Undergraduate Journal of Experimental Microbiology and Immunology (UJEMI) Vol. 26:1-12

The effects of alcohol consumption and increased body mass on the gut microbiota of Parkinson's Disease patients

Jared Dutra, Madeline Fung, Michelle Ling, Rui Lin Zhi

Department of Microbiology and Immunology, University of British Columbia, Vancouver, British Columbia, Canada

SUMMARY Parkinson's disease, alcohol abuse, and obesity are all associated with changes in the gut microbiome composition and are among common health issues in the elderly. Lower levels of butyrate-producing bacteria, which may be linked to disease pathophysiology, have been identified in Parkinson's patients and individuals dependent on alcohol. Parkinson's patients may also have altered abundances of the phyla Firmicutes and Bacteroidetes, and individuals of higher body mass have been reported to have an increased Firmicutes to Bacteroidetes ratio. Through bioinformatics analysis, our study explored the impact of alcohol consumption and body mass on Parkinson's, specifically focused on the gut microbiota. Beta diversity metrics suggested no direct relationship between alcohol consumption or body mass with gut microbiome composition. While alcohol consumption did not impact the relative abundance of butyrate-producing genera, the relative abundance of three anti-inflammatory genera were significantly lower in Parkinson's groups, proposing possible risk-reducing roles of these genera. An elevated Firmicutes to Bacteroidetes ratio was not seen with increased body weight in Parkinson's patients, suggesting it to be an unsuitable obesity biomarker. However, the family Veillonellaceae, producers of a metabolite previously associated with Parkinson's and obesity, was more abundant in overweight Parkinson's patients. This study elucidated taxa relevant to further investigation that may be important in the interplay between Parkinson's disease, alcohol abuse, and obesity.

INTRODUCTION

Parkinson's disease (PD) is a chronic neurodegenerative disorder that primarily affects the elderly (1). Although a multitude of risk factors and gene mutations have been linked to the development of PD, no singular root cause has been identified (1). One main characteristic of this disease is the lack of dopamine in the brain, leading to the death of dopaminergic neurons (2). The pathological progression of PD is well understood and is separated into distinct stages which correlate with cognitive status and symptom severity (2, 3). Signs of illness include decline of motor functions, autonomic dysfunction, and neuropsychiatric symptoms (1). Because no definitive test exists, PD is typically diagnosed based on the evaluation of symptoms and history of clinical, imaging, biochemical, and genetic biomarkers (1, 2).

In PD, gastrointestinal symptoms appear early, and research has proposed that the disease may start in the gut (3). As dysbiosis of the gut microbiome has been linked to many inflammation-driven diseases, investigations have also been performed to determine if these alterations are related to PD (4). A 2020 study by Cirstea et al. explored how the microbiota and its metabolism are associated with gut function in PD patients (5). 16S rRNA-based sequencing of the microbiota and untargeted metabolomics were performed on fecal and serum samples from a large cohort of 300 PD patients and controls (5). Using this dataset and its associated metadata, taxonomic differences and microbial metabolites were investigated in PD and control patients (5). Significant shifts in taxonomy away from short-chain fatty acid (SCFA) producers and elevated levels of proteolytic metabolites were observed in PD patients and were associated with differences in gut function (5).

Lifestyle factors play a crucial role in the risk and progression of PD. Data collected from the University of Rochester Medical Center found that alcohol abuse and obesity are among the top ten most common health issues in those aged 65 years or older, which is the

Published Online: September 2021

Citation: Jared Dutra, Madeline Fung, Michelle Ling, Rui Lin Zhi. 2021. **The effects of alcohol consumption and increased body mass on the gut microbiota of Parkinson's Disease patients**. UJEMI 26:1-12

Editor: Daniela Morales, Stefanie Sternagel and Brianne Newman, University of British Columbia

Address correspondence to: https://jemi.microbiology.ubc.ca/

main demographic of PD patients (1, 6). These two health concerns increase the risk of or are more prevalent in PD patients, while also being associated with changes in the gut microbiome composition.

The exact impact of alcohol consumption in PD patients has not been well-characterized, despite previous studies in related areas. PD patients who consume very high or low quantities of alcohol have been reported to have accelerated disease progression compared to those who consume moderate amounts (7). Yet, a recent study found no correlation between alcohol intake and PD (8). Additionally, different types of alcohol appear to have different effects in PD patients (9). Regardless, alcohol has been shown to alter the gut microbiome. It was reported that both PD patients and individuals highly dependent on alcohol have lower levels of butyrate-producing bacteria in their gut microbiota (5, 10). Butyrate is a metabolite of intestinal bacteria and has a prominent role in intestinal homeostasis and gut metabolism, which may be how butyrate levels are linked to PD pathophysiology via the gut-brain axis (11). Further studies are needed to verify potential links between alcohol consumption and PD, and to determine if alcohol-induced gut microbiome changes significantly impact PD progression.

Multiple studies have discovered that elevated alcohol consumption is linked to weight gain and the risk of becoming overweight or obese (8, 12). This is of interest as changes in body mass index (BMI), a measure of body fat calculated with weight and height, have been associated with the risk and pathogenesis of PD (13, 14). Individuals of different BMI have also been shown to have significantly different gut microbiota profiles. Notably, increased BMI may lead to increased bacterial diversity and changes in abundance compared to those with a normal BMI (15). Furthermore, multiple studies have proposed an increased ratio of Firmicutes to Bacteroidetes (F/B ratio) as a hallmark of obesity after investigating the relationship between obesity and the abundance of these two phyla (16). In PD, it is possible that this ratio is altered as well, as some families within the Firmicutes phylum, such as Erysipelotrichaceae, have been found to be elevated, while some families within the Bacteroidetes phylum, such as Prevotellaceae, have been found to be lowered (4). While the influence of increased BMI and PD on the composition of the gut microbiome have been separately considered, no study has considered how the microbiome is affected by these two variables together.

Despite recent strides in research into the progression, symptoms, and physiological changes related to PD, no successful cure has been established, and only treatments in reducing and managing symptoms are available (1). Using bioinformatics to analyse the data and metadata from Cirstea et al. 2020, this study seeks to explore the impact of two common health concerns on PD, with a specific focus on the gut microbiota. In particular, the relationship between alcohol consumption and PD patient gut microbiota composition and symptom severity will be examined. Based on previous literature, we hypothesize that distinct bacterial compositions will be observed between patients with different alcohol consumption levels. We predict that PD patients consuming more alcohol will have lower abundance of butyrate-producing bacteria and more severe PD symptoms. Furthermore, the combinatorial effect of BMI and PD on microbiome composition will be studied. It is expected that patients of different BMI will have different microbiota, and that increased BMI will lead to an increased F/B ratio. Examining the effects of these two common health concerns on the composition of gut microbiota in PD patients is crucial in developing a deeper understanding of the microbiome's role in the root causes, risk factors and associations of neurodegenerative diseases. Moreover, this understanding may be beneficial in defining future health guidelines to reduce PD risk, as well as the development of appropriate treatments and cures.

METHODS AND MATERIALS

Refer to Script S1 for all QIIME2 commands, and Script S2 for all R commands.

Processing raw 16S rRNA sequences. QIIME2 (v2020.8), a package for microbiome analysis, was used for this stage (17). The 16S rRNA sequences from the original study "Microbiota Composition and Metabolism Are Associated with Gut Function in Parkinson's

Disease" by Cirstea et al. (5) were first demultiplexed, then quality controlled using DADA2 (18). The resulting feature table was used for further analysis, and to generate a tree for phylogenetic diversity analyses.

Analysis of relationship between alcohol consumption and gut microbiome composition in PD patients. Subjects were grouped into "none", "low", "moderate", and "high" alcohol consumption groups according to guidelines from the Canadian Centre on Substance Use and Addiction (Table S1). Using QIIME2, the data was then filtered to exclude any subjects who had taken more than five doses of antibiotics in the previous five years, and this dataset was used for subsequent analysis for alcohol consumption. Beta diversity analysis between subjects of different alcohol consumption groups was performed using QIIME2.

Data outputs from QIIME2 were exported into R (v4.0.3) and R studio (v1.3.1093) (19, 20). The packages used in this analysis were *tidyverse* (21), *vegan* (22), *phyloseq* (23), *DESeq2* (24), *dplyr* (25), *ggplot2* (26), *data.table* (27), *rstatix* (28), and *ggpubr* (29). The relative abundance (RA) for each amplicon sequence variant (ASV) was calculated, and those with less than 0.1% RA were filtered out. Features that were statistically insignificant at an α value of 0.05 were also filtered out, and the remaining features were sorted based on their adjusted p-value. Significant features were then merged with taxonomic classification data and the data frame was subset to exclude subjects who did not have data available for their daily alcohol consumption. The *rstatix* package was used to determine statistical differences between groups by calculating an adjusted p-value via the Bonferroni method. RA plots displaying adjusted p-values were created using *ggplot2* and *ggpubr* packages as described by Albouk (30).

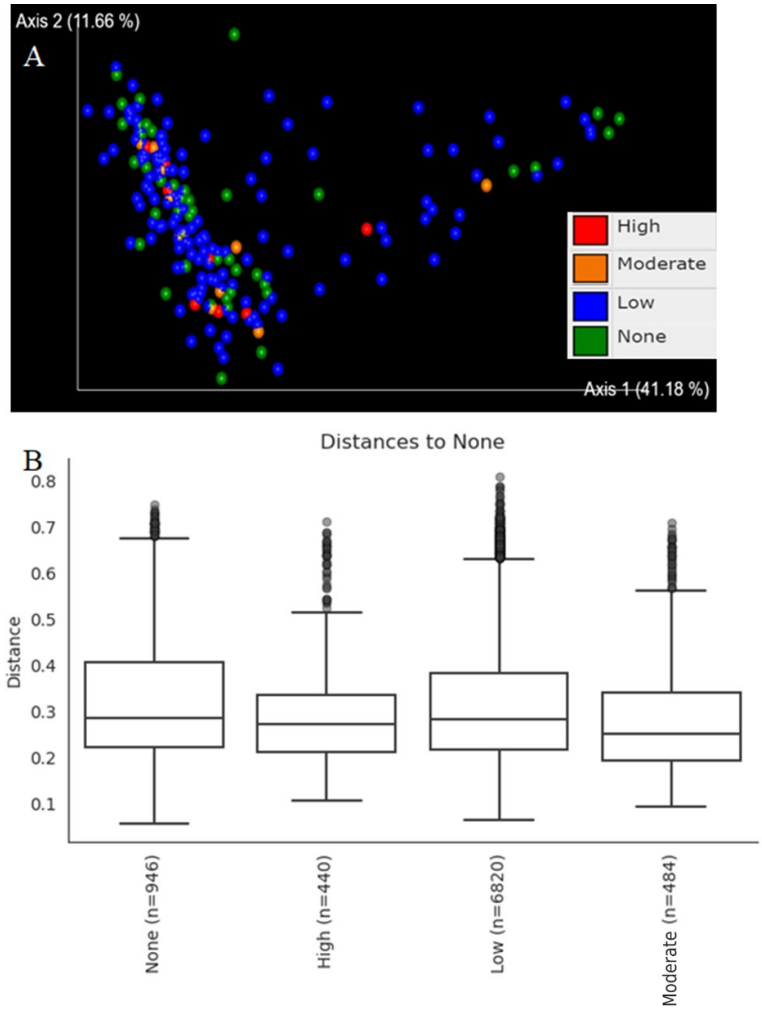

FIG. 1 Alcohol consumption does not affect gut microbiome composition according to Weighted UniFrac beta diversity metric. (A) Microbial communities did not separate based on high (red), moderate (orange), low (blue), and no (green) alcohol consumption in PD patients. (B) Box and whisker plot indicate that Weighted UniFrac distances of gut microbiome members do not differ between no alcohol consumption and all other groups. Corresponding pairwise PERMANOVA statistics can be found in Table S3.

Linear regression analysis. Any subjects with no data for their alcohol consumption or BMI were removed from the analysis, and the remaining data points were plotted on a scatterplot in Microsoft Excel (2019) (31). Linear regression was performed between Unified Parkinson's Disease Rating Scale (UPDRS) 1/2/3/4/Total scores and BMI versus alcohol consumption.

Analysis of relationship between BMI and gut microbiome composition in PD patients. Subjects were grouped into "control-normal", "control-overweight", "PD-normal", and "PD-overweight" categories based on their disease status and BMI (Table S2). Individuals who fell into the other BMI categories (underweight, obese, morbidly obese) were filtered out from analyses using QIIME2 due to low number of samples. Beta diversity analysis between subjects of different disease status and BMI groups was performed using QIIME2. Data outputs from QIIME2 were exported into R for analysis, and the same packages were used as above, with the addition of *indicspecies* (32). RA analysis was performed to compare the RA of Firmicutes and Bacteroidetes phyla between "PD-overweight" and "PD-normal" or between "control-overweight" and "control-normal" categories. Differential abundance analysis was done at the family level to assess differences in taxonomic composition between "PD-overweight" and "PD-normal" categories. Feature filtering was performed as above. Indicator taxa analysis was performed at the family level to find taxa that are present at different levels in the "PD-overweight" group.

RESULTS

Weighted UniFrac beta diversity metric revealed no differences in gut microbiome composition between PD patients with different alcohol consumption levels. Beta diversity metrics were analysed to determine if the microbiome composition was significantly different between PD patients who consumed different levels of alcohol. Multiple beta diversity metrics were run in an exploratory approach to see if the differences in composition were more driven by abundance or phylogenetic relatedness. To visualize differences in beta diversity metrics, Jaccard, Bray-Curtis, Weighted UniFrac and Unweighted UniFrac analyses were displayed on principal coordinates analysis (PCoA) plots (Figure 1 & Figure S1). Across the four beta diversity metrics, Bray-Curtis (Figure S1) and Weighted UniFrac (Figure 1A) exhibited microbial community similarities of the majority of samples in one area, while Jaccard and Unweighted UniFrac (Figure S1) showed no microbial community similarities at all. Axis 1 on the Weighted UniFrac plot explained the highest percent variance (41.18%), compared to all other axes of all other PCoA plots. Despite microbial communities showing similarity by clustering in one area of the Bray-Curtis and Weighted UniFrac plots, no distinct clustering was seen based on the four categories of daily alcohol consumption. This is emphasized by the box and whisker plots (Figure 1B & Figure S2), which demonstrates the lack of significant difference in distance between the gut microbiome composition of no alcohol consumption and all other groups, measured by any of the four beta diversity metrics. Further, q-values from PERMANOVA analysis confirmed that there were no significant differences between alcohol consumption groups across the four beta diversity metrics (Tables S3 - S6). From this data, there appears to be no apparent community structure similarities of alcohol consumption groups based on any of the beta diversity metrics, indicating that alcohol consumption does not directly drive any changes in gut microbiome composition.

Alcohol consumption did not affect the relative abundance of genera associated with butyrate production, but overall, PD subjects showed depletion of butyrate-producing genera. Previous research by Cirstea et al. identified several genera of butyrate-producing bacteria that were significantly less abundant in PD subjects relative to controls (5). RA analysis was carried out in our study to validate these findings and investigate further if alcohol consumption similarly leads to changes in abundance of butyrate-producers. RA analysis of these genera revealed three were differentially abundant between PD and controls, while the remaining two genera were not. *Roseburia*, *Faecalibacterium*, and *Lachnospira* (Figure 2C - E) appeared to be present at higher levels in healthy controls relative to subjects with PD, while there was no difference in the RA of *Blautia* and

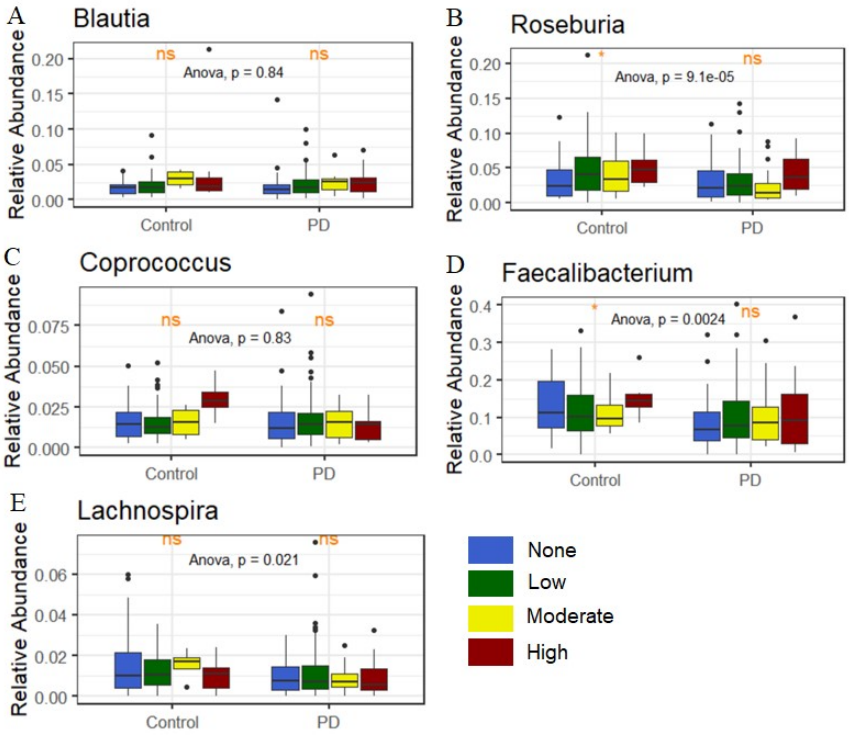

FIG. 2 Relative abundance of several important butyrate producers varies between control and PD patients, but not between alcohol consumption groups in control or PD subjects. Differential abundance analysis was performed on the (A) *Blautia*, (B) *Coprococcus*, (C) *Faecalibacterium*, (D) *Lachnospira*, and (E) *Roseburia* genera. $\alpha = 0.05$. **p*<0.05, *** *p*<0.001, ns = not significant.

Coprococcus (Figure 2A - B). None of the five genera analysed (*Roseburia*, *Blautia*, *Coprococcus*, *Faecalibacterium*, *Lachnospira*) were differentially abundant within alcohol consumption groups (Figure 2A - E, Tables S7 - S11). Therefore, alcohol consumption does not change the RA of these five genera of butyrate producers, but *Roseburia*, *Faecalibacterium*, and *Lachnospira* are significantly less abundant in the gut microbiomes of PD subjects.

Average alcohol consumption did not significantly correlate with BMI or PD symptom severity. To determine if a relationship between alcohol consumption and BMI exists within this dataset, linear correlation analysis was performed. No significant correlation was seen, as indicated by the low R^2 value of 0.0015 (Figure 3A). In order to verify claims of correlation between PD symptom severity and alcohol consumption, the UPDRS score was used as a measure of PD symptom severity and linear regression was performed to assess the correlation with average alcohol consumption. For all 4 subscores (Figure S3) as well as total UPDRS score (Figure 3B), there was no significant correlation between alcohol intake and PD symptoms severity, as indicated by low R^2 values (UPDRS 1 = $8x10^{-6}$, UPDRS 2 = 0.0047, UPDRS 3 = 0.0027, UPDRS 4 = 0.0006, UPDRS Total = 0.022). Altogether, no direct relationship between alcohol consumption and BMI or PD progression is supported by our data.

Weighted UniFrac beta diversity metric revealed no differences in gut microbiome composition between participants with different PD disease statuses and BMI. Beta diversity was calculated via Weighted UniFrac analysis (Figure 4A). This metric was chosen as it best represented the data for alcohol consumption and results from related past literature. The PCoA plot of Weighted UniFrac explained a high percent variance at 56.75%. The four categories based on PD disease status and BMI score did not reveal distinct microbial communities. A subset of patients, unrelated by the disease-BMI groups, had similarities in microbial communities. Box and whisker plots of Weighted UniFrac distances showed similar findings as the PCoA analysis, since distances between gut microbiomes of overweight PD patients and three other groups do not appear to differ (Figure 4B). These observations were supported by the q-values from PERMANOVA analysis (Table S12), which confirmed that there were no significant differences. Overall,

this data suggests that neither PD disease status nor BMI drives changes in gut microbiome composition.

Abundance of Firmicutes and Bacteroidetes did not differ for overweight PD patients. The F/B ratio was investigated in overweight PD patients, as previous literature has cited a higher F/B ratio as a possible hallmark of obesity in animal and human gut microbiomes (16). The analysis showed no significant differences (adjusted p-values > 0.05) in the RA of Firmicutes or Bacteroidetes between overweight individuals and normal controls (Figure 5). This was true for both participants with or without a PD diagnosis, questioning the validity of an increased F/B ratio as a suitable hallmark of obesity.

The family Veillonellaceae was differentially abundant and an indicator taxon in overweight PD patients. Further investigation into species that are differentially abundant between PD and BMI categories was conducted to fully examine the research question. Differential abundance analysis on the family level revealed that Veillonellaceae, Lachnospiraceae, Desulfovibrionaceae and Alcaligenaceae appeared to be more abundant in overweight PD patients, while Victivallaceae appeared to be more abundant in PD patients with a normal weight range (Figure S4). Further analysis found that Veillonellaceae was also an indicator family of overweight PD patients, along with another family, Peptococcaceae (Table S13). Veillonellaceae appeared to be not as specific for the "PD-overweight" group but had high fidelity, and hence was present in the majority of these patients. Differential abundance analysis and indicator taxa analysis successfully identified potential families of interest associated with PD patients or changes in BMI.

DISCUSSION

Microbiota composition of alcohol consumption groups. We hypothesized that PD patients consuming different levels of alcohol would differ in microbiota composition, and that higher alcohol consumption would be associated with lower abundance of butyrate-producing bacteria and more severe PD symptoms. Based on the analyses performed on "none", "low", "moderate", and "high" alcohol consumption groups, neither one of these hypotheses were supported. It was seen that the beta diversity of these groups did not differ, and that no significant relationship existed between alcohol consumption and butyrate-producing bacteria or PD symptom severity.

While no significant differences were present in the microbiome composition between the groups of different alcohol consumption levels investigated, as measured by beta diversity, some unrelated similarities between patients were seen. This similarity in microbial structures of patients appeared to be more driven by abundance than phylogenetic distance. The two metrics showing a pattern in microbial community similarity, Bray-Curtis and Weighted UniFrac, consider abundance, while the other two with no pattern, Jaccard and Unweighted UniFrac, take into account phylogenetic distance.

No significant correlation between alcohol consumption and PD symptom severity was suggested by our study. This is consistent with many other sources, such as the Million Women Study, which found no correlation between alcohol consumption and PD risk (8). Interestingly, some studies claim that alcohol acts as a protective agent against neurological degeneration associated with PD (33). Yet other sources claim that alcohol consumption can indeed increase the chances of PD diagnosis (34). The diverse findings of these studies are unusual, but recent review papers have attempted to explain these inconsistencies. A meta-analysis was performed on studies that examined links between PD risk and alcohol consumption (35). It was determined that the studies which found correlations between alcohol intake and PD risk were more likely to have weaknesses in their methodologies, namely increased selection bias and recall bias issues (35). Overall, this shows that while it is currently difficult to make definitive claims regarding the correlation between PD risk and symptom severity in relation to alcohol intake, it is clear that future studies must have rigorous study design to avoid these biases.

Butyrate is a SCFA molecule with a variety of roles in the gut, including inducing regulatory T cell (Treg) activation, acting as a nutrient source for gut epithelial cells and being involved in anti-inflammatory signaling pathways (36). Many other studies have

found that genera of butyrate producers such as *Roseburia*, *Faecalibacterium*, and *Lachnospira* are less abundant in the gut microbiota of PD patients (4, 5). Butyrate producers are generally believed to reduce inflammation in the gut (4), which may explain the increased gut inflammation and opportunistic infections in subjects with less butyrate producers in their gut microbiomes (37). *Faecalibacteria*, being one of the most important contributors to butyrate production in the gut, is believed to mediate their anti-inflammatory effects through maintaining gut homeostasis and thereby the integrity of the gut epithelial layer (38, 39). Similarly, the *Roseburia* genus has been shown to promote anti-inflammatory signalling, in part by promoting Treg proliferation in the gut (40). The *Lachnospira* genus, in addition to containing many crucial butyrate-producing species, has also been correlated with butyrate, vitamin B3, and B5 synthesis (41). Interestingly, these two vitamins may have antioxidative and anti-inflammatory properties, along with protective capabilities against neurodegenerative diseases (41). Vitamin B3 levels have been shown to be much lower in PD patients, which may be due to *Lachnospira* depletion in the gut (42).

Recent studies have begun to link intestinal inflammation to immunological dysregulation, and in turn to neurodegenerative disease progression. Essentially, changes in the composition of the gut microbiota can lead to increased local and systemic inflammation, which then increases blood-brain permeability (43). This, along with other factors, increases neuroinflammation, leading to neurodegeneration that drives PD progression (43). Therefore, it is possible that the observed decreases in the butyrate-producing and anti-inflammatory genera are causing increased intestinal inflammation which subsequently drives PD neurodegeneration.

While Cirstea et al. found significantly reduced levels of all five butyrate-producing genera investigated in PD patients, our study found significant reductions in only three of them. This may be due to differences in filtering prior to DESeq2 analysis, as well the removal of subjects who had taken more than five doses of antibiotics in the last five years.

Relationship between alcohol consumption and BMI. While previous literature indicates that alcohol consumption may be linked to changes in BMI, no correlation was observed between average alcohol consumption and BMI of patients in our study. Alcohol consumption may play a role in influencing BMI, but other factors can affect weight as well. PD patients can experience changes in appetites, changes in motor function, depression, and medical complications, all of which may lead to weight changes (44). These variables were not controlled for in the analysis performed and may explain why no correlation was observed.

Microbiota composition of disease BMI groups. We hypothesized that PD patients of different BMI would have different microbiota, and that increased BMI would be associated with an increased F/B ratio. Based on the analyses performed on “PD-overweight”, “PD-normal weight”, “control-overweight”, and “control-normal” patients, this hypothesis was not supported. From the analyses, the diversity of these groups did not differ, and no significant relationship existed between these groups and the abundance of Firmicutes and Bacteroidetes phyla. However, interestingly, the family Veillonellaceae was found to be both differentially abundant and an indicator taxon for “PD-overweight” individuals.

Beta diversity analysis was also performed for disease-BMI groups, using the Weighted UniFrac beta diversity metric which best represented the alcohol consumption data. This metric takes into account both phylogenetic distance and abundance, and was chosen as previous literature described changes in both the abundance and the composition of the microbiome (15). While there were no significant differences between groups, a similar pattern of unrelated microbial community similarity was observed in the Weighted UniFrac plot for alcohol consumption. The fact that this pattern appears twice, despite looking at two different variables, suggests that it is not by chance and there is in fact another variable driving this likeness in microbial community composition. This variable may also be linking daily alcohol consumption and BMI scores in some way.

Early experiments in animal models found consistent increased Firmicutes and decreased Bacteroidetes in obese mice compared to wildtype, and this was supported by

research in the gut microbiota of humans with obesity (45, 46). Many studies since have also found a correlation between the F/B ratio and BMI, while other studies exist where no trend was found (47). Recently, a meta-analysis was performed on many research studies on this topic to investigate these contradictory results. These conflicting findings may be due to varying methodology in sequencing and quantifying taxa as well as differences in sample handling, which raises doubts of the existence of a relationship between F/B ratio and BMI (47). Additionally, as this index considers the relatively high taxonomic rank of phyla, the complexity of the microbiota may not be well explained by the ratio alone (48). The results from our study indicate no relationship between overweightness and the abundance of Firmicutes or Bacteroidetes, supporting the increasing literature that is calling into question the use of this ratio as a possible hallmark of obesity.

While no relationship was observed between the F/B ratio and BMI, a family in the Firmicutes phylum, Veillonellaceae, was found to be more abundant in overweight PD patients through both differential abundance and indicator taxa analysis. Veillonellaceae are obligate anaerobes that can commonly be found in the gut of humans and some other animals (49). This family has previously been correlated with obesity (50), high fat diets (51), and the conversion of lactate to produce propionate (52), a SCFA which may be associated with weight changes (53). Notably, propionate is one of the SCFAs elevated in the microbiota of PD patients (54). Veillonellaceae may also be of note as this taxon has been found to be more prevalent in patients with liver cirrhosis, a condition which can be caused by overconsumption of alcohol (55). The increased abundance seen here may further link the two health concerns investigated in our study.

Limitations. Several limitations restricted the scope of our study, including the origin of the metadata used in all analyses. The collection of metadata was done with the purpose of supplementing the Cristea et al. study with covariate information, rather than focusing on a main research question investigating them. Therefore, a wide representational range of alcohol consumption levels or BMI was not covered by the metadata. For example, alcohol consumption numbers were skewed to the lower side, with a majority of the samples falling in the “low” category, thus not having an equal number of samples in each category. This calls into question the validity of the findings from the alcohol section, and further examination into the specific relationship between gut microbiome composition and alcohol consumption is needed. In previous investigation of BMI, many studies tend to compare the extremes and present a more evident pattern. However, for our study, “underweight”, “obese”, and “morbidly obese” groups were excluded from analyses due to the low number of patients belonging to these groups. It is possible that trends exist at the extremes of BMI but were not observed due to the constraints of the dataset. Due to the nature of this study, no further collection of samples was possible to supplement the analysis.

Additionally, limitations of the Cirstea et al. paper also extend to our study. Firstly, the existing metadata only allows for correlational studies, thus no causal conclusions could be drawn. Moreover, the gender ratio of the samples was skewed to males, as they are disproportionately affected by PD diagnoses. Accordingly, since a number of the control individuals were spouses, there was a higher number of females in the control group. In the original Cirstea et al. study, they considered the gender imbalances in their analysis and it had no major impact on their findings. Although our study did not account for the role of gender, that is a valuable consideration in future studies.

Quantification of intestinal inflammation at the cellular or tissue-level was also not possible due to limited expertise, as well as resource and time constraints. Butyrate is one of the primary indicators of gut inflammation. Investigating immune cell responses or gene expression changes of intestinal epithelia in response to changing microbiome composition would have provided valuable information on the relationship between alcohol consumption and microbiota composition. Moreover, previous studies have suggested the type of alcohol consumed mediates the impact of alcohol intake on PD risk (9). Since the metadata from Cirstea et al. did not specify the type of alcohol in their alcohol intake measures, it was impossible to further explore this claim.

Conclusions The *in silico* analysis of metadata collected by Cirstea et al. gave insight on putative associations between gut microbiome composition in PD patients with daily alcohol consumption and BMI. These findings suggest no correlation between alcohol consumption levels and PD progression, measured through symptom severity. Furthermore, alcohol was not found to affect the relative abundance of butyrate-producing genera in the gut microbiome in control or PD patients. The relative abundances of the genera *Roseburia*, *Lachnospira*, and *Faecalibacterium* were significantly lower in PD groups. The anti-inflammatory properties of these genera suggest that they may play a role in reducing PD risk in healthy individuals. Additionally, this study shows no association between changes in gut microbial composition and BMI or PD status. These findings indicate the abundance of Firmicutes and Bacteroidetes is not linked to increased body weight, suggesting that the F/B ratio is not a suitable biomarker for obesity. Lastly, the family Veillonellaceae was found to be more abundant in overweight PD patients compared to normal weight controls.

Future Directions The data analyses performed in this study lays a foundation for multiple future directions. A repeat analysis of the association between alcohol consumption and BMI in PD patients, with other relevant variables such as mental health, changes in appetite, and changes in motor function matched between groups, may lead to more significant results consistent with suggestions from previous literature. As both the alcohol consumption and BMI analyses revealed an unexplained microbial community composition pattern via Weighted UniFrac beta diversity analysis, it is very likely that another factor, related to the abundance of microbiota species, is driving this observation. Future studies can either use the same metadata set from Cirstea et al. to analyse if any collected variables drive this community structure or repeat a similar sample collection protocol to analyse new data in order to examine possible relationships with the gut microbiome composition. A repeated data collection on another group of PD patients would also allow a wider and more even distribution of alcohol consumption or BMI among individuals, in order to validate our findings.

Specifying the type of alcohol consumed would allow more specific hypotheses to be tested regarding the relationship between alcohol consumption and PD symptom severity. Future work can also aim to examine intestinal inflammation markers via identifying and quantifying immune cells in the gut, analysing inflammation-associated gene expression changes in the host, and performing proteomic analysis to identify secreted pro-inflammatory cytokines and signalling proteins. Additionally, the role of the family Veillonellaceae can be further investigated in overweight and alcoholic PD patients, as a link between a metabolite produced by this taxon and these health concerns may exist. It would also be interesting to evaluate if gender has a mediating role on how alcohol consumption and BMI can influence the gut microbiome composition of PD patients.

ACKNOWLEDGEMENTS

This study was completed with resources and funding support from the Department of Microbiology & Immunology at the University of British Columbia (UBC). We thank Dr. David Oliver and Dr. Stephan Koenig, who provided intellectual support and data analysis support, respectively. We would like to show our gratitude to Emily Adamczyk, who provided immense help with experimental design, data analysis support, writing review and revision that greatly improved the manuscript. We are grateful to Mihai Cirstea for offering experimental design help and for generating the metadata set that was analysed in this study along with Adam Yu, Ella Golz, Kristen Sundvick, Daniel Kliger, Nina Radisavljevic, Liam Foulger, Melissa Mackenzie, Tau Huan, Brett Finlay, and Silke Appel-Cresswell. We thank Ilan Rubin and the rest of the UBC EDUCE teaching team for their invaluable data analysis support.

CONTRIBUTIONS

This study was co-authored by all team members. Data analysis in QIIME2 was performed by Madeline Fung. Data analysis in R was performed by Jared Dutra, Michelle Ling, and Rui Lin Zhi. All members contributed equally to the written composition of each section of the manuscript.

REFERENCES

1. **DeMaagd G, Philip A.** 2015. Parkinson's Disease and Its Management: Part 1: Disease Entity, Risk Factors, Pathophysiology, Clinical Presentation, and Diagnosis. *P T* **40**:504–532.
2. **Emamzadeh FN, Surguchov A.** 2018. Parkinson's Disease: Biomarkers, Treatment, and Risk Factors. *Front Neurosci* **12**:612.
3. **Braak H, Tredici KD, Rüb U, de Vos RAI, Jansen Steur ENH, Braak E.** 2003. Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of Aging* **24**:197–211.
4. **Haikal C, Chen Q-Q, Li J-Y.** 2019. Microbiome changes: an indicator of Parkinson's disease? *Transl Neurodegener* **8**:38.
5. **Cirstea MS, Yu AC, Golz E, Sundvick K, Kliger D, Radisavljevic N, Foulger LH, Mackenzie M, Huan T, Finlay BB, Appel-Cresswell S.** 2020. Microbiota Composition and Metabolism Are Associated With Gut Function in Parkinson's Disease. *Mov Disord* **35**:1208–1217.
6. Top 10 Most Common Health Issues - Common Senior Health Issues - Senior Health - *University of Rochester Medical Center.*
7. **Paul KC, Chuang Y, Shih I, Keener A, Bordelon Y, Bronstein JM, Ritz B.** 2019. The association between lifestyle factors and Parkinson's disease progression and mortality. *Mov Disord* **34**:58–66.
8. **Kim IY, Yang TO, Heath AK, Simpson RF, Reeves GK, Green J, Floud S, Brown A, Hunter DJ, Beral V, Sweetland S, on behalf of the Million Women Study Collaborators.** 2020. Alcohol Intake and Parkinson's Disease Risk in the Million Women Study. *Mov Disord* **35**:443–449.
9. **Liu R, Guo X, Park Y, Wang J, Huang X, Hollenbeck A, Blair A, Chen H.** 2013. Alcohol Consumption, Types of Alcohol, and Parkinson's Disease. *PLoS One* **8**:e66452.
10. **Dubinkina VB, Tyakht AV, Odintsova VY, Yarygin KS, Kovarsky BA, Pavlenko AV, Ischenko DS, Popenko AS, Alexeev DG, Taraskina AY, Nasyrova RF, Krupitsky EM, Shalikiani NV, Bakulin IG, Shcherbakov PL, Skorodumova LO, Larin AK, Kostryukova ES, Abdulkhakov RA, Abdulkhakov SR, Malanin SY, Ismagilova RK, Grigoryeva TV, Ilina EN, Govorun VM.** 2017. Links of gut microbiota composition with alcohol dependence syndrome and alcoholic liver disease. *Microbiome* **5**:141.
11. **Liu H, Wang J, He T, Becker S, Zhang G, Li D, Ma X. 2018.** Butyrate: A Double-Edged Sword for Health? *Advances in Nutrition* **9**:21–29.
12. **Wang L, Lee I-M, Manson JE, Buring JE, Sesso HD.** 2010. Alcohol consumption, weight gain, and risk of becoming overweight in middle-aged and older women. Arch *Intern Med* **170**:453–461.
13. **Chen J, Guan Z, Wang L, Song G, Ma B, Wang Y.** 2014. Meta-Analysis: Overweight, Obesity, and Parkinson's Disease. *Int J Endocrinol* **2014**.
14. **Chen H.** 2004. Obesity and the Risk of Parkinson's Disease. *American Journal of Epidemiology* **159**:547–555.
15. **Castaner O, Goday A, Park Y-M, Lee S-H, Magkos F, Shiow S-ATE, Schröder H.** 2018. The Gut Microbiome Profile in Obesity: A Systematic Review. International *Journal of Endocrinology* **2018**:1–9.
16. **Magne F, Gotteland M, Gauthier L, Zazueta A, Pesoa S, Navarrete P, Balamurugan R.** 2020. The Firmicutes/Bacteroidetes Ratio: A Relevant Marker of Gut Dysbiosis in Obese Patients? *Nutrients* **12**:1474.
17. **Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F, Bai Y, Bisanz JE, Bittinger K, Brejnrod A, Brislawn CJ, Brown CT, Callahan BJ, Caraballo-Rodríguez AM, Chase J, Cope EK, Da Silva R, Diener C, Dorrestein PC, Douglas GM, Durall DM, Duvallet C, Edwardson CF, Ernst M, Estaki M, Fouquier J, Gauglitz JM, Gibbons SM, Gibson DL, Gonzalez A, Gorlick K, Guo J, Hillmann B, Holmes S, Holste H, Huttenhower C, Huttley GA, Janssen S, Jarmusch AK, Jiang L, Kaehler BD, Kang KB, Keefe CR, Keim P, Kelley ST, Knights D, Koester I, Kosciolek T, Kreps J, Langille MGI, Lee J, Ley R, Liu Y-X, Loftfield E, Lozupone C, Maher M, Marotz C, Martin BD, McDonald D, McIver LJ, Melnik AV, Metcalf JL, Morgan SC, Morton JT, Naimey AT, Navas-Molina JA, Nothias LF, Orchanian SB, Pearson T, Peoples SL, Petras D, Preuss ML, Pruesse E, Rasmussen LB, Rivers A, Robeson MS, Rosenthal P, Segata N, Shaffer M, Shiffer A, Sinha R, Song SJ, Spear JR, Swafford AD, Thompson LR, Torres PJ, Trinh P, Tripathi A, Turnbaugh PJ, Ul-Hasan S, van der Hooft JJJ, Vargas F, Vázquez-Baeza Y, Vogtmann E, von Hippel M, Walters W, Wan Y, Wang M, Warren J, Weber KC, Williamson CHD, Willis AD, Xu ZZ, Zaneveld JR, Zhang Y, Zhu Q, Knight R, Caporaso JG.** 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat Biotechnol* **37**:852–857.
18. **Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJA, Holmes SP.** 2016. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat Methods* **13**:581–583.
19. **R Core Team.** 2018. R: a language and environment for statistical computing. 4.0.3. R Foundation for Statistical Computing, Vienna, Austria.
20. **RStudio Team.** 2020. RStudio: Integrated Development for R. 1.3.1093. RStudio, Inc., Boston, MA.
21. **Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen T, Miller E, Bache S, Müller K, Ooms J, Robinson D,**

Seidel D, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H. 2019. Welcome to the Tidyverse. *JOSS* **4**:1686.
22. **Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, Minchin PR, O'Hara B, Simpson GL, Solymos P, Stevens MHH, Szoecs E, Wagner H.** 2020. vegan: Community Ecology Package. 2.5-7.
23. **McMurdie PJ, Holmes S.** 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. *PLoS ONE* **8**:e61217.
24. **Love MI, Huber W, Anders S.** 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* **15**:550.
25. **Wickham H, Francois R, Henry L, Müller K.** 2020. dplyr: a grammar of data manipulation. R package version 1.0.2.
26. **Wickham H, Navarro D, Pedersen TL.** 2016. ggplot2: elegant graphics for data analysis, **3rd ed**, p 3–10. Springer, New York, NY.
27. **Dowle M, Srinivasan A, Gorecki J, Chirico M, Stetsenko P, Short T, Lianoglou S, Antonyan E, Bonsch M, Parsonage H, Ritchie S, Ren K, Tan X, Saporta R, Seiskari O, Dong X, Lang M, Iwasaki W, Wenchel S, Broman K, Schmidt T, Arenburg D, Smith E, Cocquemas F, Gomez M, Chataignon P, Blaser N, Selivanov D, Riabushenko A, Lee C, Groves D, Possenriede D, Parages F, Toth D, Yaramaz-David M, Perumal A, Sams J, Morgan M, Quinn M, @javrucebo, @marc-outins, Storey R, Saraswat M, Jacob M, Schubmehl M, Vaughan D, Hocking T, Silvestri L, Barrett T, Hester J, Damico A, Freundt S, Simons D, de Andrade ES, Miller C, Meldgaard JP, Tlapak V, Ushey K, Eddelbuettel D.** 2020. data.table: Extension of 'data.frame'. R package version 1.13.4.
28. **Kassambara A.** 2020. rstatix: Pipe-Friendly Framework for Basic Statistical Tests. R package version 0.6.0.
29. **Kassambara A.** 2020. ggpubr: 'ggplot2' Based Publication Ready Plots. R package version 0.4.0.
30. **Albouk A**. 2020. How to Add P-values to GGPLOT Facets: Step by Step Guide. Datanovia.
31. **Microsoft Corporation.** 2019. Microsoft Excel 2019. Microsoft Corporation, Redmond, WA.
32. **De Cáceres M, Jansen F, Dell N.** 2020. indicspecies: Relationship Between Species and Groups of Sites. 1.7.9.
33. **Ishihara L, Brayne C.** 2005. A systematic review of nutritional risk factors of Parkinson's disease. *Nutr Res Rev* **18**:259–282.
34. **Eriksson A-K, Löfving S, Callaghan RC, Allebeck P.** 2013. Alcohol use disorders and risk of Parkinson's disease: findings from a Swedish national cohort study 1972–2008. *BMC Neurol* **13**:190.
35. **Bettiol SS, Rose TC, Hughes CJ, Smith LA.** 2015. Alcohol Consumption and Parkinson's Disease Risk: A Review of Recent Findings. *JPD* **5**:425–442.
36. **Cantu-Jungles TM, Rasmussen HE, Hamaker BR.** 2019. Potential of Prebiotic Butyrogenic Fibers in Parkinson's Disease. *Front Neurol* **10**:663.
37. **Wallen ZD, Appah M, Dean MN, Sesler CL, Factor SA, Molho E, Zabetian CP, Standaert DG, Payami H.** 2020. Characterizing dysbiosis of gut microbiome in PD: evidence for overabundance of opportunistic pathogens. *npj Parkinsons Dis* **6**:11.
38. **Weis S, Schwiertz A, Unger MM, Becker A, Faßbender K, Ratering S, Kohl M, Schnell S, Schäfer K-H, Egert M.** 2019. Effect of Parkinson's disease and related medications on the composition of the fecal bacterial microbiota. *npj Parkinsons Dis* **5**:28.
39. **Gerhardt S, Mohajeri M.** 2018. Changes of Colonic Bacterial Composition in Parkinson's Disease and Other Neurodegenerative Diseases. *Nutrients* **10**:708.
40. **Patterson AM, Mulder IE, Travis AJ, Lan A, Cerf-Bensussan N, Gaboriau-Routhiau V, Garden K, Logan E, Delday MI, Coutts AGP, Monnais E, Ferraria VC, Inoue R, Grant G, Aminov RI.** 2017. Human Gut Symbiont Roseburia hominis Promotes and Regulates Innate Immunity. *Front Immunol* **8**:1166.
41. **Vascellari S, Palmas V, Melis M, Pisanu S, Cusano R, Uva P, Perra D, Madau V, Sarchioto M, Oppo V, Simola N, Morelli M, Santoru ML, Atzori L, Melis M, Cossu G, Manzin A.** 2020. Gut Microbiota and Metabolome Alterations Associated with Parkinson's Disease. *mSystems* **5:**mSystems.00561-20, e00561-20.
42. **Bender DA, Earl CJ, Lees AJ.** 1979. Niacin Depletion in Parkinsonian Patients Treated with L-dopa, Benserazide and Carbidopa. *Clinical Science* **56:**89–93**.**
43. **Houser MC, Tansey MG.** 2017. The gut-brain axis: is intestinal inflammation a silent driver of Parkinson's disease pathogenesis? *npj Parkinson's Disease* **3**:3.
44. **Kashihara K.** 2006. Weight loss in Parkinson's disease. *J Neurol* **253**:vii38–vii41.
45. **Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI.** 2005. Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences* **102**:11070–11075.
46. **Ley RE, Turnbaugh PJ, Klein S, Gordon JI.** 2006. Human gut microbes associated with obesity. *Nature* **444**:1022–1023.
47. **Walters WA, Xu Z, Knight R.** 2014. Meta-analyses of human gut microbes associated with obesity and IBD. *FEBS Letters* **588**:4223–4233.
48. **Tseng C-H, Wu C-Y.** 2019. The gut microbiome in obesity. *Journal of the Formosan Medical Association* **118**:S3–S9.
49. **Singleton P, Sainsbury D.** 2006. Dictionary of microbiology and molecular biology. 3rd ed. rev. Wiley, Chichester, West Sussex ; Hoboken, NJ.

50. **Yun Y, Kim H-N, Kim SE, Heo SG, Chang Y, Ryu S, Shin H, Kim H-L.** 2017. Comparative analysis of gut microbiota associated with body mass index in a large Korean cohort. *BMC Microbiol* **17**:151.
51. **Lecomte V, Kaakoush NO, Maloney CA, Raipuria M, Huinao KD, Mitchell HM, Morris MJ.** 2015. Changes in Gut Microbiota in Rats Fed a High Fat Diet Correlate with Obesity-Associated Metabolic Parameters. *PLoS ONE* **10**:e0126931.
52. **Flint HJ, Scott KP, Louis P, Duncan SH.** 2012. The role of the gut microbiota in nutrition and health. *Nat Rev Gastroenterol Hepatol* **9**:577–589.
53. **Dinerstein C.** 2019. Propionate, A Common Food Preservative, Alters Our Metabolism. Does That Make It A Disruptor? *American Council on Science and Health.*
54. **Campos-Acuña J, Elgueta D, Pacheco R.** 2019. T-Cell-Driven Inflammation as a Mediator of the Gut-Brain Axis Involved in Parkinson's Disease. *Front Immunol* **10**:239.
55. **Chen Y, Yang F, Lu H, Wang B, Chen Y, Lei D, Wang Y, Zhu B, Li L.** 2011. Characterization of fecal microbial communities in patients with liver cirrhosis. *Hepatology* **54**:562–572.
56. **Canadian Centre on Substance Use and Addiction.** 2018. Canada's Low-Risk Alcohol Drinking Guidelines. Canadian Centre on Substance Use and Addiction. Ontario, ON.