.1. Characteristics of participants

Table 1 shows that there were no statistically significant differences in the distribution of the characteristics of study participants based on alcohol use. One woman included in the analysis was a current light drinker. Daily alcohol use ranged from 1.6 grams to 28 grams for current light drinkers and 41 grams for current heavy drinkers. Wine and beer were the most frequently used alcoholic beverages.

3.2. Biodiversity

We identified 120 OTUs with a relative abundance of $> 0.05\%$. We found similarities in alpha diversity indexes (Shannon and Simpson) in ND, FD, and LD. HD had the lowest alpha-diversity indices (Fig. 2). Beta diversity, i.e., the community composition, of the gut bacteria in HD had the highest dissimilarity from the other groups (P value = 0.002) (Fig. 3). The sensitivity analysis based on 23 sigmoid samples showed a nonsignificant lower alpha diversity indices and the dissimilarity of community composition in HD than in other groups (data not shown).

3.3. Relative abundance at the phylum and family level

At the phylum level, LD had the highest, while HD had the lowest relative abundance of Verrucomicrobia (q value = 0.001); HD had the highest relative abundance of the Proteobacteria (q value = 0.007), and LD had the lowest abundance of Actinobacteria (q value = 0.02) (Fig. 3). At the family level, LD had the highest, while HD had the lowest relative abundance of Verrucomicrobiaceae (q values < 0.05) (Supplemental Figure S2).

3.4. Relative abundance at the genus level

A total of 36 out of 99 genera significantly differed by the amount of alcohol use. Except for *Bacteroides* and *Fusobacterium*, all major bacteria ($> 1\%$ relative abundance) differed in relative abundance based on alcohol use. ND had the highest, while HD had the lowest relative abundance of *Faecalibacterium*, *Subdoligranulum*, *Sutterella*, *Alistipes*, and *Roseburia* (Table 2). ND also had higher *Bilophila* than other groups. LD had the highest relative abundance of *Akkermansia*, *Lachnoclostridium*, *Desulfovibrio*, and *Lachnospiraceae* (Unc94789). HD had the lowest relative abundance of *Akkermansia*, *Desulfovibrio*, but the

highest abundance of *Escherichia*, *Haemophilus*, *Lachnospiraceae* (UncO8895), *Tyzzerella*, and *Erysipelatoclostridium* (q values < 0.05) (Table 2). All the bacterial genera were further tested in the multivariable negative binomial regression models. The difference in the relative abundance of less common bacteria (relative abundance between 0.05% and 1%) is shown in Supplemental Table S1.

3.5. Multivariable analysis

The multivariable negative binomial regression analysis showed significantly higher *Akkermansia* in LD compared to ND and significantly higher *Lachnospiraceae* (UncO8895) in HD compared to ND (Table 3). Compared to ND, HD had the significantly lower incidence of having a non-zero count of *Faecalibacterium*, *Subdoligranulum*, *Sutterella*, *Roseburia*, and *Lachnospiraceae*unc91005. HD had the significantly higher incidence of having a non-zero count of *Lachnospiraceae*unc8895 (Table 3). The IRR (95% CI) for *Escherichia* was 3.78 (1.23–11.6) when HD compared to ND. However, the IRR (3.19, 95% CI: 0.91–11.3, $P = 0.07$) was attenuated when the HEI score was adjusted in the multivariable model. ND and FD had essentially similar gut bacterial profiles (Table 2 and Table 3). Only significant findings on bacterial count and alcohol use were presented in Table 3.

3.6. Sensitivity analysis and stratified analysis

The sigmoid-based study showed a similar trend of difference at the genus level based on alcohol use, as shown in the analysis based on 97 mucosal biopsies. For example, the relative abundance of *Akkermansia* was the highest in LD, but was missing in HD. The relative abundance of *Subdoligranulum* was lowest in HD (P values < 0.05). However, the relative abundance of *Sutterella* was the highest in LD in the sigmoid samples, while it was the highest in ND in all mucosal samples. HD had the lowest *Sutterella* in both analyses (Supplemental Table S2).

In the stratified analyses, more pronounced associations between alcohol use and gut bacteria were seen among ever smokers or people with lower dietary quality. HD had the lowest *Subdoligranulum* in those with a higher HEI score and never smokers. ND had the highest *Faecalibacterium* among never smokers (Supplemental Figure S3).

3.7. Tax4fun analysis

The Tax4fun analysis showed that 108 of 159 bacterial functional pathways differed by alcohol use (Supplemental Table S3). Vitamin B6 metabolism, ubiquinone synthesis, tuberculosis, chloroalkane degradation, and lipopolysaccharide (LPS) biosynthesis were the top-ranked pathways that varied by alcohol use based on the q values.

4. Discussion

In adults, the colonic mucosa's bacterial community composition and structure differed significantly based on self-reported alcohol use. Light drinkers had the highest relative abundance of *Akkermansia*. Heavy drinkers had the lowest bacterial richness and evenness, the lowest relative abundance of *Faecalibacterium*, *Akkermansia*, *Subdoligranulum*,

Roseburia, *Sutterella*, and *Lachnospiraceae* unc91005, and the highest relative abundance of *Lachnospiraceae* unc8895. These observations were independent of multiple potential confounding factors. Never drinkers and former drinkers had similar bacterial profiles. Varying amounts of alcohol use altered multiple bacterial functional pathways..

Heavy drinkers had the lowest alpha diversity. This observation was consistent with a study that showed patients with chronic alcoholic pancreatitis having the lowest alpha diversity [31]. Decreased alpha diversity has been associated with inflammatory bowel disease [32], colorectal cancer [33], and autism [34]. In addition, the microbial community composition of heavy drinkers had the most significant dissimilarity from other groups. Therefore, the biodiversity of the gut bacteria was altered in heavy drinkers.

Light drinkers had the highest relative abundance of *Akkermansia*. *Akkermansia* is generally thought to be beneficial as it is associated with lower inflammation levels, improved gut barrier function, adipose tissue homeostasis, and reduced diabetes incidence [35–37]. There is a possible link between polyphenols and *Akkermansia*. The addition of red wine grape extract containing polyphenols to *in vitro* gut models increased the number of *Akkermansia*. Polyphenols may increase the growth of beneficial bacteria and inhibit pathogenic bacterial strains, such as *C. difficile* [38]. Another mice study showed that fermented rice wine increased the abundance of *Akkermansia muciniphila*. It is likely due to the survival advantage of *Akkermansia muciniphila* in the presence of antioxidant compounds (such as benzene derivatives) in fermented rice wine [39]. On the other hand, heavy drinkers had the lowest *Akkermansia*. A study found that patients with alcoholic steatohepatitis or mice fed with ethanol had decreased fecal *Akkermansia*, which could be restored by oral *Akkermansia muciniphila* supplementation [37]. Therefore, the health benefits of light alcohol use may be attributable to the effect of *Akkermansia*. More studies should test this possibility.

Never drinkers had a higher relative abundance of the butyrate-generating bacteria, including *Faecalibacterium*, *Subdoligranulum*, and *Roseburia* [40]. Butyrate and acetate are the major short-chain fatty acids (SCFAs) produced by the gut microbiota after fermentation of carbohydrates [41]. Butyrate and acetate are beneficial to human energy metabolism by serving as the energy substrates, inhibiting histone deacetylases, and turning on G-coupled receptors [42]. *Faecalibacterium* has anti-inflammatory effects and maintains gut integrity, and it is reduced in patients with Crohn's Disease, Ulcerative Colitis, and colorectal cancer [43–45]. *Subdoligranulum* is similarly reduced in inflammatory bowel disease [46]. *Roseburia* was shown to ameliorate alcohol-related liver disease in humans [47]. Never drinkers also had a higher relative abundance of *Sutterella* for which the function is less well-known. In our study, never drinkers and former drinkers had a similar bacterial profile essentially. Likewise, a study showed that some bacteria, such as *Bifidobacterium* and *Lactobacillus*, were normalized to nonalcohol use levels after three weeks of alcohol cessation in alcohol-dependent patients [48]. Stopping alcohol use may have allowed the microbiota to normalize back to a never drinker state.

Heavy drinkers had the least *Akkermansia* and butyrate-generating bacteria, but the highest Proteobacteria/Enterobacteriaceae/*Escherichia*. Alterations in these bacteria have been

reported for alcohol-related diseases and colorectal cancer [12–14, 49]. *Escherichia* is an ethanol producer. The accumulation of endogenous and exogenous ethanol may promote the production of carcinogenic and toxic acetaldehyde [8]. In the bacterial functional pathway analysis, we found that the LPS biosynthesis pathway was upregulated in heavy drinkers. LPS is an essential component of Gram-negative bacteria, such as *Escherichia*, that can trigger inflammatory reaction through toll-like receptor 4 [50]. Nevertheless, adjusting for diet attenuated the association between *Escherichia* and heavy drinkers in the analysis. Heavy drinkers also had altered relative abundance of unclassified members of the *Lachnospiraceae* family. A metagenomics study is needed to identify the bacterial species associated with alcohol use. Overall, the reduced relative abundance of SCFA producers and the increased pathogenic bacteria could partially explain the adverse influence of heavy alcohol consumption in the body.

In our study population, most heavy drinkers had a lower HEI score or had ever smoked. In the exploratory stratified analyses, we found a significant association between gut microbiota and alcohol use in those who had a lower dietary quality or had ever smoked. The difference observed in never smokers and those who had a higher HEI score followed the same patterns but was not statistically significant. Future studies with a greater study power are needed to determine the synergistic impact of alcohol and dietary quality or smoking on the gut microbiota.

The current study provided a novel cross-sectional evaluation of the association between daily alcohol use and the mucosal adherent gut microbiota in individuals with endoscopically normal colons. The adherent microbiota was different from the fecal microbiota and may be more related to the host immunity [51]. Inclusion of individuals with grossly healthy colons minimized the potential confounding effect of colorectal diseases on the microbiota. The analysis was also less likely to be confounded by antibiotic use because we excluded participants who used systemic antibiotics within the past three years. Participants discontinued the use of non-essential drugs one week before the colonoscopy. However, the use of antihypertensive drugs may have affected the gut microbiota. We also could not exclude the residual confounding effect of smoking, diet, or other unknown factors on the association between alcohol use and gut microbiota.

Our study had a few limitations. Almost all participants were adult men, possibly impacting the generalizability of our finding to women and younger individuals. One study has observed the sex difference in the influence of alcohol on gut microbiota profile [52]. We used the V4 region for 16S rRNA gene sequencing, which has been shown to provide resolution at the phylum level as accurately as the full 16S gene [53]. The V4 region also has the greatest similarity to community profiles determined by shotgun sequencing [54] and has higher alpha diversity compared to V1-V2 and V3-V4 regions [55]. However, our results may not be generalizable to studies that target other hypervariable regions for amplicon sequencing [56]. As a secondary analysis using the available data, this study had a small sample size. The findings should be validated in larger studies. Selection bias was also likely because 35 participants who had the sequencing data did not represent the 174 polyp-free participants in terms of smoking status and alcohol use status. However, this difference would not invalid the cross-sectional examination of the association between alcohol use and

gut microbiota. Lastly, the information bias of self-reported alcohol use could not be excluded.

In summary, we accepted our hypothesis that alcohol misuse influenced the adherent gut microbiota in humans. Current heavy drinkers had distinct gut microbial community composition and structure compared to non-heavy drinkers. The reduced relative abundance of SCFA producers and the increased potentially pathogenic bacteria could partially explain the adverse influence of heavy alcohol use in inflammation, increased gut permeability, disturbed immune homeostasis, and health conditions [57, 58]. Understanding the functions of the bacteria may provide new therapeutic options for alcohol-induced diseases. Lastly, alcohol use could be a confounding factor in epidemiological studies examining gut microbiota and health outcomes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations:

ATIMA	Agile Toolkit for Incisive Microbial Analyses
CMMR	Alkek Center for Metagenomics and Microbiome Research
BCM	Baylor College of Medicine
FDR	False Discovery Rate
FFQ	Food Frequency Questionnaire
FD	Former Drinkers
HD	Heavy Current Drinkers
HEI	Healthy Eating Index
LD	Light Current Drinkers
LPS	Lipopolysaccharide

MEDVAMC	Michael E. DeBakey VA Medical center
ND	Never drinkers
OTUs	Operational Taxonomic Units
PcoA	Principal Coordinate Analysis
PERMANOVA	Permutational Multivariate Analysis of Variance
rRNA	Ribosomal RNA
SCFAs	Short-chain fatty acids; V4: hypervariable region 4

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Highlights

- The association between daily alcohol use and the colonic gut microbiota is unknown
- Light alcohol use was associated with a higher relative abundance of *Akkermansia*
- Heavy alcohol use was associated with a lowest relative abundance of *Subdoligranulum*, *Faecalibacterium*, *Roseburia*, and *Lachnospiraceae* unc91005.
- Colonic gut bacteria may partially mediate the effect of alcohol on health outcomes

Fig. 1 -
Flow chart of participate selection.

Fig. 2 -

Boxplot of alpha diversity indices of the gut microbiota based on alcohol use. The mean (standard deviation) of the Simpson index was 0.86 (0.07), 0.88 (0.04), 0.88 (0.05), and 0.79 (0.14) in ND, FD, LD, and HD, respectively (q value = 0.03, Kruskal-Wallis test). The mean (standard deviation) of the Shannon index was 2.82 (0.56), 2.76 (0.36), 2.86 (0.48), and 2.15 (0.53) in ND, FD, LD, and HD, respectively (q value = 0.002 for the Kruskal-Wallis test, q value = 0.004 for ANOVA test).

Fig. 3.

Beta diversity of gut bacteria based on alcohol use. The PCoA of weighted Unifrac distance shows the centroid of heavy drinkers was statistically significantly different from three other groups (P value = 0.001, PERMANOVA). PC1 and PC2 represent the top two principal coordinates that capture most of the diversity. The fraction of diversity captured by the coordinate was shown as a percentage in the corresponding axis.

Fig. 4. Stacked bar chart of relative abundance (%) of bacterial phyla based on alcohol use. The q value (Kruskal-Wallis test) was 0.001 for Verrucomicrobia, 0.007 for Proteobacteria, and 0.02 for Actinobacteria.

Table 1-

Basic characteristics of 34 study participants based on alcohol use

Characteristics	Never drinkers (n=9)	Former drinkers (n=10)	Light drinkers (n=9)	Heavy drinkers (n=6)	<i>P</i> value ^a
Mean (SD) or n (%)					
Current daily alcohol use (grams)			5.27 (8.98)	46.3 (3.91)	
Age, mean (SD)	60.7 (5.8)	64.6 (5.0)	61.2 (5.9)	60.9 (5.5)	0.40
Men, n (%)	9 (100)	10 (100)	8 (88.9)	6 (100)	0.71
Race, n (%)					0.97
Non-Hispanic White	6 (66.7)	6 (60.0)	8 (88.9)	4 (66.7)	
African Americans	2 (22.2)	2 (20.0)	1 (11.1)	1 (16.7)	
Hispanic	1 (11.1)	2 (20.0)	0 (0)	1 (16.6)	
BMI (kg/m ²), mean (SD)	31.8 (5.56)	35.6 (7.83)	33.8 (5.59)	34.5 (7.06)	0.65
Hypertension (yes, n (%))	7 (77.8)	6 (60.0)	8 (88.9)	4 (66.7)	0.59
Diabetes (yes, n (%))	4 (44.4)	4 (40.0)	7 (77.8)	2 (33.3)	0.70
Smoker (yes, n (%))					0.28
Never	6 (66.7)	3 (30.0)	4 (44.4)	0 (0)	
Former	2 (22.2)	5 (50.0)	3 (33.3)	4 (66.7)	
Ever	1 (11.1)	2 (20.0)	2 (22.2)	2 (33.3)	
Alcohol type (yes, n (%))					
Beer ever	0	0	8	6	>0.05
Wine ever	0	0	9	6	
Liquor ever	0	0	5	4	
HEI score, mean (SD)	66.0 (11.0)	61.3 (7.45)	60.0 (5.06)	54.0 (9.40)	0.08
Dietary quality, n (%)					0.30
HEI < 60	3 (33.3)	4 (40.0)	5 (55.6)	5 (83.3)	
HEI ≥ 60	6 (66.7)	6 (60.0)	4 (44.4)	1 (16.7)	
Segments, n (%)					0.50
Cecum	2	2	0	0	
Ascending	0	2	2	2	
Transverse	0	3	1	0	
Descending	2	1	1	0	

Characteristics	Never drinkers (n=9)	Former drinkers (n=10)	Light drinkers (n=9)	Heavy drinkers (n=6)	<i>P</i> value ^a
Mean (SD) or n (%)					
Sigmoid	4	1	3	3	
Rectum	1	1	2	1	

HEI, healthy eating index; SD, standard deviation.

^a *P* value for Fisher's exact test for categorical variables and for ANOVA for continuous variables.

Table 2-
Relative abundance (%) of major bacterial genera (relative abundance 1 %) based on alcohol use

Bacterial genera	Relative abundance (%) (mean $\pm$ standard deviation)				<i>p</i> value ^a
	Never drinkers	Former drinkers	Light drinkers	Heavy drinkers	
	34 biopsies	36 biopsies	19 biopsies	8 biopsies	
<i>Alloprevotella</i>	0 (0.01)	0 (0.02)	1.26 (0.02)	0 (0.0)	< 0.0001
<i>Subdoligranulum</i>	2.50 (0.02)	2.26 (0.02)	0.73 (0.004)	0.34 (0.60)	0.0002
<i>Prevotellaceae (Unc04zvf)</i>	0.36 (0.01)	0	2.85 (0.03)	0.74 (0.02)	0.0002
<i>Akkermansia</i>	1.34 (3.33)	1.24 (3.70)	10.7 (11.00)	0.001 (0.02)	0.0002
<i>Lachnospiraceae (Unc91005)</i>	7.52 (5.07)	8.95 (6.57)	5.08 (3.56)	0.96 (1.13)	0.0001
<i>Desulfovibrio</i>	0.46 (0.89)	0.13 (0.31)	3.13 (3.31)	0 (0.0)	0.004
<i>Erysipelatoclostridium</i>	1.98 (4.39)	0.32 (0.63)	0.20 (0.43)	6.08 (6.55)	0.004
<i>Lachnospiraceae (Unc08895)</i>	6.36 (11.82)	4.78 (6.23)	2.69 (5.21)	15.7 (11.0)	0.008
<i>Faecalibacterium</i>	11.9 (8.31)	7.86 (6.16)	8.10 (10.75)	2.26 (4.61)	0.01
<i>Lachnospiraceae (Unc94789)</i>	1.02 (1.20)	3.34 (4.76)	4.48 (4.33)	3.62 (2.96)	0.01
<i>Erysipelotrichaceae (Unc05nru)</i>	0.19 (0.05)	1.42 (2.20)	0.01 (0.09)	0	0.01
<i>Lachnospiraceae (Unc89581)</i>	0.01 (0.03)	0.1 (0.16)	0.01 (0.02)	2.99 (3.67)	0.01
<i>Escherichia Shigella</i>	2.53 (3.51)	2.97 (3.68)	8.05 (11.2)	17.2 (11.8)	0.01
<i>Bifidobacteria</i>	1.17 (1.05)	0.82 (0.94)	0.37 (0.63)	0.48 (0.92)	0.01
<i>Roseburia</i>	3.31 (2.61)	1.88 (2.62)	3.20 (3.79)	0.97 (1.61)	0.01
<i>Tyzzerella</i>	0.08 (0.19)	0.38 (1.26)	0.7 (1.07)	1.42 (1.41)	0.01
<i>Alistipes</i>	2.13 (2.57)	0.81 (1.00)	1.13 (1.55)	0.08 (0.13)	0.02
<i>Prevotellaceae (Unc00Yx7)</i>	0	1.07 (0.02)	1.11 (0.02)	0	0.02
<i>Sutterella</i>	1.75 (2.12)	1.11 (1.21)	0.6 (0.69)	0.08 (0.24)	0.02
<i>Lachnoclostridium</i>	1 (0.70)	1.94 (1.77)	3.26 (2.86)	2.03 (2.02)	0.03

^a *p* value for the Kruskal-Wallis test

Table 3-

Incident rate ratio (IRR) and its 95% confidence interval (CI) of having a non-zero bacterial count based on alcohol use

Bacterial genera	Alcohol use	IRR ^a	95% CI	P value
<i>Akkermansia</i>	Former drinkers	1.40	0.29–6.70	0.68
	Light drinkers	15.4	2.96–80.3	0.001
	Heavy drinkers	0.04	0.002–0.84	0.04
<i>Faecalibacterium</i>	Former drinkers	1.07	0.48–2.40	0.86
	Light drinkers	0.80	0.34–1.85	0.60
	Heavy drinkers	0.05	0.01–0.20	<0.001
<i>Roseburia</i>	Former drinkers	0.28	0.11–0.68	0.005
	Light drinkers	0.44	0.15–1.20	0.11
	Heavy drinkers	0.08	0.01–0.56	0.01
<i>Subdoligranulum</i>	Former drinkers	1.12	0.61–2.08	0.71
	Light drinkers	0.47	0.23–0.96	0.04
	Heavy drinkers	0.19	0.06–0.63	0.007
<i>Sutterella</i>	Former drinkers	0.14	0.04–0.48	0.002
	Light drinkers	0.27	0.07–0.94	0.04
	Heavy drinkers	0.002	0.0001–0.03	<0.001
<i>Lachnospiraceaeunc91005</i>	Former drinkers	1.03	0.61–1.73	0.92
	Light drinkers	0.67	0.40–1.11	0.12
	Heavy drinkers	0.13	0.05–0.33	<0.001
<i>Escherichia</i>	Former drinkers	1.00	0.44–2.29	0.98
	Light drinkers	0.42	0.16–1.05	0.06
	Heavy drinkers	3.19	0.91–11.2	0.07
<i>Lachnospiraceaeunc8895</i>	Former drinkers	0.93	0.37–2.34	0.88
	Light drinkers	0.87	0.34–2.21	0.77
	Heavy drinkers	6.36	1.85–21.8	0.003

CI: confidence interval. IRR: incident rate ratio. P value was bolded if < 0.05.

^aNever drinker was the reference group in the model. The multivariable negative binomial regression model using panel data was adjusted for age (continuous), ethnicity (non-Hispanic white, African American, and Hispanic), body mass index (continuous), smoking status (never, former, and current), colon segment, hypertension (yes vs. no), diabetes (yes vs. no), and HEI score (continuous). Each individual was treated as a panel if one had multiple biopsies.

Compared to never drinkers, the incidence rate of having a non-zero *Akkermansia* count was higher in light drinkers and lower in heavy drinkers. Compared to never drinkers, heavy drinkers had significantly lower incidence rate of a non-zero count of *Faecalibacterium*, *Roseburia*, *Subdoligranulum*, *Sutterella*, and *Lachnospiraceaeunc91005*; heavy drinkers had significantly higher incidence rate of a non-zero count of *Lachnospiraceaeunc8895* and an insignificantly higher count of *Escherichia*. For example, the incidence of having a non-zero *Faecalibacterium* count was 95% lower in heavy drinkers than in never drinkers.