Volume 10:1-12

The Undergraduate Journal of Experimental Microbiology & Immunology (+Peer Reviewed)

Parkinson's Disease Status but not High Sodium Consumption is Correlated with Alterations in the Gut Microbiome

Jamila Huseynova, Sana Alayoubi, Ives Chau, Alissa Gama, Ethan Wong

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

SUMMARY Globally, Parkinson's disease (PD) is emerging as the most rapidly growing neurological disorder. PD is associated with a strongly altered gut microbial composition, which may promote disease in part by increasing inflammation. A high sodium diet (HSD) has been shown to alter gut microbial composition in a pro-inflammatory manner, and thus our study aimed to determine whether people with PD who had HSDs experienced more dysbiosis than those with lower sodium intake. If a correlation between HSDs and an exacerbated PD gut phenotype exists, adopting low-sodium diets could emerge as a viable strategy to mitigate PD gut dysbiosis and alleviate associated downstream symptoms. We examined differences in gut microbial diversity and composition related to an HSD within a cross-sectional cohort of 281 individuals both with (n=182) and without PD (n=99). Our approach involved employing alpha and beta diversity analyses alongside differential abundance analyses at the amplicon sequence variant (ASV) level. An HSD was associated with significant differences in the gut microbial composition within the control subjects, but not those with PD. Thus, our findings suggest that the factors underlying the distinct gut microbial profile associated with PD appear to exert a more pronounced influence than the impact of sodium. Based on our findings, there is no compelling evidence to advise individuals with PD against consuming HSD for alleviating gastrointestinal dysbiosis. Overall, our research provides more insight into the correlation between sodium and the gut microbial composition of individuals both with and without PD and establishes novel avenues for future research.

INTRODUCTION

Globally, neurological disorders are the top cause of disability, with Parkinson's disease (PD) the most rapidly growing among them (1). As the second most prevalent neurodegenerative disorder worldwide, PD impacts over eight million individuals (2). PD encompasses a range of symptoms including tremors, rigidity, bradykinesia, postural instability, and impairment of cognitive function (1). Another defining feature of PD pathogenesis is gastrointestinal tract dysfunction, reported to arise as early as ten years before clinical diagnosis (3). Individuals with PD report having major difficulties swallowing, constipation, unexpected weight gain or loss, uncontrolled drooling, as well as oral and dental disorders (4).

Alterations in the gut microbial composition may promote oxidative stress, inflammation, and in some cases, affect gut permeability, which impairs the blood-brain barrier and leads to neural degeneration (5, 6). Recent research has shed light on a potential link between the gut microbiota and the onset of PD symptoms, particularly concerning gastrointestinal issues including constipation and slow colonic transit time (6). To explore this link between the gut microbiome and PD, Metcalfe-Roach et al. (7) collected fecal samples from a group of 197

Published Online: September 2024

Citation: Huseynova, Alayoubi, Chau, Gama, Wong. 2024. Parkinson's disease status but not high sodium consumption is correlated with alterations in the gut microbiome. UJEMI+ 10:1-12

Editor: Shruti Sandilya, University of British Columbia

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

participants with Parkinson's and 103 healthy individuals, along with comprehensive data on each individual's medication, diet, demographics, and various motor and non-motor PD symptoms. Cirstea et al. found a significant taxonomic difference in the gut microbiome in participants with PD compared to controls (8). Several studies have suggested that distinct differences in gut microbiota exist among PD patients compared to healthy controls (9-13).

Generally, Dietary Guidelines for Americans recommends consuming less than 2,300 mg of sodium per day (14). Many individuals on Western diets regularly consume excess dietary sodium, at an average of 3,500 mg per day (15). A high sodium diet (HSD) has been demonstrated to alter microbial composition and function by reducing the occurrence of short-chain fatty acid-producing bacteria such as *Lactobacillus* species in the gut microbiota, thus promoting a pro-inflammatory environment in the gut (5). Short-chain fatty acids (SCFAs) have shown promise in stimulating dopamine production and safeguarding dopaminergic neurons. This physiological effect has been shown to partially alleviate neurological damage in individuals with PD (16, 17, 18, 19). Previous studies have demonstrated that certain diets such as low-protein, high-fiber or a high-quality (characterized by a high HEI score) diet, may alter the gut microbial composition in individuals with and without PD in a way that promotes SCFA production (20, 21, 22). However, there is a lack of research on the impact of a diet that is particularly high in sodium on the gut microbiota in the context of individuals with PD. Our study aims to investigate whether high sodium intake is associated with alterations in the human gut microbiota composition and provide further insight into whether such differences vary between individuals with PD and those without PD. We hypothesize that high sodium intake correlates strongly with gut dysbiosis in individuals both with PD and without PD, and we expect to observe significant differences in the microbial gut diversity between individuals on high-sodium and low-sodium diets. Investigating the effects of high sodium content on the human gut microbiome aligns with the goal of informing lifestyle modifications, symptom management, and improving overall quality of life of individuals with PD. Additionally, exploring the correlation between an elevated sodium intake and the alterations in the gut microbial composition of individuals with PD has not been attempted before; therefore, our research could contribute to better understanding of whether a low-sodium diet could potentially be beneficial for alleviating gastrointestinal symptoms in individuals with PD.

METHODS AND MATERIALS

Dataset and metadata. In this study, we utilized data from a cohort of 300 individuals, comprising 197 PD individuals and 103 control subjects without PD or any related degenerative disorders. (7). The data, including bacterial 16S rRNA gene sequencing targeting the V4 region, was sourced from fecal samples collected and processed by Metcalfe-Roach et al. using the Illumina MiSeq platform. Additionally, we leveraged extensive metadata, which included 99 variables such as constipation severity score, body mass index (BMI), age, disease duration, education, calcium and dietary intake variables like calcium, sodium, and various food groups. Our analysis specifically focuses on sodium intake and sodium-rich food groups as predictor variables. To better suit our investigation on the correlation between sodium intake and PD gut microbiomes, participants with missing values for sodium (n=15), age (n=0), and disease (n=0) were removed from the sample. Additionally, sodium values exceeding 5000 mg/day were excluded to eliminate outliers (n=4). This filtering process resulted in final sample sizes of 281 individuals for the entire cohort, with 182 in the PD group and 99 in the control group.

The data used in our study came from food frequency questionnaire (FFQ) responses provided by participants, which were originally designed by Metcalfe-Roach et al. (7). Cirstea et al. then ran these responses through the FFQ EPIC Tool for Analysis (FETA) software (8, 23). The median participant age was 66 years in both the control and PD groups, with an overall age range from 58 to 71 years. Given the significant impact of age on gut microbial composition, age was controlled in our analyses (24).

Preliminary data processing in QIIME2 and R. We performed the preliminary data analysis on the dataset in QIIME2, which contained high-quality Amplicon Sequence Variant (ASV) reads containing 251 nucleotides. Utilizing the DADA2 pipeline (25), we filtered

ASVs without truncating sample length, resulting in the retention of all ASVs. Preliminary manipulation in RStudio (26) utilized tidyverse (27), ape (28), phyloseq (29), picante (30), and vegan (31), filtering for bacteria, excluding chloroplast and mitochondrial ASVs, eliminating rare ASVs with fewer than 5 counts, and removing samples with fewer than 100 total reads.

Statistical analysis of diversity. The phyloseq object created was rarefied in even depths of 6853 sample size, determined using a rarefaction curve of the cohort metadata. RStudio (Version: 2023.12.1+402) was used to produce alpha and beta diversity metrics. Sodium intake acted as the explanatory variable, allowing for assessment of metrics across the entire disease cohort and for disease-stratified groups. RStudio packages utilized for these calculations include tidyverse (27), ape (28), phyloseq (29), picante (30), vegan (31), and ggplot2 (32). Statistical significance, p, was set at an alpha level of less than 0.05. For alpha diversity, assessed metrics included observed richness, Faith's phylogenetic diversity and Shannon's diversity. Statistical analysis on alpha diversity was calculated using linear regression, predicting the significance of sodium intake whilst controlling for age and/or disease status. For beta diversity, assessed metrics by using Bray-Curtis, Weighted UniFrac, and Unweighted UniFrac were performed on the overall cohort and disease-stratified groups, while controlling for age and/or disease status. Permutational Analysis of Variance (PERMANOVA) from vegan (31) compared beta diversity metrics and generated principal coordinates analysis plots (PCoA). Steps are outlined in our scripts: ACGHW7523 - 024 (Alpha & Beta - Entire Cohort) and ACGHW7523 - 027 (Alpha & Beta - Disease Stratified).

Differential abundance analyses. Differential abundance analyses of microbial genera were conducted using DESeq2 in R v4.3.1 (33). The following packages were loaded into R for the differential abundance analysis: phyloseq (29), which provided a framework to organize and visualize microbiome data, and DESeq2, which allowed to model count data and apply statistical tests to identify significant changes in microbial abundance between high-sodium and low-sodium intake groups (34). As part of our analysis of the entire cohort, we controlled for both age and disease status to address potential confounding factors. Analyses of divided cohort participants with and without PD, we controlled for age as a covariate.

The following conditions were the same for all DESeq2 analyses. All reads were adjusted by one count. Units of sodium were centered and scaled to unit variance to normalize the values, and the taxonomy data were agglomerated to the genus level. The $\log_2$fold change was calculated using sodium as a continuous variable and reported as the estimated change in taxonomic abundance per unit of autoscaled sodium intake. The significance threshold for the adjusted p-values was set to <0.01 and the threshold for $\log_2$fold changes was set to greater than 2 or less than -2. The resulting significantly differentially abundant genera were visualized via bar plots and volcano plots. All steps are outlined in scripts named ACGHW7523 -038 (Differential Abundance Tax Glom), ACGHW7523-059-1 (Differential Abundance - Disease stratified - PD), ACGHW7523-059-2 (Differential Abundance - Disease stratified - Ctrl) and the ACGHW7523 - 035 (Sodium and Food Groups) directories.

RESULTS

High and low sodium intake shows statistical significance in both alpha diversity and beta diversity in the overall cohort. Alpha diversity metrics measure variation within one community from a single sample (Figure 1), while beta diversity metrics measure variation between different communities from multiple samples (Figure 2). Analysis of the entire cohort revealed significant variations in alpha and beta diversity that correlated with sodium consumption, even when controlling for age and disease status (Figure 1-2 A, C, E).

Across the entire cohort, alpha diversity metrics such as Faith's phylogenetic diversity (Figure 1A, p=0.0078), observed richness (Figure 1C, p=0.0343), and Shannon's diversity (Figure 1E, p=0.0426), showed significant differences in gut microbiota with increasing sodium consumption, even when controlling for disease status and age. This highlights a clear correlation between higher sodium intake and changes in microbial alpha diversity.

Similarly, the Unweighted Unifrac (Figure 2A, p=0.038) and Bray-Curtis plots (Figure 2E, p=0.029) indicated significant differences, whereas the Weighted Unifrac plot did not

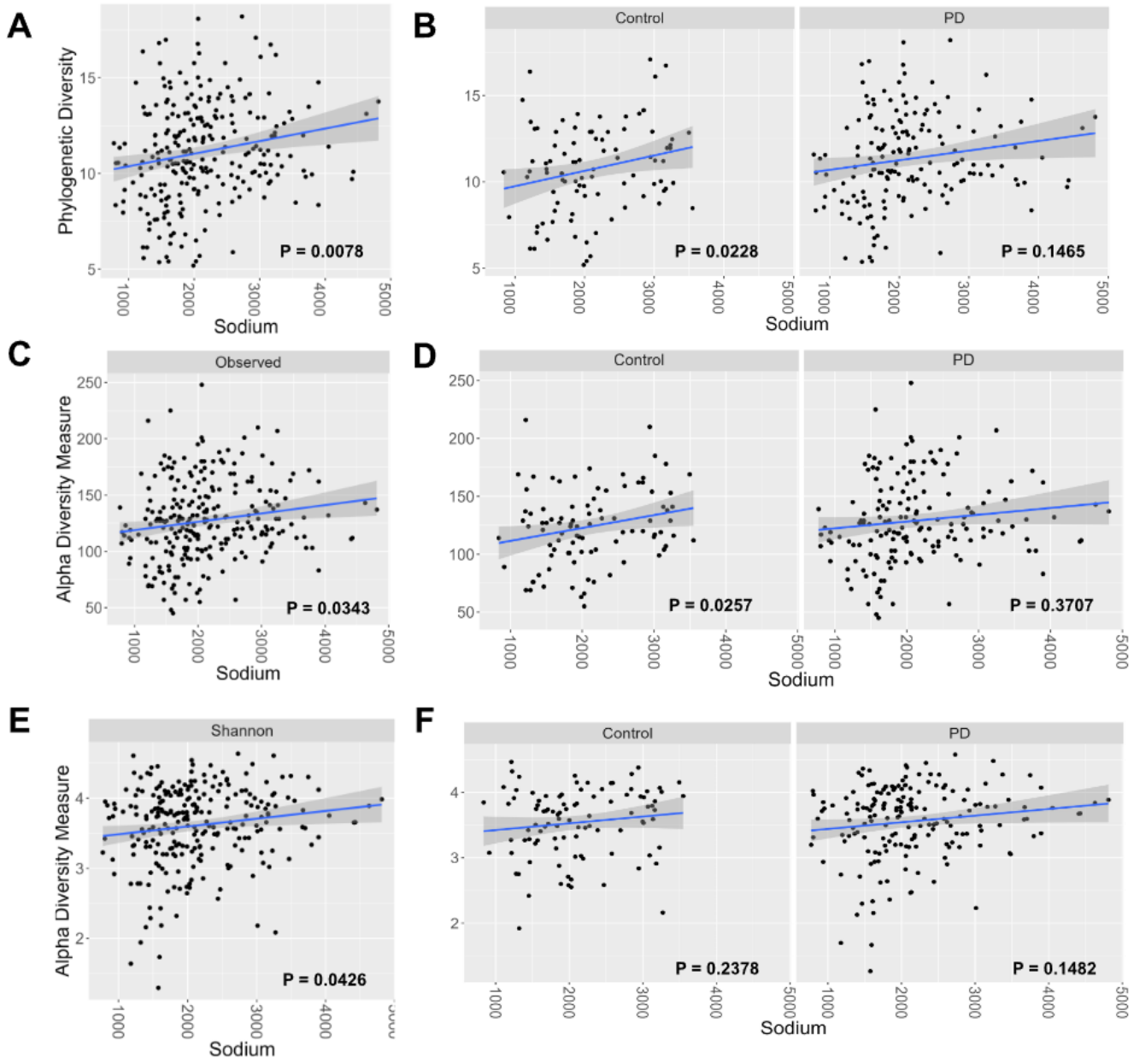

FIG. 1 Correlations between sodium intake (mg) and alpha diversity of gut microbiomes of the overall cohort and PD-stratified groups. Linear regression models of the overall cohort **(A, C, E)**, controlling for disease and age; of PD-stratified groups **(B, D, F)**, controlling for age. Faith's Phylogenetic Diversity **(A, B)**; Observed richness **(C, D)**; Shannon Diversity **(E, F)**. Significance: P* $\leq$ 0.05, p-value computed by linear regression model. Blue lines and shaded regions denote the linearized model and error distribution, respectively.

show any significance (Figure 2C, p=0.419). These findings suggest that microbial beta diversity also varies with increasing sodium consumption.

Significance of alpha and beta diversity is primarily influenced by control participants. Stratifying by disease status revealed differences in alpha and beta microbiome diversity between disease status cohorts (Figure 1-2: B, D, F), of which age is the controlling variable. When separating our dataset into non-PD participants and PD participants to investigate the effects of sodium on these two populations, we found that sodium only correlated significantly with diversity in non-PD participants.

In alpha diversity analyses, significant correlations were observed in Faith's phylogenetic diversity (control: p=0.0228; PD: p=0.1465) (Figure 1B) and observed richness (control:

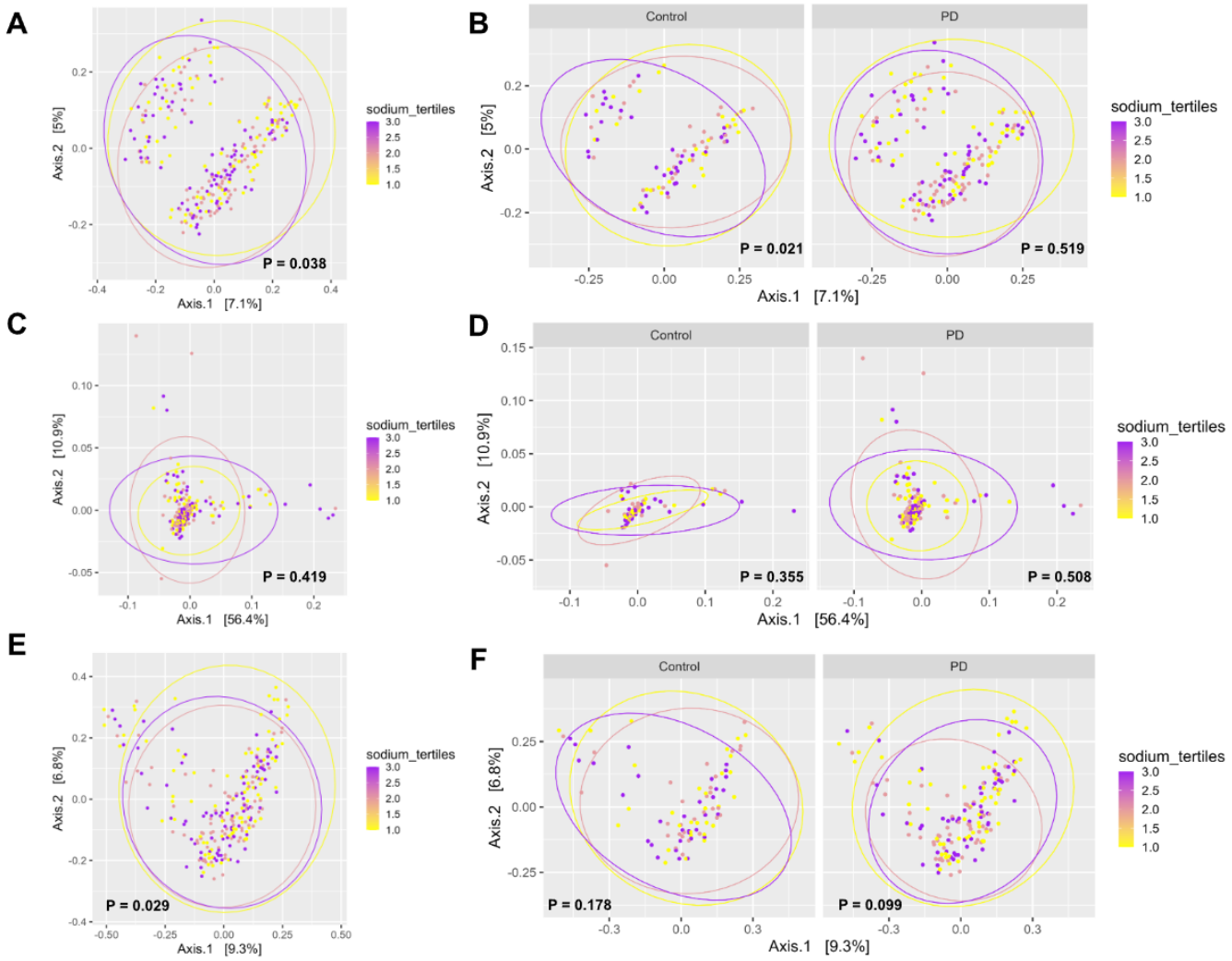

FIG. 2 Correlations between sodium intake (mg) and beta diversity of gut microbiomes in the overall cohort and PD-stratified groups. Sodium intake was separated into tertiles, with ellipses representing the 95% confidence interval of the group centroids (high sodium in purple, medium in orange, low in yellow) on the PCoA plots. **(A, C, E)** PERMANOVA test was conducted on the overall cohort, controlling for disease and age, and **(B, D, F)** on PD-stratified groups, controlling for age. PCoA plots: Unweighted Unifrac **(A, B)**; Weighted Unifrac **(C, D)**; Bray-Curtis **(E, F)**. Significance: P* ≤ 0.05, p-value indicative of PERMANOVA.

p=0.0257; PD: p=0.3707) (Figure 1D) among non-PD patients, whereas Shannon's diversity (control: p=0.2378; PD: p=0.1482) (Figure 1F) showed no significant correlation in either cohort. These results highlight a clear association between increased sodium consumption and changes in alpha microbiome diversity.

For beta diversity, Unweighted Unifrac (Control: p=0.021; PD: p=0.519) (Figure 2B) also demonstrated a distinct clustering pattern, with participants without PD and high sodium intake differing from those with low and medium sodium intake, after controlling for age. However, this pattern was not evident in the Weighted Unifrac (Control: p=355; PD: p=0.508) (Figure 2D) and Bray-Curtis plots (Control: p=0.178; PD: p=0.099) (Figure 2F), which did not account for skewed clustering effect. Thus, similar to alpha diversity, beta diversity is predominantly influenced by increased sodium consumption in control patients only.

High sodium consumption correlates with alterations in the prevalence of eight bacterial genera in the entire cohort (PD and non-PD subjects). Differential abundance analysis was performed to identify the association between elevated sodium consumption and alterations in the abundance of bacterial genera. Differential abundance analysis of the overall cohort exhibited an underrepresentation of *Treponema* (log_2fold change of -3.22, equivalent

to a 9.32-fold decrease in abundance) and overrepresentation of seven genera that include *Succinivibrio*, *Rikenellaceae RC9 gut group*, *Lactobacillus*, *Megasphaera*, *Bifidobacterium*, *Asteroleplasma*, and *CAG-873*. The most pronounced increase in abundance was observed in *Succinivibrio* (log_2fold change of 4.12, equivalent to a 17.4-fold increase in abundance) (Figure 3A).

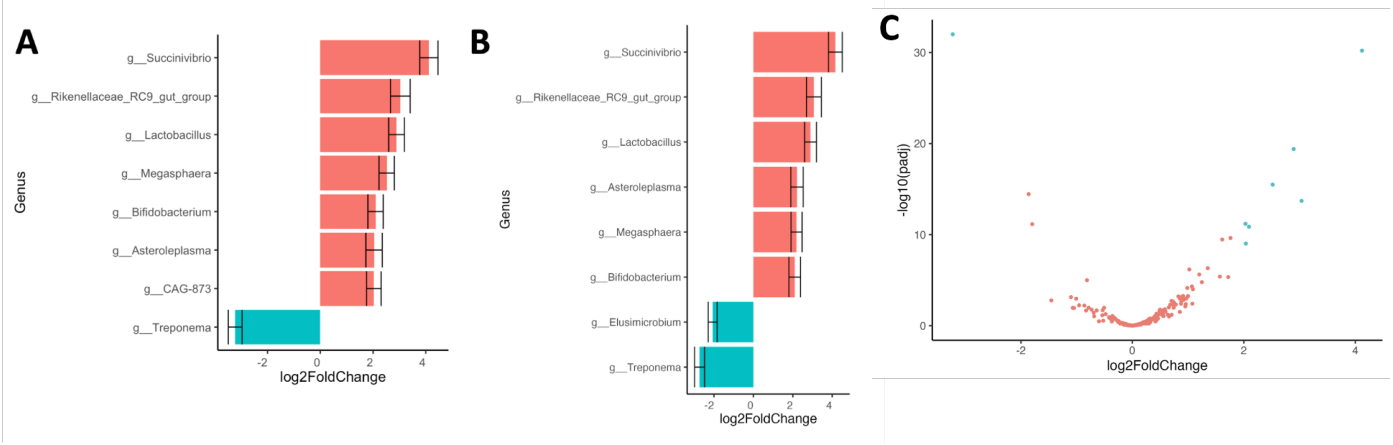

FIG. 3 DESeq2 analysis reveals that high sodium consumption in the entire cohort correlates to gut microbial alterations that are more typical of PD. Bar plots display differentially abundant genera in individuals with (**A**) high sodium consumption and (**B**) high soups and sauces consumption. Blue bars indicate decreased abundance while red bars signify increased abundance. (**C**) Volcano plot displays adjusted p-values and log_2fold changes for all genera in individuals with high sodium consumption. Significant genera are indicated in blue and non-significant genera are indicated in red. Log_2fold reflects the estimated change in taxonomic abundance per unit of autoscaled sodium. Only genera with log_2fold changes greater than 2 or less than -2 are considered significant. Significance: $p_{adj} < 0.01$.

No significant alterations in the abundance of bacterial genera were found with high sodium consumption in individuals with PD. To investigate the association of disease status and alterations in bacterial abundances with high sodium intake, we repeated the differential abundance analysis after dividing the cohort based on the disease status (PD vs. control individuals). Looking at individuals with PD, there were no differentially abundant genera found in those with an HSD; interestingly, even the eight bacterial genera that were abundantly present with elevated sodium in the overall cohort analysis (Figure 3A) were not differentially abundant in individuals with PD (Figure 4A). The differences in the abundance of *Treponema* approached significance in the non-PD group, with a log_2fold change of 1.77, equivalent to a 3.4-fold abundance increase. Differences in the abundance of *Succinivibrio* were also verging on statistical significance with a log_2fold change of -1.24, which translates to a 2.4-fold decrease in the abundance with elevated sodium intake. In individuals without PD, we identified one significantly differentially abundant genus, *Rikenellaceae RC9 gut group*, with a log_2fold change of 2.00, equivalent to a 4-fold increase in abundance with elevated sodium intake. Notably, *Rikenellaceae RC9 gut group* also showed a log_2fold change of 0.66, equivalent to a 1.6-fold increase in its abundance in individuals with PD.

Consumption of sodium-rich food groups correlates with alterations in bacterial genera abundances, mirroring alterations seen with high sodium consumption. To explore the possibility that sodium alone is not the main driving factor of the observed alterations in gut microbial composition and that constituents in sodium-rich food categories may correlate more strongly with the observed changes than sodium itself, we conducted an additional differential abundance analysis with specific food groups. First, we conducted correlation tests to determine which one of the available food groups correlated with sodium intake the most. Spearman's correlation test was selected due to a left-skewed data distribution of the food groups, as observed through the visualization of histograms (Supplemental Figure S1). As evident from the Spearman's correlation test, the food group that correlated with sodium content the most was soups and sauces (Supplemental Figure S2A). Consequently, we performed differential abundance analysis to investigate the abundance of bacterial genera associated with high consumption of soups and sauces in the overall cohort, and compared the log_2fold changes of shared genera, with those obtained from

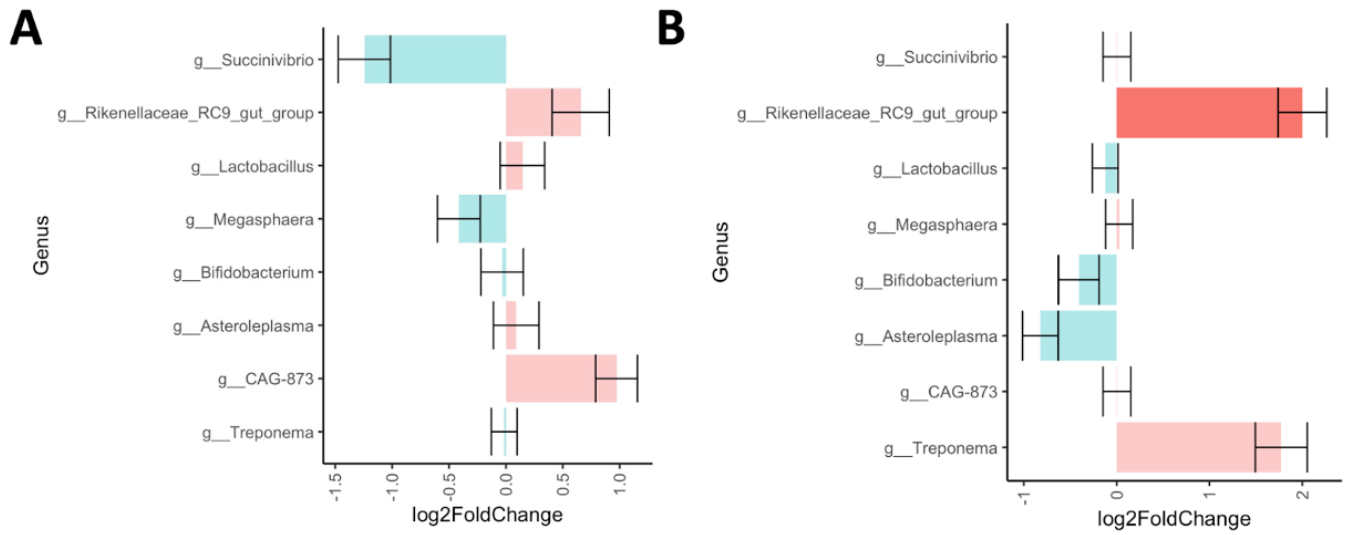

FIG. 4 High sodium consumption is not associated with changes in the gut microbial composition of PD participants. (**A**) Bar plot displays genera differentially abundant in PD participants with high sodium consumption and in (**B**) control participants with high sodium consumption. Blue bars represent genera with decreased abundance, while red bars represent genera with increased abundance. Log_2fold reflects the estimated change in taxonomic abundance per unit of autoscaled sodium. Only genera with >2 log_2fold change are significant as indicated by the darker coloured bars. Significance: $p_{adj} < 0.01$.

the analysis exclusively centered on sodium consumption (Figure 3A). There were a few notable differences between the two groups, with the soups and sauces-related analysis (Figure 3B) exhibiting non-significant results for *CAG-873* and significant changes in the *Elusimicrobium* abundances, as compared to sodium-associated changes seen in Figure 3A. Overall, however, the two predictor variables, elevated consumption of sodium and that of soups and sauces, correlate with roughly the same microbial organisms that demonstrate similar log_2fold changes (Table 1).

TABLE. 1 Log_2fold changes of differentially abundant genera for individuals consuming high amounts and sodium and high amounts of soups and sauces. Log_2fold reflects the estimated change in taxonomic abundance per scaled unit of variance for sodium. Significance: adjusted $p < 0.01$.

Genus	Log_2fold changes	
	Sodium	Soups and sauces
g__Treponema	-3.22	-2.73
g__Elusimicrobium	NA	-2.07
g__Bifidobacterium	2.10	2.10
g__Megasphaera	2.52	2.19
g__Asteroleplasma	2.04	2.21
g__Lactobacillus	2.89	2.90
g__Rikenellaceae_RC9	3.04	3.07
g__Succinivibrio	4.12	4.16

DISCUSSION

This study aimed to examine the correlation between high sodium intake and gut microbial alterations in the entire cohort, as well as between PD and non-PD participants. We investigated the gut microbiome variation through alpha and beta diversity analyses and examined differential abundance in relation to sodium intake.

In our alpha diversity analysis, we found significance in Faith's phylogenetic diversity – which measures phylogenetic relationships – for the entire cohort (Figure 1A). These results

are corroborated with a prior study in mice that examined the impact of varying NaCl concentrations in the diet (0.03% for sodium deficiency, 0.5% as the control, and 4% and 10% NaCl for elevated intake) on the gut microbial diversity (35). The study found that the 10% NaCl group had greater Faith's phylogenetic diversity compared to the 4% NaCl group, which the author claims could be ascribed to the proliferation of halotolerant species in the microbiota (35). Shannon's diversity and observed richness – which measure community richness with and without abundance respectively – were also significantly different between high-sodium and low-sodium groups for the entire cohort. These results are supported by Wilck et al.'s findings which state that high sodium consumption correlates with alterations in microbial diversity and richness (36).

When looking at beta diversity within the entire cohort, sodium intake variation was statistically significant in Bray-Curtis and Unweighted Unifrac (Figure 2A, E). Bray-Curtis is a metric that reflects compositional dissimilarity between two different sites; Unweighted Unifrac is a metric that reflects the changes in the abundance of rare genera. These results suggest a correlative relationship between high sodium intake and diversification of the overall microbiome composition, along with the presence of rare or low-abundance features in the control group. Observing the same beta diversity metric after disease status stratification, Unweighted UniFrac revealed significance in participants without PD (Figure 2B). These observations align with previous research done in healthy mice where high sodium intake was shown to lead to alterations in beta diversity of the gut microbiome (35). However, these observations in beta diversity are not reflected in our analysis of gut microbial diversity in participants with PD, potentially suggesting that the diagnosis of PD likely has a more pronounced influence on the gut microbial composition than the consumption of sodium. This phenomenon could be attributed to the disease status of individuals, as PD has an established impact on the gut microbiome (9-13). The disproportionate male representation of the PD cohort and the varied medications used to treat PD further contribute to the complex picture of microbial composition.

In our differential abundance analysis of the entire cohort, we found that eight genera had significantly increased or decreased abundance with high sodium consumption (Figure 3). Based on our results, we proposed that PD may be masking the impact of sodium on gut microbial composition. This is further supported by our findings within the PD cohort, where the eight genera that were initially differentially abundant across the entire cohort showed no significant differential abundance in participants with PD. Therefore, we propose that the influence of PD diagnosis on the composition of the human gut microbiome is likely more significant than the correlation seen with sodium.

Furthermore, an HSD has been firmly linked to the depletion of the *Lactobacillus* genus in humans (5, 36). However, our results deviate from this existing body of work, revealing an elevated abundance of *Lactobacillus* with high sodium intake. A comparable pattern was noted for *Bifidobacterium*, which exhibited increased abundance in individuals with heightened daily sodium intake, presenting a contrast to prior literature (35). One plausible explanation could be that, due to an overrepresentation of individuals with PD across our cohort, the patterns identified in our study once again align more closely with the trends observed in PD-associated gut microbiomes, wherein *Lactobacillus* and *Bifidobacterium* have been shown to have increased abundances (6, 34). Nevertheless, this pattern is reflected in our analysis of the control group that demonstrated a slight decrease in the differential abundances of *Lactobacillus* and *Bifidobacterium* in individuals without PD, with elevated sodium intake (Figure 4B). Overall, these results strengthen our hypothesis that the diagnosis of PD likely exerts a more prominent impact on human gut microbial composition than sodium.

Cirstea et al. proposed that higher levels of *Bifidobacterium* could be attributed to higher doses of levodopa (L-DOPA), prescribed with increased disease severity in participants (8, 37). L-DOPA is the gold standard prescription for individuals with PD, and *Bifidobacterium* has been shown to utilize excess L-DOPA for metabolism, leading to its proliferation in individuals with PD (38). *Bifidobacterium*-containing probiotics have proven beneficial to individuals with PD, by improving mood and alleviating gastrointestinal symptoms (39, 40). Therefore, the augmented *Bifidobacterium* abundance that we observe should not be a cause of concern considering the benefit this genus offers to individuals with PD. Also seen in

individuals with PD reporting an HSD was a notable rise in the abundance of *Rikenellaceae RC9 gut group* (Figure 4B). Consistent with our results, a previous study (35) found enriched abundances of the *Rikenellaceae* genus with elevated sodium intake.

Certain food groups such as sauces and spreads, processed meats, and salty snacks, have the highest mean sodium content among other foods (41). Our findings demonstrate comparable microbial abundances when considering sodium consumption and consumption of soups and sauces. Therefore, it is unclear if the consumption of soups and sauces confounds that of sodium in the changes observed in the microbial composition. However, since sodium is the only evident common dietary factor among all soups and sauces, sodium is the most likely determining factor for the alterations in the microbial composition observed.

Limitations Metcalfe-Roach et al. gathered data on food and nutrition values, including sodium, using the EPIC-Norfolk Food Frequency Questionnaire. The questionnaire uses algorithms to estimate the average daily nutrition intake from reported amounts or groups of food eaten (7). While some food items like snacks or prepackaged foods may have brand names or manufacturer labels to provide information, it is difficult to account for homemade foods or restaurant foods that may include more or less sodium than the average expected amount. Therefore, the method used to report dietary sodium intake introduces limitations to the dataset utilized for our study, as the values for sodium intake are estimates, and self-reporting through a questionnaire is susceptible to inaccuracies.

Additionally, the PD cohort exhibits a notable overrepresentation of males (with a distribution of 116 males and 66 females), in contrast to the control group (with a distribution of 47 males and 52 females). Therefore, sex remains a confounding variable for variations in the gut microbiome observed with elevated sodium intake between the PD and control cohorts. Differences in the gut microbiome among people, influenced by genetic background and environmental factors, could also be obscuring potential changes in the microbiome associated with sodium. Finally, due to the cross-sectional design of the study, the data are correlative; therefore, we cannot conclude cause and effect using the collected data.

In terms of the analyses conducted, one major limitation of applying DESeq2 to microbial data is the assumption that the underlying counts follow a negative binomial distribution. Microbial datasets frequently contain a large number of zeros and highly variable counts, which can lead to poor model fitting and, consequently, biased differential abundance estimates. To mitigate this issue, counts were adjusted by one, however this may not have been enough to fix the underlying issue, thus the data from this study is susceptible to bias. Future studies can employ alternative methods such as zero-inflated models to more accurately reflect the true distribution of microbial data.

Conclusions This study aimed to investigate whether high sodium intake is associated with alterations in the human gut microbial composition and provide further insight into whether such differences vary between individuals with PD and those without PD. We hypothesized that high sodium intake would strongly correlate with disturbances in the gut microbial diversity and composition in individuals both with PD and without PD in our cohort. However, while the different alpha and beta diversity metrics investigated showcased significant correlations with high sodium intake in the control group and in the overall cohort (PD and non-PD), none of the diversity metrics showcased any significant correlations in individuals with PD. Similarly, the differential abundance analysis only showcased significant results when investigating either the control group or the overall cohort, but no significant results were found when investigating solely individuals with PD. In the overall cohort, our study displayed an unexpected overrepresentation of *Lactobacillus* and *Bifidobacterium* populations with elevated sodium intake which is more consistent with the gut microbial profile of individuals with PD and inconsistent with previous literature on individuals consuming high sodium diets. Thus, based on our findings, we found no compelling evidence to advise individuals with PD against consuming high-salt diets for alleviating gastrointestinal symptoms. In this study, the correlation is more likely tied to the manifestation of Parkinson's disease itself rather than an elevated sodium intake. Overall, our research provides more insight into the gut microbial composition of individuals both with

and without PD with respect to sodium intake and identified new directions for future research.

Future Directions Our hypothesis that PD may be masking the effects of sodium on the human gut microbial composition may be further investigated in a controlled animal study using a mouse model of PD to be able to definitively conclude cause and effect. Moreover, future research could also investigate the effects of medications prescribed in PD treatment as a confounding variable on the gut microbiome in individuals with both high sodium and low sodium intake. Future studies could also repeat the investigation of the effects of sodium on the gut microbiome in a cohort of individuals with an equal distribution of individuals with and without PD. To account for sex imbalances mentioned in the limitations section, follow-up investigations could recruit more men for the control group to account for the disproportionate representation of men in PD. This would minimize the effects of individuals' sex as a confounding variable in the context of investigating the correlation between sodium intake and alterations in the human gut microbial composition.

ACKNOWLEDGEMENTS

We acknowledge that our work was conducted on the traditional, unceded and ancestral territories of the xʷməθkʷəy̓əm (Musqueam) First Nation. We would like to thank Avril Metcalfe-Roach for his continued guidance, support and feedback throughout the course of this project. We would also like to thank Dr. Evelyn Sun and Dr. Melissa Chen for their guidance on using QIIME2 and R. Finally, we would like to thank Metcalfe-Roach et al. (7) for providing us with the dataset. We also thank the Microbiology and Immunology Department for the funding of this project. We would also like to thank two anonymous reviewers for constructive feedback on this manuscript.

CONTRIBUTIONS

This paper is a collaborative effort by all co-authors, with the general contributions as follows. SA made the phyloseq object using RStudio, performed correlation analyses using Spearman's test for sodium with the different food groups using RStudio, wrote the abstract, part of the methods, study limitations, conclusions, and future directions, made the supplemental figures, and helped to edit the introduction, results, and discussion of the manuscript. IC performed preliminary QIIME2 data processing, graphical and statistical alpha and beta analyses of the overall cohort and across disease status in RStudio, and wrote parts of methods, results, discussion, and figures of the manuscript. AG performed the DESeq2 analysis for the overall cohort with sodium and for PD-stratified samples, contributed to writing and editing of the introduction, methods, figure legends and conclusions and editing of the results and discussion. JH was responsible for performing the DESeq2 analysis for the sodium intake and food groups in RStudio, writing the results and discussion of all differential abundance analyses, part of the introduction, methods and editing abstract, study limitations, conclusions, future directions and figure legends of the manuscript. EW contributed to performing supplementary alpha and beta analysis and DESeq2 interpretation, and writing and editing of introduction, methods, results, discussion, and limitations.

REFERENCES

1. **Dorsey ER, Sherer T, Okun MS, Bloem BR.** 2018. The Emerging Evidence of the Parkinson Pandemic. J Parkinsons Dis. **8**.
2. **World Health Organisation**. 2022. Launch of WHO's Parkinson disease technical brief. https://www.who.int/news-room/fact-sheets/detail/parkinson-disease
3. **Knudsen K, Fedorova TD, Bekker AC, Iversen P, Østergaard K, Krogh K, Borghammer P.** 2017. Objective Colonic Dysfunction is Far more Prevalent than Subjective Constipation in Parkinson's Disease: A Colon Transit and Volume Study. J Parkinsons Dis. **7**:359–367.
4. **Poirier A-A, Aubé B, Côté M, Morin N, Di Paolo T, Soulet D.** 2016. Gastrointestinal Dysfunctions in Parkinson's Disease: Symptoms and Treatments. Parkinsons Dis. **2016**:1–23.
5. **Smiljanec K, Lennon SL.** 2019. Sodium, hypertension, and the gut: Does the gut microbiota go salty? Am. J. Physiol. Heart Circ. Physiol. **317.**
6. **Li Z, Liang H, Hu Y, Lu L, Zheng C, Fan Y, Wu B, Zou T, Luo X, Zhang X, Zeng Y, Liu Z, Zhou Z, Yue Z, Ren Y, Li Z, Su Q, Xu P.** 2022. Gut bacterial profiles in Parkinson's disease: A systematic review. CNS Neurosci. Ther. **29**:140–157.
7. **Metcalfe-Roach A**, **Yu A**, **Golz E**, **Sundvick K**, **Cirstea M**, **Kliger D**, **Foulger L**, **Mackenzie M**, **Finlay B**, **Appel-Cresswell S**. 2020. Mind diet associated with later onset of parkinson's disease.

8. **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. J. Mov. Disord. **35**:1208–1217.
9. **Zapała B, Stefura T, Wójcik-Pędziwiatr M, Kabut R, Bałajewicz-Nowak M, Milewicz T, Dudek A, Stój A, Rudzińska-Bar M.** 2021. Differences in the composition of gut microbiota between patients with Parkinson's disease and healthy controls: A cohort study. J. of Clin. Med. **10**:5698.
10. **Romano S, Savva GM, Bedarf JR, Charles IG, Hildebrand F, Narbad A.** 2021. Meta-analysis of the Parkinson's disease gut microbiome suggests alterations linked to intestinal inflammation. npj Parkinsons Dis **7**:27.
11. **Scheperjans F, Aho V, Pereira PAB, Koskinen K, Paulin L, Pekkonen E, Haapaniemi E, Kaakkola S, Eerola-Rautio J, Pohja M, Kinnunen E, Murros K, Auvinen P.** 2015. Gut microbiota are related to Parkinson's disease and clinical phenotype. Movement Disorders **30**:350–358.
12. **Nishiwaki H, Ito M, Ishida T, Hamaguchi T, Maeda T, Kashihara K, Tsuboi Y, Ueyama J, Shimamura T, Mori H, Kurokawa K, Katsuno M, Hirayama M, Ohno K.** 2020. Meta-Analysis of Gut Dysbiosis in Parkinson's Disease. Movement Disorders **35**:1626–1635.
13. **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.
14. **U.S. Department of Health and Human Services and U.S. Department of Agriculture.** 2015. 2015 – 2020 Dietary Guidelines for Americans.
15. **Ferguson JF, Aden LA, Barbaro NR, Van Beusecum JP, Xiao L, Simons AJ, Warden C, Pasic L, Himmel LE, Washington MK, Revetta FL, Zhao S, Kumaresan S, Scholz MB, Tang Z, Chen G, Reilly MP, Kirabo A.** 2019. High dietary salt–induced DC activation underlies microbial dysbiosis-associated hypertension. JCI Insight **4**.
16. **Du X, Yu L, Ling S, Xie J, Chen W.** 2021. High-salt diet impairs the neurons plasticity and the neurotransmitters-related biological processes. Nutr. J. **13**:4123.
17. **Silva YP, Bernardi A, Frozza RL.** 2020. The Role of Short-Chain Fatty Acids From Gut Microbiota in Gut-Brain Communication. Front. Endocrinol. **11**:25
18. **Hou Y, Li X, Liu C, Zhang M, Zhang X, Ge S, Zhao L.** 2021. Neuroprotective effects of short-chain fatty acids in MPTP induced mice model of Parkinson's disease. Exp. Gerontol. **150**:111376
19. **Perez-Pardo P, Kliest T, Dodiya HB, Broersen LM, Garssen J, Keshavarzian A, Kraneveld AD.** 2017. The gut-brain axis in parkinson's disease: Possibilities for food-based therapies. Eur. J. Pharmacol. **817**:86–95.
20. **Koh A, De Vadder F, Kovatcheva-Datchary P, Bäckhed F.** 2016. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. Cell **165**:1332–1345.
21. **Garcia-Mantrana I, Selma-Royo M, Alcantara C, Collado MC.** 2018. Shifts on Gut Microbiota Associated to Mediterranean Diet Adherence and Specific Dietary Intakes on General Adult Population. Front Microbiol **9**:890.
22. **Hegelmaier T, Lebbing M, Duscha A, Tomaske L, Tönges L, Holm JB, Bjørn Nielsen H, Gatermann SG, Przuntek H, Haghikia A.** 2020. Interventional Influence of the Intestinal Microbiome Through Dietary Intervention and Bowel Cleansing Might Improve Motor Symptoms in Parkinson's Disease. Cells **9**:376.
23. **Mulligan AA, Luben RN, Bhaniani A, Parry-Smith DJ, O'Connor L, Khawaja AP, Forouhi NG, Khaw K-T.** 2014. A new tool for converting food frequency questionnaire data into nutrient and food group values: FETA research methods and availability. BMJ Open **4**
24. **Bosco N, Noti M.** 2021. The aging gut microbiome and its impact on host immunity. Genes Immun. **22**:289–303.
25. **Callahan BJ, McMurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP.** 2016. DADA2: High resolution sample inference from Illumina amplicon data. Nat. Methods **13**:581–583.
26. **RStudio Team.** 2019. RStudio: Integrated Development for R. RStudio, Inc., Boston, MA.
27. **Wickham H, Averick M, Bryan J, Chang W, McGowan LD, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM, Müller K, Ooms J, Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani AH.** 2019. Welcome to the tidyverse. JOSS **4**:1686.
28. **Paradis E, Schliep K.** 2019. APE 5.0: An environment for modern phylogenetics and evolutionary analyses in R. J. Bioinform. **35**:526–528.
29. **McMurdie PJ, Holmes S.** 2013. phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One **8**:e61217.
30. **Kembel SW, Cowan PD, Helmus MR, Cornwell WK, Morlon H, Ackerly DD, Blomberg SP, Webb CO.** 2010. Picante: R tools for integrating phylogenies and ecology. J. Bioinform. **26**:1463–1464.
31. **Oksanen J, Simpson G, Blanchet F, Kindt R, Legendre P, Minchin P, O'Hara R, Solymos P, Stevens M, Szoecs E, Wagner H, Barbour M, Bedward M, Bolker B, Borcard D, Carvalho G, Chirico M, De Caceres M, Durand S, Evangelista H, FitzJohn R, Friendly M, Furneaux B, Hannigan G, Hill M, Lahti L, McGlinn D, Ouellette M, Ribeiro Cunha E, Smith T, Stier A, Ter Braak C, Weedon J.** 2022. vegan: Community Ecology Package. R package version 2.6-4.
32. **Wickham H.** 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York.
33. **R Core Team.** 2021. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

34. **Love MI, Huber W, Anders S.** 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. **15**:550.
35. **Hamad I, Cardilli A, Côrte-Real BF, Dyczko A, Vangronsveld J, Kleinewietfeld M.** 2022. High-Salt Diet Induces Depletion of Lactic Acid-Producing Bacteria in Murine Gut. Nutrients **14**:1171.
36. **Wilck N, Matus MG, Kearney SM, Olesen SW, Forslund K, Bartolomaeus H, Haase S, Mähler A, Balogh A, Markó L, Vvedenskaya O, Kleiner FH, Tsvetkov D, Klug L, Costea PI, Sunagawa S, Maier L, Rakova N, Schatz V, Neubert P, Frätzer C, Krannich A, Gollasch M, Grohme DA, Côrte-Real BF, Gerlach RG, Basic M, Typas A, Wu C, Titze JM, Jantsch J, Boschmann M, Dechend R, Kleinewietfeld M, Kempa S, Bork P, Linker RA, Alm EJ, Müller DN.** 2017. Salt-responsive gut commensal modulates TH17 axis and disease. Nature **551**:585–589.
37. **Rivest J, Barclay CL, Suchowersky O.** 1999. COMT Inhibitors in Parkinson's disease. Can. J. Neurol. Sci. **26**.
38. **Cirstea MS, Creus-Cuadros A, Lo C, Yu AC, Serapio-Palacios A, Neilson S, Appel-Cresswell S, Finlay BB.** 2023. A novel pathway of levodopa metabolism by commensal *Bifidobacteria*. Sci. Rep. **13**.
39. **Tan AH, Lim S-Y, Chong KK, A Manap MA, Hor JW, Lim JL, Low SC, Chong CW, Mahadeva S, Lang AE.** 2021. Probiotics for constipation in Parkinson disease. J. Neurol. **96**.
40. **Tamtaji OR, Taghizadeh M, Daneshvar Kakhaki R, Kouchaki E, Bahmani F, Borzabadi S, Oryan S, Mafi A, Asemi Z.** 2019. Clinical and metabolic response to probiotic administration in people with Parkinson's disease: A randomized, double-blind, placebo-controlled trial. Clinical Nutrition **38**:1031–1035.
41. **Webster JL, Dunford EK, Neal BC.** 2010. A systematic survey of the sodium contents of Processed Foods. The American Journal of Clinical Nutrition **91**:413–420.