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The Microbiome and p-Inulin in Hemodialysis: A Feasibility Study

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Abstract

Background The intestinal microbiome is an appealing target for interventions in ESKD because of its likely contribution to uremic toxicity. Before conducting clinical trials of microbiome-altering treatments, it is necessary to understand the within-person and between-person variability in the composition and function of the gut microbiome in patients with ESKD.

Methods We conducted a multicenter, nonrandomized, crossover feasibility study of patients on maintenance hemodialysis consisting of three phases: pretreatment (8 weeks); treatment, during which the prebiotic, p-inulin, was administered at a dosage of 8 g twice daily (12 weeks); and post-treatment (8 weeks). Stool samples were collected 1–2 times per week and blood was collected weekly for 28 weeks. The gut microbiome was characterized using 16S ribosomal-RNA sequencing and metabolomic profiling.

Results A total of 11 of the 13 participants completed the 28-week study. Interparticipant variability was greater than intraparticipant variability for microbiome composition ($P<0.001$ by UniFrac distances) and metabolomic composition ($P<0.001$ by Euclidean distances). p-Inulin was well tolerated by 12 of 13 participants. Adherence to the frequent sample collection and self-aliquoting of stool samples were both 96%. A change in the microbiome composition from pretreatment to post-treatment was evident by the overall shifts in weighted UniFrac distances ($P=0.004$) and a progressive decrease in prevalence of high intraclass correlations, indicating an increase in intraparticipant microbiome diversity during and after p-inulin treatment. An effect of p-inulin on the metabolomic profile was not evident.

Conclusions The intraparticipant stability of the gut microbiome under no-treatment conditions, the tolerability of p-inulin, the signals of increased diversity of the microbiome with p-inulin treatment, and the willingness of participants to provide stool samples all support the feasibility of a larger trial to investigate interventions targeting the gut microbiome in patients with ESKD. Whether or not p-inulin has sufficient efficacy as an intervention requires evaluation in larger studies.

Clinical Trial registry name and registration number: Gut Microbiome and p-Inulin in Hemodialysis, NCT02572882

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Key Points

- Analyses of repeated samples revealed greater between-person than within-person variability for both the microbiome and metabolome.
- p-Inulin treatment was associated with an increase in microbial diversity, but an effect on the metabolome was not evident.
- p-Inulin was well tolerated by participants.

Introduction

Alterations in the composition and function of the intestinal microbiome are increasingly recognized as potentially modifiable components of chronic conditions, such as ESKD (1,2). Processes that contribute to an altered, or "dysbiotic," microbiome in ESKD include impaired protein assimilation, low dietary fiber consumption, frequent antibiotic use, urea

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accumulation, slow colonic transit, and metabolic acidosis (3–6).

Prebiotics are nondigestible food products that promote growth of beneficial gut bacteria (7). A substantial body of work suggests that prebiotics can alter the composition and metabolic function of the intestinal microbiota among healthy individuals, and among people with certain diseases, but they have not been well studied in ESKD (8,9). A single-center, nonrandomized, open-label study of patients on hemodialysis found that administration of the prebiotic, p-inulin, for 4 weeks was accompanied by reductions in the plasma concentration of p-cresyl sulfate (PCS), a microbiome-derived uremic toxin (10). The effects of prebiotics on clinical outcomes among patients with ESKD are unknown.

To design clinical trials of interventions targeting the microbiome in ESKD, several knowledge gaps need to be addressed. The purpose of the Microbiome and p-Inulin in Hemodialysis feasibility study was to address some of these gaps. Specifically, the study aimed to evaluate the intra- and interpatient variability in the composition and metabolic function of the gut microbiome to inform the sample-size requirements for a larger clinical trial. Additional objectives were to evaluate the tolerability and effects on gastrointestinal symptoms of p-inulin, to assess willingness of patients to provide stool samples, and to explore the effects of p-inulin on the composition and metabolic products of the microbiome.

Materials and Methods

Design

The Microbiome and p-Inulin in Hemodialysis study was a multicenter, nonrandomized, crossover trial that consisted of three sequential phases: (*1*) pretreatment (weeks 1–8), (*2*) p-inulin treatment (weeks 9–20), and (*3*) post-treatment (weeks 21–28). The study was conducted by the Hemodialysis Novel Therapies Consortium, which was established by the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) to conduct early-phase studies of interventions for patients receiving maintenance hemodialysis.

Participants

Participants were enrolled from dialysis units affiliated with four US academic centers. The major inclusion criteria were treatment with maintenance hemodialysis for ≥90 days, age ≥18 years, and self-reported stool frequency of at least every other day. The major exclusion criteria were (*1*) use of prebiotics, probiotics, or antibiotics during the past 8 weeks; (*2*) consumption of probiotic yogurt during the past 2 weeks; (*3*) current infection, inflammatory bowel disease, chronic diarrhea, or *Clostridium difficile* infection; and (*4*) hemoglobin levels of <9.0 g/dl within the past 4 weeks. The study was approved by the institutional review boards at each enrolling center and the data coordinating center, and the study was conducted in accordance with the Declaration of Helsinki. All participants provided written informed consent before initiating study procedures.

Biosample Collection

The schedule for study procedures is shown in Figure 1. Stool samples were provided by participants weekly during weeks 1–7, 10–18, and 21–28, and twice weekly during weeks 8, 9, 19, and 20. Participants generated ten aliquots from each stool sample at home, stored the samples at 4°C, and transported the samples in Styrofoam coolers with ice packs to the dialysis unit within 1 day. Blood was collected weekly before initiation of dialysis treatments during weeks 1–28 and stored as serum and plasma aliquots at −80°C. Participants received compensation for study participation that was based, in part, on providing the stool samples.

Intervention

p-Inulin (oligofructose-enriched inulin), manufactured by Prebiotin (Jackson GI Medical), was provided to participants in 2-g packets and administered at a dosage of 8 g twice daily. For each administration, the contents of four packets were added to approximately 200 ml of liquid. A dosage reduction to 4 g twice daily was permitted for gastrointestinal side effects.

Dietary and Gastrointestinal Symptom Assessments

The Block Food Frequency Questionnaire (11) was administered at baseline and weeks 8, 20, and 28. The Gastrointestinal Symptom Rating Scale (12) was administered at baseline and every 4 weeks thereafter.

Outcomes

Microbiome characterization outcomes included: (*1*) intraparticipant variability in the bacterial composition of the stool during the each study phase; (*2*) intraparticipant variability in the metabolomics profile and targeted metabolites for stool and plasma during each phase; and (*3*) intracohort variability in bacterial composition, metabolomic profiles, and targeted metabolites during each phase.

Tolerability and safety outcomes included: (*1*) gastrointestinal symptoms, (*2*) early discontinuation or reduction in p-inulin dose, and (*3*) adverse events. Feasibility outcomes included: (*1*) proportion of completed protocol-specified stool-sample collections; (*2*) proportion of completed blood-sample collections; (*3*) adherence to p-inulin assessed by counts of returned packets at weeks 12, 16, and 20; and (*4*) participant withdrawal during each phase of the study.

16S Ribosomal-RNA Sequencing

The microbiome profile for each sample was determined using 16S ribosomal RNA (rRNA) sequencing, performed by the Baylor College of Medicine Alkek Center for Metagenomics and Microbiome Research. Briefly, fecal bacterial genomic DNA was extracted using the DNeasy PowerSoil Kit (Qiagen). The 16S rRNA gene was amplified using degenerate primers that target the V4 hypervariable region. Primers contained molecular bar codes and adapters to allow PCR products to be pooled and sequenced directly on the Illumina MiSeq platform (2×250 bp protocol). Pooling depth targeted an average of at least 20,000 merged reads per sample. Rarefaction and collector's curves of microbial community data were constructed using sequence data for each sample to ensure sampling of the majority of microbial diversity that was present.

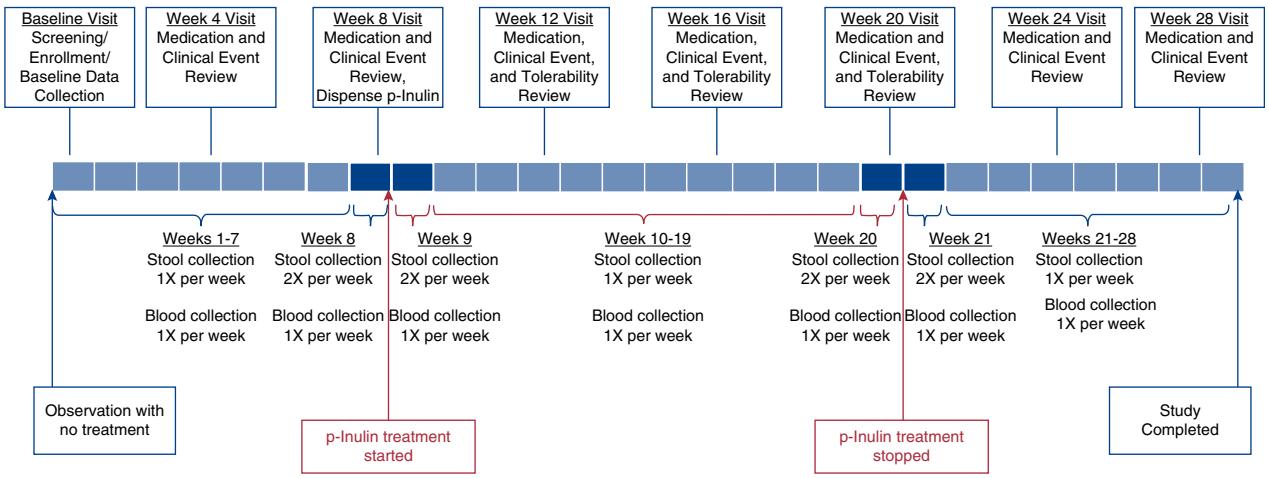

Figure 1. | **Study design.** Biosample and data-collection schedule during the pretreatment, treatment, and post-treatment phases.

Metabolomic Profiling

Metabolomic studies were performed by the West Coast Metabolomics Center at the University of California, Davis. Untargeted metabolomics profiling was performed using gas chromatography–time of flight mass spectrometry (MS) and hydrophilic interaction liquid chromatography Q-Exactive HF MS platforms that included 233 known metabolites (13,14). ChromaTOF version 4.50 and Binbase version 5.0.3 were used for gas chromatography–time of flight MS data processing (15,16). Hydrophilic interaction liquid chromatography Q-Exactive HF MS data were processed by MS-DIAL and MS-FLO (13,14). In-house retention time m/z libraries and tandem-MS spectra databases were used for compound identification (13,14). Targeted analyses were used to quantify the concentrations of PCS, indoxyl sulfate (IS), betaine, choline, and trimethylamine *N*-oxide (TMAO). Stool metabolite analyses were normalized to extract weight.

Statistical Analyses

Descriptive statistics were used to summarize the characteristics of the study population. Means and SDs were used for symmetrically distributed continuous data, medians and interquartile ranges for skewed continuous data, and frequencies and percentages for categoric data. α-Diversity, a measure of within-sample microbial diversity, was assessed using the Simpson diversity index, which captures the richness (number of types of organisms) and evenness (uniformity across organisms) for each individual at each time point. α-Diversity across the treatment phases was analyzed using a mixed-effects model, treating the Simpson diversity indices in each treatment phase as repeated measurements. β-Diversity, which depicts the dissimilarity of the composition between samples, was calculated with the weighted UniFrac distance for the microbiome data and the Euclidean distance for the metabolome data. β-Diversity was visualized using principal coordinate analysis and assessed using permutational multivariate ANOVA, with permutations constrained within time to investigate compositional changes across the treatment phases. The difference between the intra- and interparticipant variability was compared using an approximate randomization method (17). For changes in the composition of the microbiome (or metabolome) across the treatment phases, β-diversities calculated at baseline (*i.e.*, week 2) and at follow-up time points were assessed using linear mixed-effects models (18), treating the β-diversities in each treatment phase as repeated measurements. The intraclass correlation coefficient (ICC) for each taxon (or metabolite) was calculated with the interparticipant variance, and the total variance estimated using a linear mixed-effects model. To identify differentially abundant taxa (or metabolites), the abundance levels of taxa in each treatment phase were treated as repeated measurements, and their differences across the treatment phases were assessed using linear mixed-effects models. All analyses were performed with R (19).

Sample-Size Determination

The study aimed to enroll ten individuals who completed phases 1 and 2 and provided at least two stool samples during weeks 1–4, two stool samples during weeks 5–8, three stool samples during weeks 9–15, and three stool samples during weeks 15–20. On the basis of two-dimensional circle simulations using distance-based multivariate ANOVA analysis (20), the target sample size of ten participants with repeated measurements was anticipated to allow detection of moderate to large changes in bacterial community membership, evenness, richness, and lineages. For the effect of p-inulin on metabolites, a sample size of ten participants was expected to detect changes in metabolites of <1.7 SD on the basis of a paired t test, assuming that 25 metabolites were tested and that adjustment for multiple comparisons was conducted using the framework outlined by Benjamini and Hochberg (21) for controlling the false discovery rate.

Results

Participants

Between December 11, 2015 and July 7, 2017, 13 participants were enrolled from four centers. Baseline characteristics are shown in Table 1. Two participants withdrew

before completing the study. The withdrawals, both of which occurred at week 9, were because of participant preference and receipt of a kidney transplant, respectively. Samples from the participants who withdrew and one participant with antibiotic use during the study were not included in the microbiome composition and metabolomics analyses.

Adherence to Biosample Collection and p-Inulin

Table 2 shows participant-level adherence to the sample-collection and stool-aliquoting procedures. Overall, the 13 participants provided samples for 358 of the 373 expected stool collections (96%), and 3573 of the 3730 expected stool aliquots (96%). For blood collections, 329 of the 342 samples were obtained (96%). Adherence to p-inulin, calculated as (number of packets used/expected number of packets used) $\times$100%, ranged from 0% to 133% with a mean (SD) of 80% (38%). The individuals with no use of p-inulin both withdrew from the study during the first week of the treatment phase.

Tolerability and Safety of p-Inulin

One participant discontinued p-inulin after taking five doses because of personal preference. All of the other participants, with the exception of the individual who received a kidney transplant, took p-inulin for the full 12-week period. A trend toward an improvement in gastrointestinal symptoms was evident during and after the treatment period (Table 3). Dietary composition remained reasonably stable over the course of study participation, as shown in Supplemental Figure 1. There were no serious adverse events.

Variability in the Microbiome and Metabolome

As shown in Figure 2, for the stool microbiome, plasma metabolome, and stool metabolome, intraparticipant variability was lower than interparticipant variability ($P<0.001$ for each comparison), as measured by the distributions of the overall distances between the samples. Pairwise distances are commonly used in microbiome data analysis to capture the β-diversity of the microbial communities. Highly granular depictions incorporating participant, sample week, and study phase for individual participants are provided in Supplemental Figures 2–4. Principal coordinate analyses of the microbiome (Supplemental Figure 2) suggest that both the extent of intraparticipant variability and the extent of dissimilarity across the treatment phases differed across participants. Principal component analyses for the stool (Supplemental Figure 3) and plasma (Supplemental Figure 4) metabolites indicate high intraparticipant similarity over time, but variability between participants in relationships between treatment phase and metabolomic composition. Overall, within each of the three study phases, the microbiome composition and both stool and plasma metabolites were stable over time.

Response of the Microbiome to p-Inulin

Figure 3A shows distances relative to baseline composition (determined from week-2 samples) stratified by treatment phase. An effect of p-inulin on the microbiome is evident from the increased distances from baseline during the post-treatment compared with the pretreatment phases ($P=0.004$). The increase in intraparticipant variability in the microbiome is evident by examining the distributions of the ICC for the genera (Figure 4). Because the ICC is defined as the ratio of the interparticipant variance to the total variance, a lower density at high ICCs (*e.g.*, ICC >0.5) implies a larger number of microorganisms with large intraparticipant variabilities. A similar result was found from the ICCs for phyla, as shown in Supplemental Table 1. Supplemental Tables 2 and 3 suggest that the genera with the largest ICC are more abundant than those with the smallest ICCs, suggesting more measurement variability for very rare genera. For most phyla there is an apparent increase in intraparticipant variability in the post-treatment phase compared with the pretreatment and treatment phases, despite the general stability across phases in the relative abundances of the different phyla (Supplemental Figure 5). Heatmaps of the most abundant families and genera are shown in Supplemental Figure 6A. After adjustment for multiple comparisons, changes in the relative abundance of two genera were evident between pretreatment and post-treatment phases: Ruminococcaceae decreased (adjusted $P=0.003$) and Clostridiales increased (adjusted $P=0.03$) (Supplemental Figure 7A).

Response of the Metabolome to p-Inulin

In contrast to the microbiome, statistically significant changes in the intraparticipant variability of the stool or plasma metabolome were not evident (Figure 3, B and C, respectively). Heatmaps for the untargeted metabolites in the stool and plasma are provided in Supplemental Figure 6B and 6C, respectively. After adjustment for multiple comparisons, changes in the relative abundance of

Table 1. Baseline characteristics ($n=13$)

Characteristic	Value
Age (yr), mean (SD)	48.2 (12.5)
Male, *n* (%)	8 (62)
Race, *n* (%)	
Black	11 (85)
White	2 (15)
Hispanic ethnicity, *n* (%)	1 (8)
Hypertension, *n* (%)	13 (100)
Diabetes mellitus, *n* (%)	7 (54)
Duration of dialysis (yr), median (interquartile range)	2.97 (0.88–4.59)

Table 2. Biosample collections

Participant	Visit Week Completed	Stool Collection Provided/Expected	Stool Aliquots Provided/Expected	Blood Collections Performed/Expected
1	28	32/32	320/320	27/29
2	28	32/32	320/320	28/29
3	28	29/32	285/320	28/29
4	28	32/32	320/320	29/29
5	28	31/32	310/320	28/29
6	28	30/32	300/320	29/29
7	9	9/9	90/90	9/9
8	9	8/8	80/80	9/9
9	28	32/32	320/320	29/29
10	28	30/32	298/320	27/29
11	28	31/32	310/320	29/29
12	28	32/32	320/320	29/29
13	28	30/32	300/320	28/29
Total	326	358/369 (97%)	3580/3690 (97%)	329/337 (98%)

Table 3. Gastrointestinal Symptom Rating Scale score

GI Symptom Score	Visit Week							
	0	4	8	12	16	20	24	28
n	13	13	13	11	11	11	11	11
Median (IQR)	7.0 (5.0–7.0)	6.0 (5.0–7.0)	5.0 (2.0–8.0)	4.0 (1.5–6.0)	4.0 (2.0–7.5)	3.0 (2.0–8.5)	3.0 (2.5–4.0)	3.0 (2.0–5.5)
Mean (SD)	5.69 (3.35)	6.23 (3.65)	5.31 (3.50)	4.36 (3.75)	6.00 (6.47)	5.27 (5.37)	3.45 (2.77)	3.91 (2.59)

Shown are the sum of the scores for 15 questions, each with a response ranging from zero to three, with zero being least bothered and three being most bothered by the symptom (minimum score, zero; maximum score, 15). IQR, interquartile range.

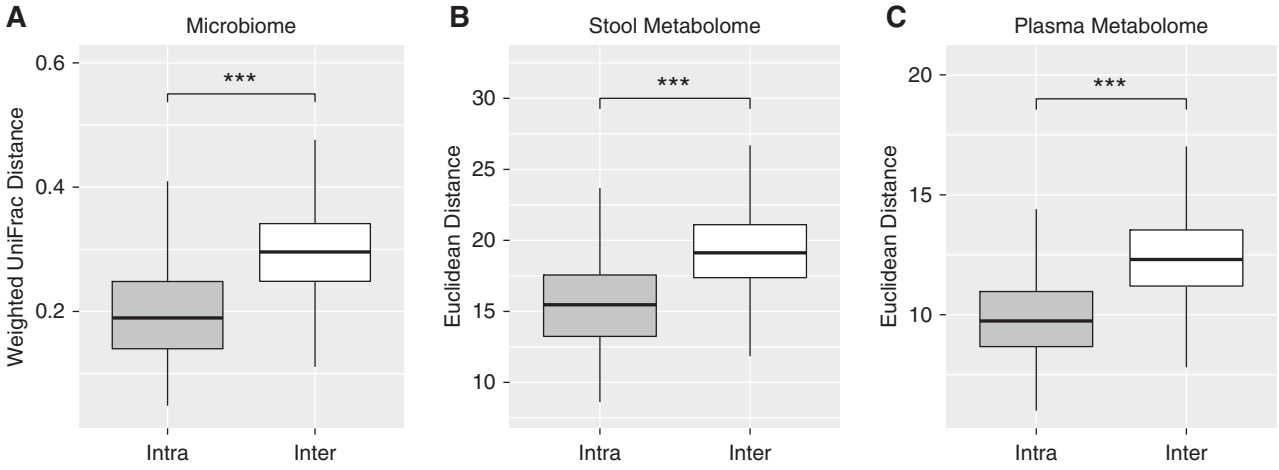

Figure 2. | **Intra- and interparticipant variability using weighted UniFrac distance for the microbiome samples and Euclidean distance for the metabolome samples.** (A) Microbiome, (B) stool metabolome, and (C) plasma metabolome. ***$P<0.001$.

uridine was evident between pretreatment and post-treatment phases (adjusted $P=0.003$ and 0.001 for plasma and stool, respectively) (Supplemental Figure 7B). The relative abundance of the targeted stool and plasma metabolites, including betaine, choline, TMAO, IS, and PCS, did not change with treatment (Supplemental Figure 8).

Discussion

In this small, nonrandomized, crossover, feasibility study of patients on maintenance hemodialysis, we found that the stool microbiome, stool metabolome, and plasma metabolome were stable over time, and that treatment with the prebiotic, p-inulin, was associated with changes in the microbiome but not with detectable changes in the metabolome. Additionally, we found that p-inulin was well tolerated and that participants were able to perform the protocol-required, frequent, stool-sample collections and aliquoting procedures with high fidelity.

The findings of this feasibility study have implications for conducting a clinical trial of agents targeting the gut microbiome in the setting of dialysis-dependent ESKD. The willingness of participants to provide and aliquot stool samples on a weekly or twice weekly basis for 28 weeks—despite the comorbidity and treatment burdens associated with dialysis-dependent ESKD—was striking, and suggests that home biosample collection and processing is an approach that could be used more broadly for studies in this population; this is an important consideration because participant visits to research centers can be challenging given

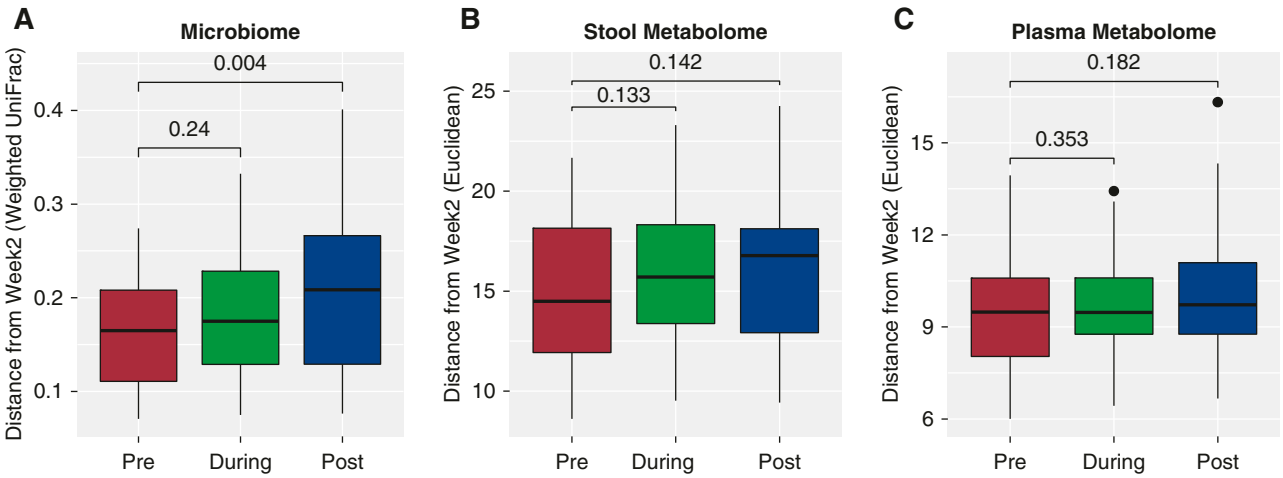

Figure 3. | **UniFrac distances from the initial measurement (week-2 samples) stratified by treatment phase.** (A) For the microbiome samples, the weighted UniFrac distance was used to compute the distances for each sample. (B and C) For the metabolome samples, the Euclidean distance was used. Numeric values within the plots are *P* values. Differences between samples were determined on the basis of distances from the initial measurements to account for participant-level differences in initial abundance of microorganisms or concentration of metabolites. Measurements in each treatment phase were treated as repeated measurements because of time variability in treatment effects. Differences across the treatment phases were tested using a linear mixed-effects model.

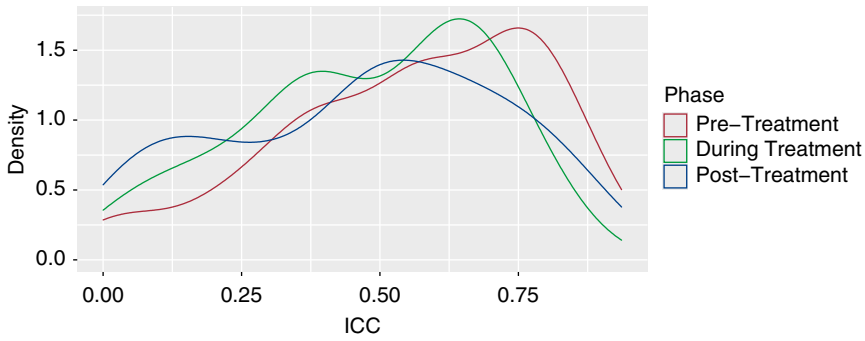

Figure 4. | **Density of intraclass correlation coefficients (ICC) for the microbiota.**

the substantial time spent at outpatient dialysis facilities, which are often geographically distant from academic institutions. The intraparticipant stability of the microbiome composition over time, as assessed with repeated samples obtained during the pretreatment phase, suggests that less frequent sampling would be sufficient to characterize the microbiome of individual participants and make comparisons across treatment conditions. Less frequent sampling is desirable to reduce participant burden and study costs for a large trial. The interparticipant variability in both the microbiome and metabolome observed in this study, both during the pretreatment phase and with respect to the response to p-inulin, is also informative for designing future trials. The pairwise distance matrix can be used to estimate power and the sample size needed to detect shifts in the microbial community using distance-based permutational multivariate ANOVA (22).

The high degree of interparticipant variability that we observed is consistent with results from a recent study of 17 patients on hemodiafiltration or hemodialysis, which also incorporated frequent collection of stool samples, and found that interparticipant variability in microbiome composition was greater among patients on hemodialysis than among age- and sex-matched controls from a population-based cohort (23). Similarly, in a study of 18 patients on hemodialysis in which serum concentrations of specific uremic toxins were measured repeatedly over a 16-week period, interparticipant variability was greater than intraparticipant variability. However, for certain bacterial-derived solutes, such as IS, intraparticipant changes were substantial and contributed importantly to the overall variability (24).

The increase in diversity of the microbiome observed in our study during and after p-inulin treatment supports the concept that short-term administration of a prebiotic can alter the intestinal microbiome in patients on maintenance hemodialysis. Although it is not possible to draw conclusions about the health implications of these changes, the increase in intrasample bacterial diversity likely reflects a positive change because greater microbial diversity is generally beneficial (25). This finding adds to results of prior work. A study of patients with nondialysis-dependent CKD found that treatment with a combination of a prebiotic and probiotic increased the relative abundance of fecal *Bifidobacterium* and decreased abundance of Ruminococcaceae bacteria (26). In a study of patients on peritoneal dialysis, treatment with inulin-type fructans had no detectable effect on the phylum-level microbiota or genus-level bacteria, but did alter *Bacteroides thetaiotaomicron* (one of 14 indole-producing bacteria evaluated) and decreased the fecal concentration of indole (27). In our study, we observed a significant reduction in the Ruminococcaceae family and an increase in Clostridiales with p-inulin treatment. Clostridiales include saccarolytic bacteria that contribute to the production of short-chain fatty acids in the intestine, a change that is viewed as advantageous (28).

Changes in the metabolomes of the stool and plasma were not evident with p-inulin treatment, and, with the exception of uridine, we also did not see changes in individual metabolites. This finding is important because any beneficial effects of changing the intestinal microbiome would presumably be mediated, at least in part, by resulting changes in bacterial or host metabolites. In addition to untargeted approaches, we performed prespecified targeted measurements of PCS (29), IS (30), and TMAO (31), which are well-recognized, intestinal bacteria–derived, uremic-retention solutes. It is not clear whether the lack of change in the metabolome or in the individual metabolites of interest indicates that p-inulin did not have the intended effect, that our approach to detecting its effect was not sufficiently sensitive, or that power was inadequate. A previous randomized, placebo-controlled trial of dietary supplementation for 8 weeks with the prebiotic high amylase resistant starch, among 50 patients on hemodialysis, found reductions in serum p-cresol but not IS (32). A randomized, crossover study of administration (for 4 weeks) of the prebiotic arabinoxylan oligosaccharides to 40 patients with nondialysis-dependent CKD found a modest decrease in TMAO with the prebiotic, but no effect on other microbiota-derived uremic solutes (33). The composition of the microbiome was not evaluated in either of these studies. The significance of the increase in stool and plasma concentrations of uridine that we observed during p-inulin treatment is not clear. Animal studies have provided some preliminary evidence that high-fat diets decrease fecal uridine concentrations, and that uridine administration can improve intestinal morphology and glucose tolerance, but the relevance of these findings to humans with ESKD is not known (34,35). Given the large medication burden that accompanies ESKD and the prevalence of gastrointestinal symptoms in this patient population, we were not certain

that participants would be willing to take the p-inulin as required by the protocol. The finding that p-inulin was well tolerated by all but one participant, and that adherence was high, suggests this agent could be successfully evaluated in a larger-scale trial. The signal, albeit nondefinitive, that gastrointestinal symptoms improved with p-inulin also supports the feasibility of more extensive investigations of this agent in this patient population. Additionally, the high tolerability and adherence suggest that, if p-inulin were found in a clinical trial to have efficacy, it would likely be acceptable to patients outside of a trial setting.

This study has several strengths. Although the target sample size could have been met by a single center, we elected to conduct a multicenter study to produce feasibility data that would be more generalizable to a future trial. The RNA sequencing and metabolite measurements were performed by core facilities using standardized protocols and attention to quality control. Importantly, there were a remarkably small number of missing biosamples, despite the intensity of the collection schedule. This study also has limitations. Although the frequent sample collection provided a rich set of data for repeated-measures analyses, the small number of participants limited the power to detect effects of the intervention. The small number of participants also limits conclusions about feasibility for a clinical trial requiring a substantially larger sample size.

In summary, this study suggests that, among patients on hemodialysis, p-inulin is well tolerated; home collection and aliquoting of stool samples is acceptable to patients; and, on the basis of the intraparticipant stability of the microbiome and metabolome, frequent collection of biosamples is not required. Taken together, the findings support the feasibility of a clinical trial evaluating interventions targeting the gut microbiome in this patient population. Whether or not p-inulin has sufficient efficacy as an intervention requires evaluation in larger studies.

Disclosures

D.M. Charytan reports receiving personal fees from AstraZeneca, Douglas and London, Fresenius, GSK, Merck, PLC Medical, and Zoll; grants and personal fees from Amgen, Gilead, Medtronic, and NovoNordisk; grants from Bioporto; other from Daichi-Sankyo; and personal fees and other from Janssen, outside the submitted work. L.M. Dember receives consulting fees from GlaxoSmithKline and Merck, and compensation from the National Kidney Foundation for her role as deputy editor of *American Journal of Kidney Diseases*, outside of the submitted work. J. Himmelfarb reports being a founder of AKTIV-X Technologies, Inc., with equity; and has received fees for acting as a consultant or scientific advisory board member for Akebia, Chinook Therapeutics, Maze Therapeutics, Pfizer, Renalytix AI, and Seattle Genetics. T.A. Ikizler received personal fees from Abbott Renal Care and Fresenius Kabi, during the conduct of the study. P.L. Kimmel is a coeditor of *Chronic Renal Disease* (Academic Press, San Diego, CA), and a member of the board of directors of the Washington Academy of Medicine. A.S. Kliger receives income from the American Society of Nephrology, the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), and Yale New Haven Hospital. H. Li receives consulting fees from Eli Lily, outside the submitted work. R. Mehrotra receives consulting fees from Baxter Healthcare, outside the submitted work. All remaining authors have nothing to disclose.

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Author Contributions

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Supplemental Material

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Supplemental Summary 1. Collaborator information.

Supplemental Table 1. ICCs for phyla at each treatment phase and the relative abundance of the corresponding phyla.

Supplemental Table 2. Five largest ICCs for genera at each treatment phase and the relative abundance of the corresponding genera.

Supplemental Table 3. Five smallest ICCs of genera at each treatment phase and the relative abundance of the corresponding genera.

Supplemental Figure 1. Dietary composition as assessed by the Block Food Frequency Questionnaire administered at baseline (week 0) and at the end of each study phase (weeks 8, 20, and 28).

Supplemental Figure 2. Principal coordinate analysis (PCoA) with the weighted UniFrac distance for the microbiome samples.

Supplemental Figure 3. Principal component analysis (PCA) for the log-transformed stool metabolome samples.

Supplemental Figure 4. Principal component analysis (PCA) for the log-transformed plasma metabolome samples.

Supplemental Figure 5. Taxonomic composition of stool.

Supplemental Figure 6. Heatmap plots of 20 most abundant (A) genera, (B) stool metabolites, and (C) plasma metabolites.

Supplemental Figure 7. (A) Differentially abundant genera across the treatment phases. (B) Differentially abundant metabolites across the treatment phases.

Supplemental Figure 8. Abundance of targeted metabolites across the treatment phases.

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