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Low Serum Saturated Fatty Acid Levels Positively Associate with Microbiota Diversity and Metabolic Pathways in Parkinson's Disease Patients

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SUMMARY Parkinson's disease is the fastest-growing neurodegenerative disease worldwide. Gut microbiome dysbiosis can precede the onset of Parkinson's disease symptoms by 20 years. The ketogenic diet has shown beneficial impacts as an intervention in the treatment and modulation of the microbiome in Parkinson's disease. While the ketogenic diet improves Parkinson's disease symptoms, it increases the serum levels of saturated, monounsaturated, and polyunsaturated fatty acids. The current literature indicates conflicting results with the increase in intake and serum levels of fatty acids and the gut microbiome in Parkinson's disease. We analyzed the data of 197 Parkinson's disease patients and 103 healthy controls to unveil associations between serum levels of saturated, mono-unsaturated, and polyunsaturated fatty acids and the microbiome. Our results indicate that saturated fatty acids have a weak but statistically significant positive relationship with the Shannon diversity of the gut microbiome in Parkinson's disease subjects. Mono-unsaturated and poly-unsaturated fatty acids were not significantly associated with the microbiome diversity. Additionally, we identified low saturated fatty acids associated with the *Akkermansia, Bifidobacterium*, *Faecalibacterium*, and *Haemophilus* genera, with implications in Parkinson's disease progression and gut dysbiosis. Our analysis also shows low saturated fatty acid positively associates with metabolic pathways such as menaquinol and L-methionine, both having been highlighted as beneficial for Parkinson's disease. Together, our study indicates that low levels of serum saturated fatty acids are associated with specific genus and pathway changes known to have a positive effect on individuals with Parkinson's disease.

INTRODUCTION

Parkinson's disease (PD) is a neurodegenerative condition characterized by resting tremors and bradykinesia, that has over last two decades risen rapidly in incidence and prevalence worldwide (1). Though PD progresses through dopaminergic (DA) neuronal degeneration, it presents with distinct gastrointestinal co-morbidities and altered bacterial abundance in the gut microbiota (2,3). Evidence has indicated that gut dysbiosis is correlated with PD onset and progression (4). However, the link between early disease processes in the gut and the following neural degeneration is not fully understood (1). Cristea *et al.* analyzed fecal samples from a cohort of 197 PD patients and 103 healthy controls, finding associations between the microbiome and PD with implications in gastrointestinal (GI) dysfunction, serum metabolites, and disease etiology (2). The study found significant taxonomic abundance differences between the cohorts, notably an increased *Akkermansia* and *Bifidobacterium* genera abundance and a decreased *Faecalibacterium* and *Lachnospiraceae* genera abundance in PD patients compared to the healthy control (2).

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While diet is a known pivotal factor in microbiome composition and diversity in humans, the value of the ketogenic diet (KD) which contains a high fat, moderate protein, and low carbohydrate macronutrient profile presents a dichotomy (5). KD has been shown to modulate the microbiota by decreasing the *Bifidobacterium* abundance in PD patients while clinical studies have demonstrated KD to help with the Movement Disorder Society-Sponsored Revision of the Unified Parkinson's disease Rating Scale (MDS-UPDRS) (5-8). However, KD's utilization of a high fat intake (55-60% of total daily calories) raises serum levels of fatty acids including saturated fatty acids (SFA), monounsaturated fatty acids (MUFA), and polyunsaturated fatty acids (PUFA), the elevation of which has been shown to have mixed results for health (5,7-12).

SFA, MUFA and PUFA each have unique and complex associations with PD, which can all influence disease prevention or progression in distinct ways. Higher SFA intake has been associated with an increased risk of PD with longitudinal studies reporting up to a 41% increased incidence of PD with higher SFA intake (9, 13). High serum SFA has also been associated with higher levels of lipopolysaccharides (LPS which are linked with inflammation (9). Despite this, SFA intake is also associated with increases in the abundance of *Faecalibacterium* (genus level) and *Lachnospiraceae* (family level), both members of the Firmicutes phylum, which are decreased in PD patients based on the data utilized by Cristea *et al.*, indicating a potentially positive of higher SFA intake restoring a healthier microbiota composition in PD (2,9). Higher MUFA intake has been associated with a lower risk of PD, demonstrating a 0.68 adjusted hazard ratio for each standard deviation (SD) increase in intake (10). However, in vitro studies indicate oleic acids and other MUFA to be neurotoxic and elevating the levels of alpha-synuclein proteins (11). Studies exploring the serum levels of MUFA and associations in PD patients have yet to be explored in the literature. Higher PUFA intake is found to promote diversity and enhance neuronal growth (9). However, studies have also shown a significantly increased risk of PD onset with increased arachidonic acid 20:4 (a type of PUFA) intake (2). Other studies examining the effect of omega-3 (ω3) supplements, another type of PUFA, found it decreased *Faecalibacterium*, which correlates with the microbiota changes that precede PD (14,15). However, omega-3 has also been associated with improving brain health and reducing inflammation (16). Lower serum levels of alpha-linolenic acid and linoleic acid were associated with more severe motor symptoms in PD patients, while higher levels of plasma docosahexaenoic acid and arachidonic acid were associated with more severe non-motor symptoms in PD (12).

Overall, given this gap in the literature surrounding the specific associations of serum FAs and the gut microbiome for PD, our study aimed to build off the dataset generated by Cristea *et al.* to unveil associations between serum levels of SFA, MUFA, and PUFA and the microbiome diversity in PD patients (5-7,17).

METHODS AND MATERIALS

Dataset collection. The dataset used for this research came from Cirstea *et al.* which is comprised of 300 participants (197 with PD and 103 healthy controls) ranging from 40-80 years old (2). The goal of the study was to determine how intestinal microbiota plays a role in gastrointestinal disturbances seen in PD patients (2). 16S rRNA sequencing was done on fecal samples collected from participants (2). Our research focused on the serum SFA, MUFA, and PUFA data provided by Cirstea *et al*. (2).

16S rRNA sequence processing via QIIME2. Using QIIME2 (v2023.7), the 16S rRNA V4 sequences provided by Cirstea *et al*. were imported and demultiplexed. Subsequently, Divisive Amplicon Denoising Algorithm 2 (DADA2) was used to denoise the sequences to attain the feature table of the Amplicon Sequence Variants (ASVs) (2,18,19). The trimming and truncation parameters were set to 8 and 251 respectively based on the quality scores. The silva-138-99-515-806-nb-classifier was used. The QIIME2 output was used for downstream analysis in R.

Data filtering and rarefaction. R 4.3.1 was used to analyze the outputs generated from QIIME2. Using the tidyverse package, the outputs from QIIME2 were imported and modified for the conversion into a phyloseq object using the phyloseq, ape (v5.7.1), and vegan packages

(20-23). Before doing so, any ASVs belonging to the Archaea domain, chloroplast order, or mitochondria family were filtered out. After creating a phyloseq object, any ASVs with less than 5 total counts, then any samples with less than 100 ASVs were filtered out. Then any samples with NaN values in their SFA, MUFA, and PUFA columns were filtered out, leaving behind only 285 samples from the original 300. A rarefaction sampling depth of 3000 was chosen based on where the samples seemed to plateau, and a rarefied phyloseq object was created. This object contained 184 PD and 101 control subjects.

Alpha-diversity and multivariate linear regression. Within the R environment, the tidyverse and phyloseq packages were used to load in and manipulate the previously created phyloseq object (20,21). The data was divided into PD and control patients to enable separate statistical analyses on PD and control subjects only. To visualize the relationship between each FA type and Shannon diversity of both PD and control subjects, a scatter plot containing a best-fit line was created, and the Spearman test was used to find their correlation coefficients because it is able to assess monotonic relationships with order-based associations. To identify which predictor variable has a significant association with Shannon diversity, a multiple linear regression model was conducted using the stats package that is pre-installed in R (24). This analysis contained age, sex, and farm residency as confounding variables, while SFA, MUFA, and PUFA were our predictor variables.

Binning of Low, Medium, and High Fat Groups. FA values were categorized into “low”, “medium” and “high” based on quartile separations. “Low” was below the 25th percentile, “high” was above the 75th percentile and “medium” was in between.

Indicator Species Analysis and Core Microbiome Analysis. The indicspecies package was used to perform indicator species analysis (ISA), while the microbiome and ggVennDiagram packages were used to perform core microbiome analysis (25-27). Furthermore, they both utilized relative abundance to perform the analysis. For ISA, the PD_Binned and Control_Binned data were analyzed separately, and the analysis was done only on SFA. Only bacterial genera with a p-value <0.05 were considered. In the core microbiome analysis, only the high and low SFA levels were analyzed for the PD_Binned and Control_Binned subjects. The evaluation thresholds were set to 0.0 and 0.8 for detection and prevalence, respectively. To visualize the results of core microbiome analysis, a Venn diagram that compared the high and low SFA levels between the PD and control patients was created.

Differential Expression Sequence analysis and visualization. DESeq2 package was used to conduct the differential expression sequence analysis (DESeq) (28). DESeq analysis compared low SFA condition to high SFA condition in PD patients, and low SFA in PD versus control patients. Only ASVs with an absolute $\log_2$ Fold change of 2 and p-value<0.05 were kept. To visualize the results of the aforementioned conditions, a bar plot was created that showed the increase/decrease in abundance relative to the reference point. These reference points were high SFA values in PD patients and low SFA values in control patients for the two different conditions.

Functional analysis of the microbiome and visualization. Functional analysis was performed using QIIME2 and PICRUSt2, with the final output being analyzed in R using the following packages: readr, ggpicrust2, tibble, tidyverse, ggprism, DESeq2, "ggh4x”, and patchwork (18,20,28-40). A representative sequences fasta and a biological observation matrix (BIOM) feature table, were filtered in QIIME2 to remove anything with counts lower than five and then were plugged into the PICRUSt2 pipeline (29-34). This pipeline uses marker gene sequences to predict functional abundances (29-34). Visualization of the picrust2 pipeline outputs used the ggpicrust2 package (36). The data was filtered to contain only the binned low SFA level samples for PD patients and controls. It was further filtered to exclude SFA levels that had no inputted values, remove any abundances less than 1000, and any pathway counts less than 100. The DESeq2 method was chosen to perform Differential abundance analysis (DAA) (36). The resulting DAA pathways were annotated using MetaCyc

(36). Abundances were graphed in a heatmap and principal component analysis (PCA) plot while the annotated pathways were graphed in a pathway error bar plot.

RESULTS

Weak positive significant association between serum SFA levels and the gut microbial diversity of Parkinson's disease patients. Based on the statistical summary of the multivariate regression model, which evaluated the relationships between the Shannon diversity of the human gut microbiome and the serum levels of SFA, MUFA, and PUFA, only SFA was shown to have a statistically significant association (*p*-value = 0.035) (Supplementary Figure S1). This association was absent in healthy controls and appeared to be exclusive to PD patients (Table 1). Our scatterplot revealed a weak positive association (correlation coefficient = 0.0372, Supplementary Table S1) between the Shannon diversity and serum SFA in PD patients, despite its statistical significance (Supplementary Figure S1B).

TABLE. 1 Statistical summary of Shannon diversity multiple linear regression model in PD subjects. *p*-value for the independent effect each variable has on Shannon diversity was computed using a linear regression model. **p*-value < 0.05, ***p*-value<0.01

Variable	*p*-value
Age	0.00365**
Sex	0.30525
Farm Residency	0.83059
SFA	0.03500*
MUFA	0.05836
PUFA	0.74235

2 indicator genera for PD in the low SFA group. ISA showed 2 ASV hits to be statistically significant indicators of the low SFA PD group (*p*-value of 0.001 and 0.03) (Table 2). Using The Basic Local Alignment Search Tool (BLAST), the nucleotide sequences of the ASVs were linked to the *Haemophilus* genus and an uncultured bacterium (Table 2). Despite their statistical significance, the indicator values of these genera were low at 0.3202 and 0.2659 (Table 2), suggesting that they are poor indicators of PD in low SFA conditions. There were no statistically significant ASV hits associated with the medium or high SFA groups among PD subjects. Furthermore, these 2 ASV hits were exclusive to the low SFA PD group and absent from the low SFA control subjects.

TABLE. 2 ISA results of different SFA levels in Parkinson's disease patients. Tabular summary visualizing the bacterial genus, associated SFA level, indicator value, and *p*-value of all statistically significant indicator genera for the low, medium, and high SFA groups of PD patients.

Genus	Low SFA	Med SFA	High SFA	Indicator Value	*p*-value
Haeomophilus	1	0	0	0.3202	0.001
Uncultured bacterium	1	0	0	0.2659	0.030

Low SFA PD subjects contain 3 exclusive core taxa and high SFA PD subjects contain none. Core microbiome analysis was run to identify the SFA level that is best associated with PD. Low SFA PD patients contained 3 exclusive core taxa (Figure 1). These 3 ASVs belonged to the *Alistipes*, *Bacteroides*, and *Agathobacter* genera. However, no exclusive core taxa belonged solely to the high SFA PD subjects, nor were any core taxa shared only between high and low SFA levels in PD subjects (Figure 1). Furthermore, 7 core taxa members were shared between the 4 different condition combinations (Figure 1).

25 and 47 genera had a significant increase and decrease in their expression levels in their respective analyzed cohorts. Since low SFA was a better indicator of PD (Table 3 and Figure 1), only the low SFA PD subjects were chosen as the comparison group for DESeq analysis. The outcome of the analysis showed that there are 25 significant genera with a *p*-

value <0.05 and absolute value of $\log_2$ fold change > 2 in low versus high SFA conditions in PD patients (Figure 2A). While 47 genera had a significant change in their expression level in PD subjects with low SFA levels relative to control subjects with low SFA (Figure 2B).

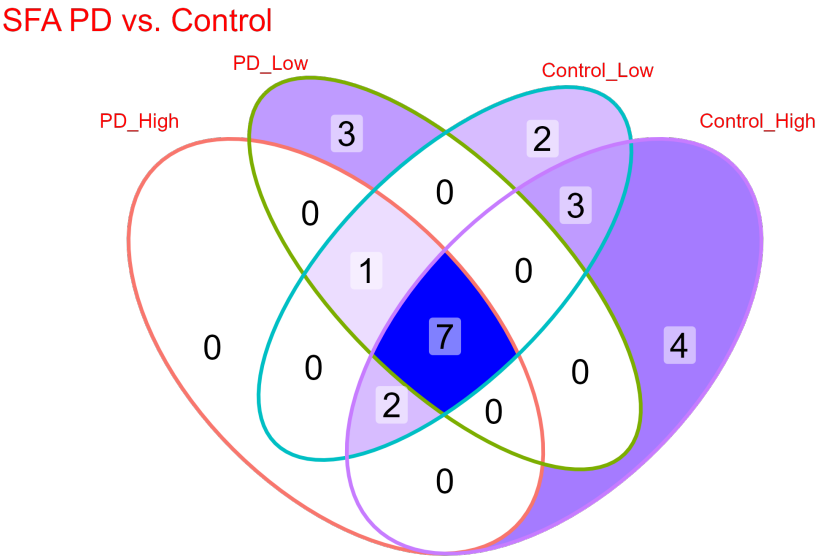

FIG. 1 Low SFA levels contain 3 exclusive core taxa. Core microbiome analysis was done on binned SFA in both PD and control subjects. Relative abundance values were used to conduct the analysis. Detection and prevalence thresholds were set to 0.0 (presence/absence) and 0.8 (presence in 80% of samples) respectively.

TABLE. 3 Statistical summary of Shannon diversity multiple linear regression model in control subjects. *p*-value for the independent effect each variable has on Shannon diversity was computed using a linear regression model.

Variable	*p*-value
Age	0.517
Sex	0.796
Farm Residency	0.224
SFA	0.330
MUFA	0.418
PUFA	0.233

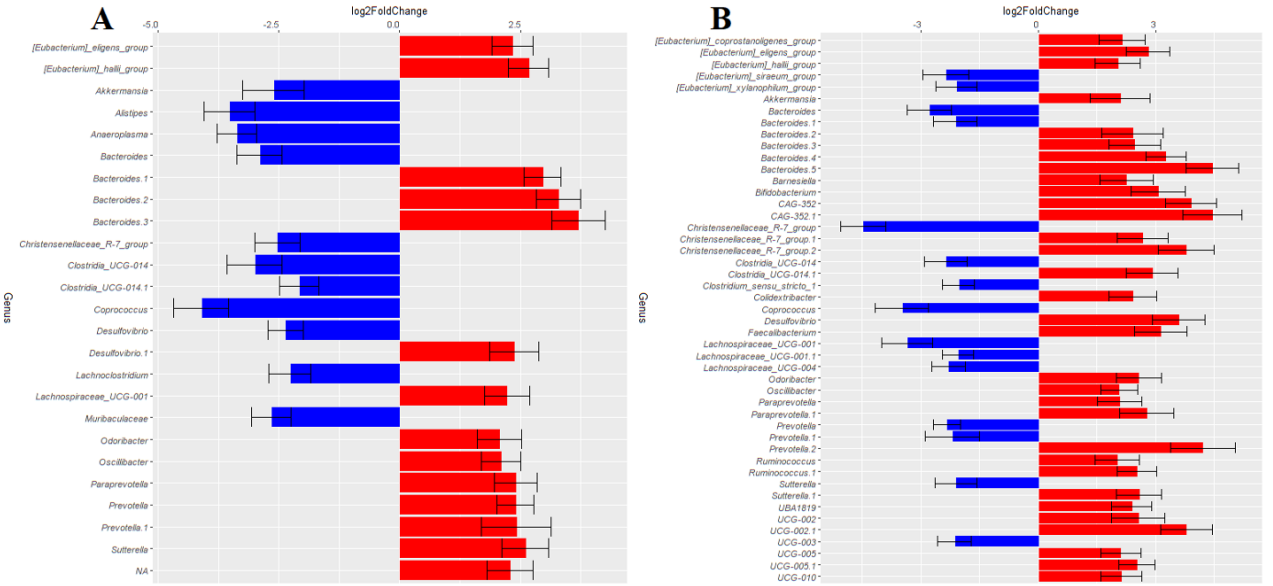

FIG. 2 DESeq analysis shows an increase in *Akkermansia*, *Bifidobacterium*, and *Faecalibacterium* genera only in PD versus control subjects with low SFA. Red bars indicate a decrease in abundance, while green bars indicate an increase. The error bands are $\log_2$ fold change standard error values. Threshold values were set to *p*-value<0.05 and an absolute value of $\log_2$ fold change>2. **A)** DESeq analysis on low (comparison group) versus high (reference group) SFA in PD subjects. **B)** DESeq analysis on PD (comparison group) versus control (reference group) subjects with low SFA levels.

Out of these 25 genera, 11 have decreased, while 14 have increased in abundance relative to high SFA (Figure 2A). Additionally, out of the 47 genera significant genera, 15 have decreased, while 32 have increased in abundance (Figure 2B). Interestingly, the *Akkermansia*, *Bifidobacterium*, and *Faecalibacterium* genera, which are commonly reported in PD patients, have increased in abundance relative to control subjects with low SFA levels (Figure 2B). However, only the abundance change in the *Akkermansia* genus was considered significant in the low versus high SFA levels in PD patients. Albeit, this genus had decreased in abundance relative to high SFA PD subjects (Figure 2A).

Low SFA levels upregulate 21 pathways in the gut microbiome. Functional analysis of pathway regulation through PICRUSt2 found significant pathway regulation changes for 21 pathways in low SFA levels between PD patients and controls (Figure 3A, B). Fifteen of these pathways are upregulated in PD patients and related to biosynthesis, while four are upregulated in PD patients and are related to degradation (Figure 3). The remaining two pathways are superpathways, which include the regulation of energetic pathways like glycolysis and the tricarboxylic acid (TCA) cycle are also upregulated in PD patients (Figure 3). A form of menaquinol biosynthesis spans six of the pathways, while L-methionine biosynthesis is involved in two pathways. L-ornithine and L-arginine are both involved in two degradation pathways (Figure 3). The rest of the pathways involve unique metabolites except for the TCA cycle and glyoxylate bypass occurring in two separate super-pathways (Figure 3).

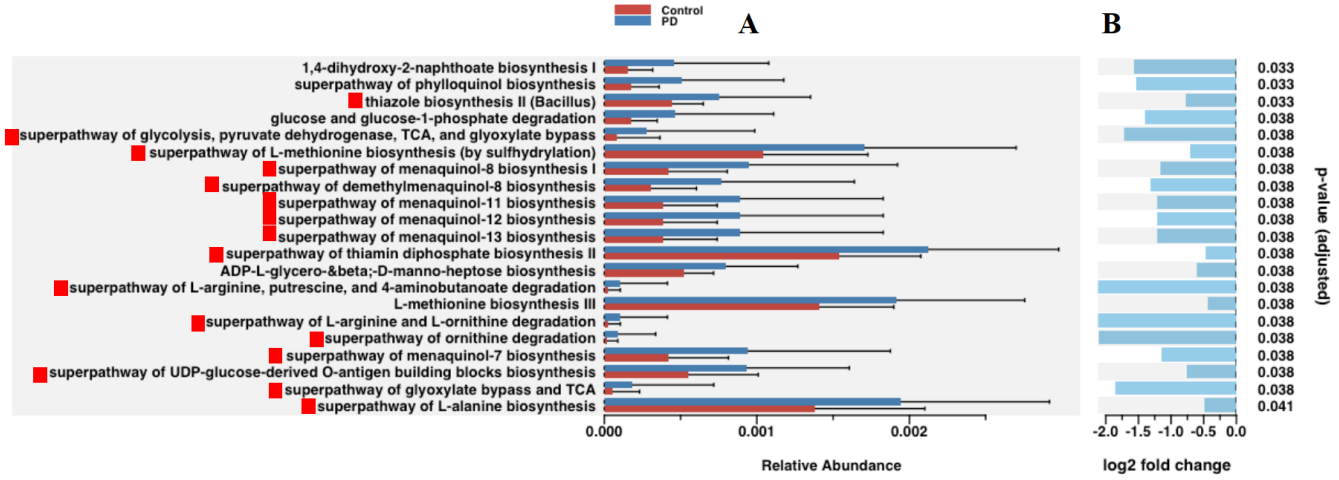

FIG. 3 Low SFA levels upregulate 21 pathways in the PD gut microbiome. Functional analysis of low SFA levels, produced using PICRUSt2, reveals an upregulation of 21 pathways in the PD microbiota (Blue) compared to controls (Red). The 21 pathways are evaluated on: **A)** relative abundance, **B)** log2 fold change, and adjusted *p*-value. An adjusted *p*-value is the smallest possible *p*-value. The Log2 fold change represents the controls in relation to PD. Comparing, log fold compared to, what are the pathways, what is an adjusted *p*-value, generated using PICRUSt. Red boxes indicate the 15 super pathways with altered regulation.

DISCUSSION

Parkinson's disease (PD) is the fastest-growing neurodegenerative disease affecting 0.3% of the general population (41,42). With an aging global population, the number of PD patients worldwide is projected to double by the year 2040 (42). Disease management is complicated and involves utilizing pharmaceuticals, exercise therapy, and lifestyle interventions (41). Dopaminergic medication such as levodopa (L-DOPA) can also cause dyskinesia and motor fluctuations (41). Prior to the onset of PD, there is a prodromal period that can precede the disease by up to 20 years (43). This period is characterized by constipation, possible rapid eye movement (REM) sleep behavior disorder (RBD), depression, anxiety disorder, and cognitive impairments (43). Interestingly, recent studies have pointed to microbiota changes of prodromal PD lying along a continuum between healthy and PD cohorts (44). This calls for a more comprehensive understanding of the associations between various biomarkers and the microbiome composition and diversity which can allow for improvements in the treatment of PD (41,42,44).

The ketogenic diet has shown promise as a beneficial dietary intervention in PD (45). A study looking specifically at very low-calorie KD found an increase in the number of bacteria that produce short-chain fatty acids (SCFA), a type of SFA (46). KD has also been shown to directly reverse the dysbiosis thought to be conducive to PD in mouse models by significantly changing the abundance of various genera in the microbiota (47). Currently, there are human clinical trials underway utilizing KD as a microbiota-targeted dietary intervention measuring the changes in the microbiota longitudinally in British Columbia, Canada (48).

Although KD has shown benefits as an intervention, the side effects can include hypertriglyceridemia and increased serum FA including SFA, MUFA, and PUFA (5,7,9,45). Given that longitudinal studies have indicated an increased incidence of PD with higher SFA intake, while effects of increased PUFA and MUFA are yet to be shown on the microbiome of PD patients (2,10,12,13); we aimed to unveil the association of SFA, MUFA, and PUFA with the microbiome in PD patients.

Multiple linear regression association between SFA levels and gut microbial diversity. To run the multivariate linear regression model, we chose to control for age, sex, and farm residency when analyzing the 3 FA types. Only SFA was associated with Shannon diversity in PD patients. Despite the statistically significant relationship found in SFA, the association is extremely weak. Although our finding from the regression model alone is insufficient to conclude the role of SFA levels in relation to PD outcomes, previous research has found that increased dietary intake of SFA is linked to elevated risk of PD development (9,49). Based on the weak correlation coefficient, we cannot definitely determine any directional associations.

ISA indicated the genus *Haemophilus* and an uncultured bacterium in the low SFA group. ISA revealed *Haemophilus* and an uncultured bacterium as possible indicators of PD in patients with low SFA levels (Table 2). The *Haemophilus* genus is associated with a plethora of neurological disorders (50-52). Of note, *Haemophilus* was positively correlated with the negative psychiatric symptoms of Schizophrenia (50). This is significant since the prevalence of schizophrenia spectrum disorder later in life (53). *Haemophilus* is underrepresented in PD cohorts compared to healthy controls (51). Systematic reviews have confirmed these findings with *Haemophilus* being lower in abundance in PD patients as well (52). Given the low indicator value of 0.3202, we cannot confidently state that *Haemophilus* is a good indicator of PD in subjects with low SFA.

Core microbiome showed that low SFA in PD patients has 3 exclusive core taxa. The core microbiome analysis conducted on low SFA subjects in both PD and control categories showed that low SFA PD subjects contain 3 exclusive core taxa. These hits belonged to the *Alistipes*, *Bacteroides*, and *Agathobacter* genera. One study has shown that *Alistipes* genus increases in abundance in PD patients (52). Additionally, upon further analysis of the DESeq results, none of these specific ASVs were seen to decrease/increase significantly in either of the two conditions. However, similar to the ISA findings, the low SFA was the only SFA level in PD patients that contained any exclusive core taxa; this possibly alludes to the fact that the 3 exclusive ASVs are potentially a good indicator of PD in patients with low serum SFA.

DESeq2 revealed major changes in the microbiota composition. Our DESeq analysis revealed several genera abundance differences between low and high SFA in PD subjects, and PD versus control subjects with low SFA. These notable genera include *Akkermansia* a mucin-degrading, *Faecalibacterium* which is a main butyrate-producing (a type of SCFA) bacteria, and *Bifidobacterium* (52,54,55).

The *Akkermansia* genus is thought of as a healthy bacteria genus as it has been associated with enhanced wound healing, protection against obesity, and more (4). However, it has also been seen to have a greater abundance in the stool sample of Parkinson's disease patients relative to healthy control patients (4). Furthermore, it is thought that the increase in this genus alongside the decrease in SCFA-producing bacteria, can lead to an increase in intestinal permeability and inflammation which can facilitate the exposure of the enteric nervous system to toxins such as LPS (4). This can lead to an abnormal aggregation of alpha-synuclein proteins, which is thought to be a contributor to PD pathogenesis (4,56). However, we can see that relative to PD subjects with high SFA, there has been a decrease in its abundance

(Figure 2A). This is a possible indicator of the beneficial role that low SFA levels can have in PD patients.

Faecalibacterium genus is a butyrate-producing bacteria that has been linked with potential positive effects on the intestinal mucosa and PD (4,55,57). Based on the DESeq results, we can see that it had an increase in abundance in low SFA PD subjects relative to low SFA control subjects (Figure 2B). Furthermore, *Bifidobacterium* is another healthy bacterium that was shown to protect dopaminergic neurons in PD mice (58). While the increase in its abundance in PD versus control patients with low SFA is congruent with established literature, it has not been consistently shown in PD-related changes of gut microbial composition unlike the *Akkermansia* genus (4).

Overall, the changes we see associated with low SFA in both *Akkermansia* and *Faecalibacterium* genera, potentially allude to the fact that this level of serum SFA can have a positive outcome for PD subjects. However, since the increase in abundance of *Bifidobacterium* may be associated with PD medication (52), we won't be able to state the same for this genus.

PICRUSt2 highlighted menaquinol and L-methionine as metabolites upregulated in Low SFA. The functional analysis of the gut microbiome revealed significant changes between PD patients and controls with low SFA levels. Specifically, 21 pathways were upregulated in PD patients compared to controls (Figure 3A). Eight of the 21 pathways are related to the biosynthesis of menaquinol (Vitamin K_2) suggesting that the gut microbiome produces more vitamin K_2 for PD patients rather than controls with low SFA levels. Current literature relating to vitamin K describes it as beneficial to PD patients (59,60). It has the ability to suppress neuroinducers like rotenone and paraquat, and vitamin K is currently being used in clinical trials as a treatment for PD (59,60). Furthermore, it repairs nerve cells using the mitochondrial quality control loop (61). In Mice models, administrations of 500 mg/kg of Vitamin K_2 per day orally, for a 2-week period, induced higher levels of Firmicutes (62). This is significant as Firmicutes are decreased in PD patients (2). Two L-methionine pathways are upregulated in PD patients as well. Previous studies depict L-methionine as beneficial to PD patients (63,64). Cantesi *et al*. found L-methionine to protect against both oxidative stress and mitochondrial dysfunction in PD patients (65). Furthermore, L-methionine was seen to have therapeutic effects in clinical trials, indicating improvements in tremors and rigidity (63). Moreover, one of the L-methionine biosynthesis pathways occurs using sulfhydration which has been seen to benefit neuro-protective processes of parkin, a ligase that removes damaged mitochondria (64). In terms of the gut microbiome methionine, an enantiomer of L-methionine, has been investigated (66). A high methionine diet (2g/kg of body weight) was found to increase the abundance of *Faecalibacterium* while previous PD studies claim *Faecalibacterium* decreases in abundance for PD patients (66,67). Since *Faecalibaculum* positively influences PD, the increase in L-methionine biosynthesis further supports the association of low SFA with beneficial metabolites in PD patients (57).

With around half of the significant regulation changes seeming to benefit PD patients, the data suggest that low SFA levels are associated with beneficial pathways for PD patients (Figure3A, B). However, it is important to note that not all of the changes support this conclusion. Both L-arginine and L-ornithine degradation-related pathways are upregulated despite both of them being proposed as beneficial for PD patients (Figure 3A, B) (68,69). Despite these three conflicting pathways, at least ten of the 21 pathway changes support that low SFA levels are associated with beneficial pathways in PD patients (Figure 3A, B).

Limitations The dataset by Cirstea *et al*. presents several limitations for our research. Given the cross-sectional nature of the data, no causal relationships can be made. There could also be a better balance between healthy and PD patients (n=103 and n=197 respectively) (2). Finally, the time of blood sample collection with regard to the subject's mealtimes was not standardized, which can impact the binning process of the FAs (2). FAs are known to take up to 4 hours to digest, meaning standardizing data collection to 4 hours after meals could have improved the quality of the data (70). This can lead to false findings in the ISA, core microbiome, DESeq, and PICRUSt2 analyses since they utilized FA levels to conduct their respective analysis.

Within our group's work, it is possible that we did not account for all relevant confounding variables in our multivariable linear regression model. While we accounted for variables such as age, location (farmlands), and sex; elements of diet (e.g., caffeine), PD therapeutics, and important biomolecules (e.g., vitamin D) were not considered. This is relevant since literature findings have shown that caffeine can significantly affect the gut microbiome composition (71). Alternatively, this may have obscured relationships between Shannon diversity and MUFA and PUFA, as our analysis revealed no correlation.

Conclusions The aim of our study was to examine the effects of serum SFA, MUFA, and PUFA levels on PD patients' microbiome diversity and functional pathways. Overall, we found that SFA showed a weak positive relation with microbiome Shannon diversity, while MUFA and PUFA showed no significant correlations. We identified several bacterial genera which are associated with low levels of SFA. *Haemophilus* genus is associated with low SFA and is a bacterium that is linked with poor prognosis in schizophrenia and other neurological disorders. Core microbiome analysis showed that the 3 exclusive core taxa in low SFA PD patients are potentially an indicator of PD in low SFA subjects. DESeq results indicated a relative decreased abundance of *Akkermansia* in the low vs high SFA PD cohort, a genus persistently upregulated in the PD microbiome. With that, *Faecalibacterium*, a butyrate-producing bacteria, had a significant increase relative to low SFA control subjects. This highlights the potential beneficial outcome of low serum SFA levels for PD patients. Pathway analysis also revealed that menaquinol (Vitamin K_2) and L-methionine are up-regulated in low SFA, both are linked with protective anti-inflammatory properties, while menaquinol (Vitamin K_2) is currently being investigated as a potential therapeutic drug for PD. Together, serum SFA levels are positively associated with gut microbiome diversity, while low serum SFA is associated with several specific bacterial abundance changes and metabolites beneficial to PD. This indicates low SFA as a promising potential treatment for PD. However further research should look into determining the impact and comparative importance of each factor we have illustrated here before further conclusions can be made.

Future Directions The uncultured bacterium found in our ISA analysis was just recently identified as the novel species *Merdimmobilis hominis* in February 2023 (72) (Table 3). Given our discovery of its link with low SFA in PD patients, it would be prudent to see what systems and pathways it is associated with, and if it affects PD prognosis.

In addition, future Microbiota-targeted dietary interventions utilizing KD can investigate serum SFA levels longitudinally and find more robust associations between low and high SFA and the gut microbiome. With that, utilizing a random sampling of the population instead of choosing spouses of PD patients can ensure a more diverse microbiome analysis.

Future studies could look into the interplay between PD medications and abundance changes in the *Bifidobacterium* genus, as well as a more comprehensive study controlling for other confounders such as PD medications and diet would make sure that the findings are statistically more rigorous. Given the dataset from Cirstea *et al.* came from a cross-sectional study, longitudinal studies should be done to see the long-term changes in the gut microbiome of PD patients with different levels of SFA (2). This could paint a more holistic picture of SFA's association with the microbiome of PD patients.

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CONTRIBUTIONS

This study is the result of the collaborative effort of all authors. AT wrote the scripts for all the analyses up to the PICRUSt pipeline, wrote the methods, results, figures, discussion, conclusion, limitations, future directions, and acknowledgment; revised and formatted the manuscript, created, and organized the references. PK came up with the research idea, wrote the abstract, introduction, discussion, limitations, conclusion, and future directions, and revised and formatted the manuscript. JS worked on the PICRUSt2 analysis, wrote the binning script, and was responsible for methods, results, and discussion; He also contributed to manuscript revision. DL helped out with revision and references and was also responsible for the methods, results, figures and tables, and discussion. JZ wrote the outline for limitations, conclusion, and future direction, and worked on revision. Co-authorship should not be considered equal for all authors.

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