Gut Microbiome and p-Inulin in CKD
TarGut CKD study

A multi-center study to characterize the gut microbiome of individuals with chronic kidney disease, and to explore effects of p-inulin on the gut microbiome

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Table of Contents

10 mlList of Abbreviations

AE	Adverse event
CBC	Complete blood count
CMP	Comprehensive metabolic panel
CKD	Chronic kidney disease
CRF	Case report form
CVD	Cardiovascular disease
DCC	Data Coordinating Center
DSMB	Data and Safety Monitoring Board
ESRD	End stage renal disease
FGF-23	Fibroblast growth factor-23
HD	Hemodialysis
HIPAA	Health Insurance Portability and Accounting Act
IDS	Investigational Drug Service
IRB	Institutional Review Board
MOP	Manual of Procedures
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
OHRP	Office of Human Research Protections
PHI	Protected health information
PTH	Parathyroid hormone
SAE	Serious adverse event
SAP	Statistical analysis plan
UAE	Unanticipated adverse event

Study Summary

Title	Gut Microbiome and p-Inulin in CKD
Short Title	TarGut CKD
Protocol Number	
Phase	Phase I
Methodology	Non-randomized, 3-period crossover trial, with repeated measures within period
Study Duration	28 weeks for participant; 15 months for full study (projected)
Study Center(s)	George Washington University University of Utah Cleveland Clinic (Data Coordinating Center)
Objectives	• To evaluate intra- and inter-patient variability in the composition and function of the gut microbiome • To evaluate the tolerability of p-inulin administration • To assess the feasibility of sample collection in this population
Number of Participants	10 individuals who satisfactorily complete the first 20 weeks of the study
Diagnosis and Main Inclusion Criteria	Chronic Kidney Disease with a estimated glomerular filtration rate (eGFR) 15.0 to 50.0 ml/min/1.73 m^2
Study Product, Dose, Route, Regimen	p-inulin 16 g orally, daily
Duration of administration	12 weeks
Major Outcomes	• Microbiome intra-subject variability • P-inulin tolerability

Statistical Methodology	• Alpha-diversity of bacterial genera will be derived at each time point, and intra-class correlation coefficients, using mixed models, will be estimated to evaluate diversity of multiple observations within each individual. • UniFrac distances will be derived as beta diversity measures of inter-patient variability to characterize the compositional dissimilarity between two individuals. A repeated measures MANOVA of UniFrac distances will be used to assess the changes of the overall microbial compositions over time • Longitudinal mixed-effect models will be applied to study change of metabolites before and after p-inulin treatment, and to identify the metabolites that change after p-inulin treatment. • Longitudinal analyses of metabolites, with time-varying covariates (microbiome diversity or microbial genera) will be conducted to identify the microbial genera associated with changes of metabolites due to p-inulin treatment

1 Introduction

This document is a protocol for a human research study. This study is to be conducted according to US and international standards of Good Clinical Practice, applicable government regulations and the research policies and procedures in effect at the institutions where the study is implemented.

1.1 Background

A substantial body of work implicates inflammation as an important contributor to the morbidity and mortality associated with chronic kidney disease (CKD) and, in particular, to accelerated cardiovascular disease.[1-3] However, the etiology of unprovoked inflammation in CKD is not well understood. Proposed etiologic factors include chronic sub-clinical infections,[4] volume overload,[5] oxidative stress,[6] sympathetic activation,[7] malnutrition, and vitamin D deficiency.[8] Alterations in the intestinal microbiome (dysbiosis) is increasingly recognized as a potential cause of inflammation in CKD and end-stage renal disease (ESRD) patients.[9-13]

The human gut harbors ~10^{14} bacteria, and the metabolic potential of the gut microbiota is enormous.[14-16] The gut microbiota performs a multitude of functions, and can be considered a metabolically active endogenous "organ".[17] Under physiological conditions, the microbiota provides a complementary role by participating in metabolic activities that are not fully evolved in the human host such as break down of undigestable plant polysaccharides,[18] synthesis of certain vitamins,[19] biotransformation of conjugated bile acids[20] and degradation of dietary oxalates.[21] Additionally, postnatal colonization of the intestine educates the immune system and reduces allergic responses to food and environmental antigens.[22]

Alterations in the gut microbiome have been demonstrated in patients with CKD and ESRD.[11;13] Individuals with uremia have greatly increased counts of both aerobic (~10^6 bacteria/ml) and anaerobic (~10^7 bacteria/ml) organisms in the duodenum and jejunum.[23] The microbial flora of the lower intestinal tract has also been shown to be altered in patients with non-dialysis dependent chronic kidney disease – the most notable changes are reductions in both the Lactobacillaceae and Prevotellaceae families.[13] Hida *et al.* studied the colonic composition of microbiota in healthy controls and hemodialysis patients.[24] The number of aerobic bacteria such as *Enterobacteria* and *Enterococci* was found to be approximately 100 times higher in patients treated with maintenance hemodialysis than in controls. Among anaerobic bacteria, hemodialysis patients had significantly lower organism counts for *Bifidobacteria* and higher organism counts for *Clostridium perfringens*.[24] Vaziri *et al* showed significant differences in the abundance of 190 microbial operational taxonomic units (OTUs) between the ESRD and the normal control individuals.[13] The microbiome profile in CKD patients, not yet on dialysis has not been studied. In order to isolate the effect of kidney failure, the investigators examined the gut microbiota in nephrectomized rats.[13] The study revealed substantially lower "species richness" as measured by the number of OTUs in the nephrectomized rats compared to the controls. The intestinal dysbiosis may be a direct or indirect effect of uremia.[25;26] For example, loss of kidney function leads to secretion of urea into the gastrointestinal tract. The hydrolysis of urea by bacterial urease results in the formation of large quantities of ammonia, which has important effects on the growth of commensal bacteria.[25;26] Other contributing indirect factors relevant to patients with kidney failure include decreased consumption of dietary fiber,[27;28] frequent use of antibiotics,[29;30] slow colonic transit,[31;32] metabolic acidosis,[12] intestinal wall edema,[5;12;26] and possibly oral iron intake.[33]

There are multiple mechanisms by which such alterations in the gut microbiota might contribute to CKD-associated inflammation and cardiovascular disease including 1) increased production of bacteria-derived solutes that are retained in kidney failure (e.g., para (p) Cresol sulfate[34;35]), and 2) increased translocation into the circulation of bacteria-derived endotoxin across an impaired intestinal barrier.[9;10;35-37] Elevated serum p-Cresol sulfate concentration is associated with cardiovascular events and mortality among patients with CKD,[38;39] and experimental as well as clinical studies indicate that endotoxin is involved in multiple steps of atherogenesis.[9;10;36;40-42;42;43;43;44] Endotoxin provokes a host of responses by binding to the CD14 receptor.[9;10] Elevation in the concentration of soluble CD14 (sCD14), an indicator of CD14 receptor activation, is an independent predictor of mortality among patients treated with maintenance hemodialysis.[9;10]

Prebiotics are microbial feed supplements that beneficially affect the host by improving its intestinal microbial balance and re-establishing symbiosis. Treatment with the pre-biotic, p-inulin, increases intestinal growth of beneficial bacteria such as bifidobacteria.[45] Increases in bifidobacteria are associated with attenuation of inflammation and improvement in intestinal barrier function.[46-49] Other reported effects of prebiotic supplementation include optimizing the immune response, decreasing endotoxin generation, and enhancing metabolic capabilities.[18;49-52] Preliminary evidence indicates that prebiotics are effective in reducing the plasma concentration of p-Cresol sulfate in CKD and hemodialysis patients.[53;54] In a single center, non-randomized, open-label phase I/II study of hemodialysis patients, treatment with p-inulin for 4 weeks was accompanied by a reduction in the p-Cresol sulfate generation rate and a 20% decrease in its serum concentration.[54]

There are important potential benefits of intervening to restore symbiosis to the gut microbiota in patients with CKD. However, before conducting clinical trials of such interventions important gaps must be filled in the understanding of the composition, function and stability of the gut microbiota in CKD. The purpose of the Microbiome and P-Inulin in CKD Study is to fill some of these gaps in anticipation of ultimately investigating the therapeutic potential of altering the gut microbiome in this patient population. Intensive biosample collection from a small number of CKD patients before, during, and after treatment with p-inulin will be performed in order to generate information about within-patient and between-patient variability in the composition and function of the gut microbiome. The study will also provide information about the tolerability of p-inulin and feasibility of biosample collection.

1.2 Study Agent

p-Inulin (oligofructose-enriched inulin) is a prebiotic.[55;56] Prebiotics are non-digestible food ingredients that selectively stimulate growth and/or activity of beneficial bacteria in the colon. Inulin-type prebiotics are members of a larger group of compounds called “fructans,” which encompasses all naturally occurring plant oligo- and polysaccharides in which one or more fructosyl-fructose linkages comprise the majority of glycosidic bonds.[55] Inulin is present in vegetables with high fiber content such as sugar beets, leeks, onions, garlic and asparagus. The basic inulin, derived from chicory root, is treated with specific bacterial enzymes that break down the long chain inulin to the short chain oligofructose in 95% pure form. Short-chain oligofructose is mixed with inulin in equal parts to prepare the final product, p-inulin.[57;58] This composition allows the short-chain oligofructose to be fermented in the proximal (right) colon and the long-chain molecule, inulin, to be fermented in the distal colon.[60] The end products of fermentation are gases (such as carbon dioxide and hydrogen), lactate, and short-chain fatty acids.

Preliminary evidence in individuals with preserved kidney function indicates that p-inulin promotes growth of bifidobacteria, reduces endotoxin generation, attenuates inflammation, decreases generation of uremic toxins and improves metabolic function.[11;45;56;59]

Inulin-type prebiotics received the designation of "generally recognized as safe" in 1992.[60]

1.3 Clinical Data to Date on Prebiotics and p-Inulin

A large number of studies have been performed examining the effect of prebiotics in a variety of conditions including obesity,[61;62] HIV,[63;64]and gastrointestinal diseases.[65] Bouhnik et al showed that four weeks of treatment with short-chain fructo-oligosaccharides increases fecal bifidobacteria counts significantly.[66] In a small randomized double-blind, placebo-controlled trial, 31 patients who had received radiotherapy were randomized either to p-inulin or maltodextrin for 4 weeks.[67] *Lactobacillus* and *bifidobacterium* counts decreased significantly with radiotherapy, but recovered in the treatment arm. In another study, 49 diabetic participants were randomized either to inulin 10 g/day or maltodextrin for 8 weeks.[68] Inulin treatment improved insulin resistance and reduced plasma levels of lipopolysaccharide, hs-CRP and TNF-α significantly compared to the maltodextrin group.

There are limited data on prebiotic treatment of patients with CKD and ESRD (**Table 1**). In a pilot study involving 22 maintenance hemodialysis patients, treatment with 20g of p-inulin for 4 weeks reduced the generation and serum concentration of p-cresol.[54] The authors reported that p-inulin was well tolerated and that there was excellent adherence with treatment. Nakabayashi showed that symbiotic treatment (*Lactobacillus casei* Shirota strain and *Bifidobacterium* breve Yakult strain as probiotics and galacto-oligosaccharides as prebiotics) three times per day for 2 weeks reduced p-cresol levels in maintenance hemodialysis patients.[69]

Table 1: Effect of pro- and pre-biotics on uremic toxins and inflammation

Reference	Patient type (n)	Intervention	Comments
De Preter et al.[70]	Healthy participants (50)	Oligofructose-enriched inulin	↓ Urinary excretion of p-cresol
Dewulf et al[61]	Obese women (30)	Inulin/oligofructose	↓Endotoxaemia
Schiffrin et al[71]	Elderly participants (74)	Oligosaccharides	↓TNF-alpha mRNA and IL-6 mRNA ↓serum sCD14
Kotzampassi et al.[72]	Trauma patients (65)	Probiotics along with inulin, oat bran, pectin, and resistant starch	↓ Rate of systemic inflammatory response, syndrome, infections, severe sepsis, and mortality
Pavan et al[73]	CKD stage III to V (24)	Synbiotic: Probiotic and prebiotic	Slowed the progression of CKD
Guida et al[53]	CKD stage III and IV (30)	Synbiotic: probiotic + inulin and tapioca-resistant starch	↓plasma p-cresol
Rossi et al[74]	CKD stages III and IV	Probiotic + prebiotic (inulin and	Ongoing

Table 1: Effect of pro- and pre-biotics on uremic toxins and inflammation

Reference	Patient type (n)	Intervention	Comments
	(37)	galactooligosaccarides)	
Meijers et al.[54]	HD patients (22)	Oligofructose-enriched inulin	↓ Serum p-Cresol sulfate and generation rate
Nakabayashi et al.[69]	HD patients (7)	Galacto-oligosaccharides and *L. casei*, and *B. breve*	↓ Serum p-Cresol

1.4 Dose Rationale and Risk/Benefits

A dose-dependent bifidogenic effect has been observed with p-inulin.[45;75;76] Bouhnik et al examined the dose dependent effect of 8 weeks treatment with short-chain fructo-oligosaccharides (SC-FOS).[62] Forty healthy volunteers were randomized to one of five groups: G_0 group (SC-FOS 0 g and saccharose 20 g) used as placebo; $G_{2.5}$ group (SC-FOS 2.5 g and saccharose 17.5 g); G5 group (SC-FOS 5 g and saccharose 15 g); G_{10} group (SC-FOS 10 g and saccharose 10 g); and G_{20} group (SC-FOS 20 g and saccharose 0 g). Bifidobacteria counts at Day 8 were greater in groups G_{10} and G_{20} compared with G_0 and $G_{2.5}$. Total anaerobes increased at the 10-g daily dose, but not at lower doses, while no significant differences were found for Bacteroides, Lactobacillus, or Enterobacteriaceae.[75] Excess flatus was significantly more frequent in participants consuming G_{20}. The authors concluded that for healthy individuals consuming their usual diets, 10 g/day is the optimal dose in terms of tolerability and efficacy with respect to increasing fecal bifidobacteria. Gibson et al reported that FOS at 15 g/day in three divided doses increased the proportion of Bifidobacteria from 6% to 22% in healthy adults during two weeks of use. The effect on Bifidobacteria was accompanied by significant decreases in Bacteroides, Clostridia, and Fusobacteria.[45] Meijers et al showed that a dose of 20 g/day is tolerated by ESRD patients, but with a large number of them reporting abdominal symptoms.[77] Thus, the doses of 10 g and 20 g daily resulted in significant increases in fecal bifidobacteria compared to lower doses, but with more abdominal symptoms at the higher dose.[45;57;75]

In the current study, all participants will receive p-inulin at a dose of 8 g two times per day for a total daily dose of 16 g. Based on experience in non-CKD populations, it is anticipated that this dose will have acceptable tolerability and sufficient efficacy.[45;76] However, dose reduction for intolerability will be permitted if necessary.

1.4.1 Risks

The common side effects of p-inulin include gastrointestinal symptoms such as flatulence, bloating, abdominal distension, loose stools, and increased stool frequency.[54;56] These symptoms have been more frequently reported with inulin doses in the range of 15 to 30 g per day than with lower doses.[77;78] Gastrointestinal symptoms, particularly constipation is common in patients with CKD. [79;80]Ascertainment of gastrointestinal symptoms using the Gastrointestinal Symptom Rating Scale will be performed in this study.[81]

Participants are not expected to benefit from participating in this study. The data obtained from this study will be used to conduct a larger study to assess the therapeutic potential of altering the gut microbiome in CKD patients.

2 Study Objectives

The overarching hypothesis motivating this exploratory study of variability is that treatment with oligofructose enriched inulin (p-inulin) will alter the composition and/or function of the gut microbiome, and thereby reduce the generation of gut-derived uremic toxins, improve gut barrier function and attenuate systemic inflammation in CKD patients. In order to design a future clinical trial the following parameters from CKD subjects are needed:

1) Intra-patient variability in the composition and function of the gut microbiome
2) Inter-patient variability in the composition and function of the gut microbiome
3) Impact of p-inulin on the composition and function of the gut microbiome
4) Tolerability of p-inulin administration
5) Feasibility of collecting stool samples in this patient population

2.1 Primary Objectives

- To evaluate the within-patient variability of gut bacteria-derived metabolites and gut bacterial composition over an 8-week period during which there is no study-driven manipulation of diet, medication, or treatment
- To evaluate, using each patient as his/her own control, whether treatment with p-inulin for 12 weeks alters gut bacteria-derived metabolites and gut bacterial composition

2.2 Secondary Objectives

- To evaluate, using each participant as his/her own control, whether p-inulin alters levels of selected circulating inflammatory markers and mediators
- To assess within participant variability of gut bacteria-derived metabolites and gut bacterial composition during an 12-week period of treatment with p-inulin
- To assess between-participant variability of gut bacteria-derived metabolites and gut bacterial composition during an 12-week period of treatment with p-inulin
- To examine, using each patient as his/her own control, whether gut bacterial composition and gut bacteria-derived metabolites return to pre-treatment findings during 8 week observation period of post-treatment.
- To evaluate the tolerability of p-inulin administered at a total daily dose of 16 g
- To evaluate the short-term safety of p-inulin administered at a total daily dose of 16 g
- To assess adherence to p-inulin treatment
- To explore the willingness of CKD patients to enroll in a study requiring repeated collection of stool samples
- To explore participant adherence to the stool sample collection requirements
- To identify barriers to adhering to stool sample collection

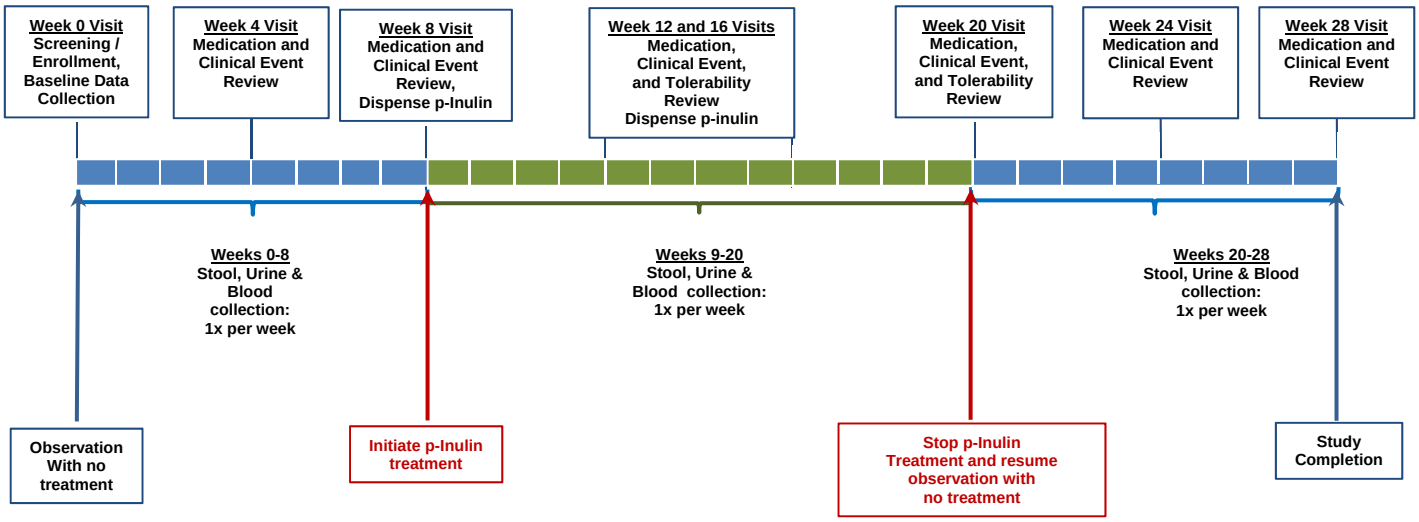
3 Study Design

3.1 General Design

This is a non-randomized, open-label, crossover study. Participants will be followed during 3 sequential phases: 1) no intervention (8 weeks observational period), 2) p-inulin administration (12 weeks treatment period), and 3) no intervention (8 weeks observational period). The visit and stool and blood collection

schedule for each phase is depicted in Figure 1 and a detailed table of study procedures is provided in **Section 15.1**. Study participants will be recruited from two sites in the U.S- George Washington University and University of Utah clinical sites**.**

Figure 1. Visit and Biosample-Collection Schedule



3.2 Study Outcome

3.2.1 Primary Outcome

Microbiome characterization primary endpoints include:

1. Within patient variability in the gut microbiome composition, metabolomic profile, targeted metabolites and inflammatory markers during the no treatment phases
2. Between -patient variability in the gut microbiome composition, metabolomic profile, targeted metabolites, inflammatory markers during the p-inulin treatment phase

3.2.2 Secondary Outcome

Microbiome characterization secondary outcomes include:

1. Within patient change in the microbiome composition, metabolomic profiles, targeted metabolites, inflammatory markers after p-inulin treatment compared with pre-treatment
2. Within-patient variability in the bacterial composition of the stool during the no treatment phase
3. Within-patient variability in the bacterial composition of the stool during the p-inulin treatment phase
4. Within-patient change in the bacterial composition of the stool after p-inulin treatment compared with pre-treatment
5. Differences in the above measures between diabetic and non-diabetic participants
6. Within-cohort variability in the metabolomic profile and targeted metabolites / inflammatory markers during the no treatment phase
7. Within-cohort variability in the metabolomic profile and targeted metabolites / inflammatory markers during the p-inulin treatment phase

8. Within-cohort change in the metabolomic profile and targeted metabolites / inflammatory markers after p-inulin treatment compared with pre-treatment

Tolerability and Safety

The major safety and tolerability outcomes include:

1. Gastrointestinal symptoms
2. Early discontinuation of p-inulin
3. Reduction in p-inulin dose
4. Adverse events

Feasibility

Feasibility outcomes include:

1. Enrollment refusal rate
2. Proportion of completed stool sample collections
3. Proportion of completed blood sample collections
4. Adherence to p-inulin assessed by sachet counts
5. Participant withdrawal during each phase of the study

4 Participant Selection and Withdrawal

4.1 Inclusion Criteria

a) Subjects with eGFR 15.0 to 50.0 ml/min/1.73 m^2 as estimated by the CKD-EPI equation
b) Albuminuria greater than 300 mg/g creatinine (by spot urine test) if eGFR is ≥45 ml/min/1.73 m^2
c) Age ≥ 18 years
d) For women of childbearing potential, willingness to use a highly effective method of birth control for up to 4 weeks after the last dose to study drug. See **Section 4.2.1** for definition of childbearing potential and acceptable methods of birth control
e) Ability to provide informed consent

4.2 Exclusion Criteria

a) Use of pre- or pro-biotics during the past 2 months
b) Consumption of probiotic yogurt during the past 2 weeks
c) Use of antibiotics within the past 3 months if the patient received a single course of antibiotic. If the patient received more than one course of antibiotic treatment, we will wait for 6 months prior to inclusion.
d) Presence of HIV infection, chronic wound infection and osteomyelitis
e) Presence of or treatment for periodontal infection
f) Inflammatory bowel disease, chronic diarrhea, current C. difficile infection
g) Cirrhosis or chronic active hepatitis
h) Treatment with immunosuppressive medications in the past 6 months or more than a week of treatment with prednisone >10 mg in the last 3 months
i) Treatment with proton pump inhibitors within the last one month
j) Anticipated initiation of dialysis or kidney transplant within 9 months
k) Acute on chronic kidney disease
l) Expected survival < 9 months

m) Pregnancy, anticipated pregnancy, or breastfeeding
n) Incarceration
o) Participation in another intervention study
p) Severe anemia defined as hemoglobin <9.0 g/dl any time during the last 3 months
q) Patients in whom frequent blood sampling may be difficult

4.2.1 Women of Childbearing Potential

Women of childbearing potential include any female who has experienced menarche and who has not undergone successful surgical sterilization (hysterectomy, bilateral tubal ligation, or bilateral oophorectomy) or is not postmenopausal. Postmenopausal is defined as:

- Amenorrhea for ≥12 consecutive months without another cause or
- Women with irregular menstrual periods and a documented serum follicle stimulating hormone (FSH) level > 35 mIU/mL or
- Women on hormone replacement therapy (HRT)

Women who are using oral contraceptives, other hormonal contraceptives (vaginal products, skin patches, or implanted or injectable products), or mechanical products such as an intrauterine device or barrier methods (diaphragm, condoms, spermicides) to prevent pregnancy, or who are practicing abstinence, or have a partner who is sterile (e.g., vasectomy) should be considered to be of childbearing potential. Acceptable methods of highly effective birth control include:

- Condom with spermicide
- Diaphragm and spermicide
- Cervical cap and spermicide
- Hormonal contraception

4.3 Recruitment

Participants will be recruited from two Clinical Centers with a general goal of enrolling 6-10 participants at each site. We will recruit total of 15 subjects and employ the recruit and replace strategy until a minimum of 10 participants have satisfactorily completed the first 20 weeks of the study protocol. Approximately, equal number of diabetics and non-diabetics will be recruited. Participants at outpatient clinics affiliated with investigator and co-investigator practices will be screened for eligibility. In addition to active screening at the outpatient clinics by study personnel, informational handouts and brochures may be disseminated at the clinics in order to allow potential participants to learn about the study and to contact investigators if interested. All study materials must be approved by local IRBs before dissemination to potential study participants.

Outpatient laboratory studies, medical records at the investigator's institution will be reviewed to assess eligibility for enrollment. No study-specific testing is required to confirm eligibility, except a serum pregnancy test for women of childbearing potential. Prior to approaching potential participants, the treating nephrologists will be contacted to assess suitability for enrollment.

Once preliminary eligibility is confirmed, informed consent will be obtained by a qualified investigator or study site designee during an in-person visit. This visit will take place at the investigator's institution.

We will recruit total of 15 subjects and recruitment will continue until a minimum of 10 participants have satisfactorily completed the first 20 weeks of the study protocol. Anticipating drop outs, we will recruit 15 patients.

Participants will be compensated for providing stool, blood, and urine samples. Each Clinical Center is responsible for developing a compensation plan and schedule, and distributing payment.

4.4 Early Withdrawal of Participants and Early Study Termination

4.4.1 Early Withdrawal of Participants

Reasons for premature withdrawal include pregnancy, death, and withdrawal of consent. Participants who are not able or willing to continue taking the study agent will be encouraged to remain in the study and continue biosample collection.

4.4.2 Early Termination of Study Medication

Participants who are unable to tolerate p-inulin at a total dose of 16 g per day (8 g twice daily) will have the dose reduced to a total dose of 8 g per day (4 g twice daily). If the participant is unable to tolerate a total daily dose of 8 g, p-inulin will be discontinued.

If p-inulin is discontinued, study visits and procedures will continue unless the participant withdraws consent for follow-up.

Participants whose dose is reduced or who stop taking p-inulin are permitted, at the discretion of the site investigator, to resume taking p-inulin within the 8 week dosing phase.

5 Study Procedures

5.1 Study Visit Schedule

A schedule of study visits and procedures is provided in the Study Procedures Table in **Section 15.1**.

5.1.1 Pre-screening Activities

Participants at outpatient clinics affiliated with investigator and co-investigator practices will be screened for eligibility. Clinical center study personnel will review medical records to assess eligibility. The treating nephrologist for a potentially eligible patient will be contacted to further assess eligibility and obtain permission for the study coordinator to contact the patient.

5.1.2 Screening/Baseline Visit

Patients who appear eligible based on pre-screening will be approached in person to determine interest in participation and confirm eligibility. Study personnel will discuss the study goals and procedures with the potential participant in detail. If the patient is agreeable to participating in the study, study personnel will review and assess understanding of the entire informed consent form prior to obtaining written informed consent from the participant. The consenting process will be done by a qualified investigator or study site designee. Informed consent will be obtained and documented before any study procedure is performed.

The screening and baseline activities may take place over one or two visits.

Screening/Baseline Visit Activities

- Informed consent process
- Collection of baseline data including demographics, medical history, etiology of kidney disease, and medication use including recent and current use of prescription products, over-the-counter products, herbal supplements, vitamins, oral or intravenous iron, proton pump inhibitors, artificial sweeteners, and pre- and probiotic use in any form
- Screening for periodontal infection (bad breath, bleeding/inflamed gum, tooth decay). Patients will also be asked about history of gum disease or treatment for gingivitis.
- Determination of eligibility, including measurement of urine protein creatinine ratio.
- Blood draw for baseline measurements (13 mL)
- Urine sample will be obtained for pregnancy test for women of childbearing potential
- Instruction for stool and urine sample collection and storage
- Dietary assessment using food frequency questionnaire
- Gastrointestinal symptom assessment questionnaire
- Blood samples collected for complete blood count (CBC), comprehensive metabolic panel (CMP), cystatin C, phosphorous, magnesium, fibroblast growth factor-23 (FGF-23), parathyroid hormone (PTH) and uric acid assays.

Enrolled participants will be instructed not to change their dietary patterns or add new medications, if possible, during the study.

See **Section 5.2** for details regarding the blood and stool sample collection schedule.

5.1.3 Week 4, 8, 12, 16, and 20, 24 and 28 Visits and Weekly Telephone Contacts

Participants will have in-person study visits at Weeks 4, 8, 12, 16, 20, 24 and 28. At each study visit, participants will be asked about emergency room visits, hospital admission or any significant clinical events that occurred since the last visit. On a weekly basis, participants will be queried on antibiotic use in the previous week, and medical records will be reviewed for antibiotics use. During the dosing phase, participants will also be asked questions to assess adherence with p-inulin.

Dietary assessment using the Block Food Frequency Questionnaire (https://nutritionquest.com) will be administered at W1 and Weeks 8 (pre-treatment phase), 20 (treatment phase), and 28 (post-treatment phase).

Week 4 Visit

- Information on clinical events and current medications will be collected.
- Gastrointestinal symptom assessment questionnaire will be administered.

Week 8 Visit

- Information on clinical events and current use of medications will be collected.
- Gastrointestinal symptom assessment questionnaire will be administered.
- Dietary assessment using a food frequency questionnaire will be administered to assess dietary patterns prior to starting p-inulin.
- A four week supply of p-inulin will be dispensed to the participant along with instructions for its use.

- To assess adherence to the study protocol, participants will be instructed to keep all unused study agent packets and bring them to the Week 12 visit.
- Blood samples collected for CBC, CMP, cystatin C, phosphorous, magnesium, FGF-23, PTH and uric acid assays.
- Patient will bring the 24 hour urine collection. Study staff will record the 24-hour urine volume and save aliquots for the estimation of urea, creatinine, uric acid, calcium, phosphorous and magnesium concentrations.

Week 12 Visit

- Information on clinical events and current medications will be collected.
- Gastrointestinal symptom assessment questionnaire will be administered.
- Tolerability of p-inulin will be assessed. If participant has not been able to tolerate 16g total daily dose, the dose may be reduced to a total daily dose of 8 g.
- Study personnel will collect, count and record the p-inulin packets.
- A four week supply of p-inulin will be dispensed.
- To assess adherence to the study protocol, participants will be instructed to keep all unused study agent packets and bring them to the Week 16 visit.

Week 16 Visit

- Information on clinical events and current medication use will be collected.
- Gastrointestinal symptom assessment questionnaire will be administered.
- Tolerability of p-inulin will be assessed by participant self-report. If participant has not been able to tolerate 16 g total daily dose, the dose may be reduced to a total daily dose of 8 g.
- Study personnel will collect, count and record the number of unused p-inulin packets.
- A four week supply of p-inulin will be dispensed.
- To assess adherence to the p-inulin treatment schedule, participants will be instructed to keep all unused study agent packets and bring them to the Week 20 visit.
- Blood samples collected for CBC, CMP, cystatin C, phosphorous, magnesium, FGF-23, PTH and uric acid assays.

Week 20 Visit

- Information on clinical events and current medication use will be collected.
- Gastrointestinal symptom assessment questionnaire will be administered.
- Dietary assessment using a food frequency questionnaire will be administered to assess dietary patterns during the p-inulin treatment period.
- Tolerability of p-inulin will be assessed.
- Study personnel will collect, count and record the number of unused p-inulin packets.
- Blood samples collected for CBC, CMP, cystatin C, phosphorous, magnesium, FGF-23, PTH and uric acid assays.
- Patient will bring the 24 hour urine collection. Study staff will record the 24-hour urine volume and save aliquots for the estimation of urea, creatinine, uric acid, calcium, phosphorous and magnesium concentrations.

Week 24 Visit

- Information on clinical events and current medication use will be collected.
- Gastrointestinal symptom assessment questionnaire will be administered.

5.1.4 Week 28 Visit (Study Completion)

- Information on clinical events and current medication use will be collected.
- Gastrointestinal symptom assessment questionnaire will be administered.
- Dietary assessment using a food frequency questionnaire will be administered to assess dietary patterns during the p-inulin post-treatment phase
- Blood samples collected for CBC, CMP, cystatin C, phosphorous, magnesium, FGF-23, PTH and uric acid assays.
- Patient will bring the 24 hour urine collection. Study staff will record the 24-hour urine volume and save aliquots for the estimation of urea, creatinine, uric acid, calcium, phosphorous and magnesium concentrations.

5.2 Biosample Collection Schedule

Stool samples will be collected at baseline and then once weekly during the study period. The sample collection during weeks 21-28 (after p-inulin treatment) will allow detection of delayed alterations in response to p-inulin and will provide information about the durability of p-inulin effects

Stool specimens will be obtained by study participants. Participants will be provided a commercial "toilet hat" stool specimen collection kit (specimen container, shipping box, styrofoam cooler, and cold packs; Fisherbrand Commode Specimen Collection System; Thermo Fisher Scientific, Waltham, MA, USA) at the Baseline visit and as needed for resampling.

Participants will bring the stool samples on ice packs in the closed styrofoam cooler to the outpatient clinic. They will also be given the option of shipping it overnight to the clinical site. Research staff will process samples according to Core Laboratory specifications and store at -80°C for future shipment to the Core Laboratory. The aliquoting of samples is performed prior to freezing in order to minimize the number of freeze-thaw cycles. Detailed information regarding stool sample collection, storage, shipping, and participant training is provided in the Manual of Procedures (MOP).

Participants who do not provide the required number of stool samples will continue to be followed for the full duration of the study and will continue to be encouraged to provide samples in accordance with the protocol schedule.

Participants who are unable to provide the required number of stool samples will continue to be followed for the full duration of the study and will not be withdrawn.

Urine samples will be collected for metabolomics studies every week (10 ml). 24 hour urine samples will be collected at 8, 20 and 28 weeks of the study.

All blood samples will be obtained by an experienced phlebotomist keeping in mind to preserve veins for future vascular access. Study staff will review the most recent hemoglobin lab results in the medical record. If this result is less than 9.0 g/dl, blood will not be collected until the hemoglobin value is 9.0 g/dl or greater.

Blood specimens will be processed and aliquoted according to the Core Laboratory specifications. Samples will be stored at -80° C for future shipment to the Core Laboratory. The MOP provides detailed information on blood specimen processing.

5.3 Stool, Urine and Blood Analyses

Metabolites and inflammatory markers of interest that provide functional information about the gut microbiota or the host response are shown in **Table 2**. Both non-biased and targeted metabolomic profiling of the stool, blood and urine will be performed at the West Coast Metabolomics Center at the University of California Davis. Filtered fecal water or plasma samples will be used for the mass spectrometry-based metabolomic profiling. The process of metabolite extraction will include internal standards and follow standard protocol. The resulting extract will be divided into a liquid chromatography fraction and a gas chromatography fraction.

Liquid chromatography/Mass spectrometry (LC/MS): A 6590 Triple Quadrupole mass spectrometer (QQQ, Agilent Technologies, Santa Clara, CA) connected in the front end to Ultra Performance Liquid Chromatography will be used for Multiple Reaction Monitoring-based target metabolic quantification.1 Data acquisition in QQQ will be controlled using the Mass Hunter data acquisition software. Findings will be confirmed using selected reaction monitoring (SRM).

Gas chromatography/Mass spectroscopy (GC/MS): The derivatized sample will be injected into GC-MS, which is run using electron impact as the ionization source. Similar to the LC-based platform, detection of the metabolite would involve detection of its precursor followed by additional accurate mass detection of the product ions for the precursor by SRM. Each of the controls (standard mixture and liver pool) will be included multiple times in the randomization scheme such that sample preparation and analytical variability are constantly monitored. Furthermore, analysis of each clinical sample will be followed by at least two blank runs to prevent any carryover of metabolites between samples.

16S rRNA Sequencing: The composition of the gut microbiota will be characterized by sequencing 16S rRNA genes from genomic DNA extracted from the stool at the Alkek Center for Metagenomics and Microbiome Research at the Baylor College of Medicine. The bacterial genomic DNA from feces will be extracted using MO BIO PowerSoil DNA Isolation Kit (MO BIO Laboratories). DNA extraction and 16S rRNA gene sequencing will be performed with methods consistent with those developed for the NIH-Human Microbiome Project. Briefly, the microbiome will be measured by deep sequencing of 16S rRNA genes on the Illumina MiSeq platform. 16S rRNA genes contained in every sample will be isolated by amplifying multiple regions of the 16S rRNA genes with barcoded, degenerate primers that target the V4 hypervariable domain of the gene. These primers also contain adapters for MiSeq sequencing so that the PCR products may be pooled and sequenced directly. Pooling will enable us to achieve a depth of sequencing of at least 5,000 reads per sample. Rarefaction and collector's curves of microbial community data will be constructed using sequence data for each sample to ensure that we are sampling the majority of the diversity present.

Inflammatory markers will be measured in the plasma samples. The urine samples will be stored at -80^{O}C as frozen 1 ml aliquots for future biomarker and metabolomics studies as described above.

A purposeful approach to analyzing the stored blood and stool samples will be used. Initial measurements will be made on stool and blood samples obtained from a subset of the time points and selection of

additional time points for samples analysis will be made based on the findings. For example, if measurements made on samples obtained at weeks 0, 4 and 8 indicate that there is limited within-patient variability over an 8-week period, additional measurements using samples from weeks 1, 2, 3, 5, 6, and 7 will have low priority. However, if within-patient variability is evident using the samples from weeks 0, 4 and 8, performing measurements on the weekly samples, and/or the within-week samples will be of greater interest.

As noted above, CBC, CMP, cystatin C, phosphorous, magnesium, FGF-23, PTH and uric acid will be measured at Screening/Baseline, 8, 16, 20 and 28 weeks. Twenty four hour urinary excretion of urea, creatinine, uric acid, calcium, phosphorous and magnesium will be quantitated at 8, 20 and 28 weeks of the study.

Table 2. Stool, Urine and Blood Analytes

	Stool	Blood	Urine
Short-chain fatty acids (butyrate, propionate, acetate)	*	*	*
Trimethylamine N oxide	*	*	*
Choline	*	*	*
Betaine	*	*	*
Phenols			
p-cresol sulfate	*	*	*
p-Cresol glucuronide	+	+	+
Phenyl sulfate	+	+	+
Phenyl glucuronide	+	+	+
α-N-phenylacetyl-L-glutamine	+	+	+
Phenylpropionylglycine	+	+	+
Hippuric acid	+	+	+
4-hydroxybenzoate	+	+	+
Phenylacetylglycine	+	+	+
Indoles			
Indoxyl sulfate	*	*	*
Indoxyl glucuronide	+	+	+
5-hydroxyindole	+	+	+
Indole-3-prioonic acid	+	+	+
Indole-3-acetic acid	+	+	+
Polyamines	*	*	*
Metabolites of urea metabolism	+	+	+
Metabolites of creatinine metabolism	+	+	+
Allantoin	+	+	+
Fructose	+	+	+
Cytokines			
IL-1β		‡	
IL-2		‡	
IL-4		‡	
IL-6		‡	
IL-10		‡	
IL-17		‡	
IL-22		‡	
TNFα		‡	
Endotoxin		‡	
Myeloperoxdase (MPO)		‡	
hsCRP		‡	
HMGB1		‡	
TNF-R1		‡	
TNF-R2		‡	
Lipopolysaccharide binding protein (LBP)		‡	
sCD14		‡	

* captured by untargeted metabolomics and quantitated by targeted metabolomics
+ captured by untargeted metabolomics, ‡ standard ELISA

6 Study Agent

6.1 Description

p-inulin (oligofructose-enriched inulin) is a pre-biotic. p-inulin will be provided in individual packets that each contain 4 g of powder.

6.2 Treatment Regimen

All participants will take a p-inulin dose of 8 g two times per day for a total daily dose of 16g. The contents of two packets will be added by the participant to 100-200 ml of water and taken orally. Patients will be asked to take the medication in the morning before meals and at night before retiring to bed. Treatment duration is 12 weeks. If down-titration of the study agent dose is required during the treatment phase, the Clinical Center investigator will instruct the participant to use only 1 packet (4 g) twice a day for a total daily dose of 8 g per day.

6.3 Administration of Study Agent

Study agent will be prepared by the Investigational Drug Service (IDS) which will be the Central Pharmacy for this study. Each study agent kit will contain a 4-week supply. The participant will receive a 4-week supply of study agent at the Week 8, 12, and 16 visits.

6.4 Receiving, Storing, Dispensing and Returning Study Agent

6.4.1 Receiving Study Agent Supplies

Each Clinical Center research pharmacy will be responsible for maintaining detailed records regarding the receipt of study agent. General Study Product Accountability, Patient Specific Study Product Accountability, and if necessary, Shipment Tracking Accountability Logs will be maintained by the site pharmacist to document study agent use. Documentation includes study product receipt, storage, dispensing, and final disposition.

6.4.2 Storage

The boxes containing p-inulin will be stored at room temperature in a locked cabinet at the University of Pennsylvania IDS pharmacy until shipment to the Clinical Center. Once at the Clinical Center, the study agent will be stored at the research pharmacy until distribution to the participant.

The study agent will be stored at 25°C (77°F) with excursions permitted to 15-30°C (59-86°F).

6.4.3 Dispensing Study Agent

Upon enrollment of a participant, study personnel will request from the Central Pharmacy that three study agent kits are sent to the Clinical Center research pharmacy. The study agent will be dispensed by the Clinical Center research pharmacy in 4-week supplies at the Week 8, 12, and 16 visits to either an appropriate member of the research team or to the participant in accordance with Clinical Center policies and preferences.

6.4.4 Return or Destruction of Study Agent

Participants will return any unused study agent at the Week 12, 16, and 20 visits.

Returned product will be counted and recorded by study staff and documented on the Study Product Accountability log. Unused supplies will be destroyed by study staff once the reconciliation is completed and documented.

At the completion of the study, there will be a final reconciliation of study agent shipped, study agent consumed, and study agent remaining. This reconciliation will be logged on the study agent reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study agent. Study agent destroyed on site will be documented in the study files.

6.5 Participant Adherence Monitoring

6.6 Adherence to the study agent will be assessed by counts of returned packets at the Week 12, 16, and 20 visits and from participant self-report. Concomitant Therapy

Medication use (both oral and intravenous) will be collected at baseline and throughout the course of the study. Information about over the counter medications, herbal supplements, and pre/probiotic supplements in any form will also be collected. Appropriate sources for obtaining this information include the participant, the medical record, and treating clinicians.

Participants will be asked to discontinue probiotic yogurt at least 2 weeks prior to enrollment in the study and throughout study participation. Participants will continue their regular medications including anti-hypertensive agents, phosphate binders, vitamin D preparations, erythropoietin stimulating agents, proton pump inhibitors (PPI) and oral or intravenous iron as prescribed by the treating physician. However, PPI and iron therapy will not be initiated or discontinued during the study period without a compelling reason. Any such change will be carefully documented.

7 Statistical Plan

Bioinformatics data preparation and biostatistical analyses will be conducted by a technical team led by Professor Hongzhe Li, PhD at the University of Pennsylvania Perelman School Of Medicine. Dr. Li is internationally acclaimed in statistical genetics, genomics and the analysis of high-dimensional data, and has published path-breaking work in both methodological and collaborative aspects of the microbiome. http://statgene.med.upenn.edu/ .

7.1 Sample Size Determination

We will recruit total of 15 subjects and employ the recruit and replace strategy until a minimum of 10 participants have satisfactorily completed the first 20 weeks of the study protocol. “Sufficient” number of stool samples is defined as 2 samples during Weeks 1-4, 2 samples during Weeks 5-8, 3 samples during Weeks 9-14, and 3 samples during Weeks 15-20. Additional participants will be enrolled (up to 10 additional participants) as needed to obtain a total of 10 participants who meet the follow-up duration and sample sufficiency criteria. Participants who do not meet the sample sufficiency criteria will continue in the study and will continue to be encouraged to provide samples in accordance with the protocol schedule Because of the high prevalence of diabetes mellitus among patients with CKD and potential differences in

the gut microbiome between patients with and without diabetes, the study will aim to have approximately 50% of participants with diabetes and 50% of participants without diabetes.

This repeated measures study design will provide important preliminary data on week-to-week variability of gut microbiome and on the effects of p-inulin on microbiome composition. Referring to relevant simulations,[82] the power for detecting various changes in microbiome composition based on 10 participants, before and after p-inulin treatment, can be estimated. In addition to alpha- and beta-diversity, detailed investigations focusing on specific taxa is also valuable. This is achieved by determining the relative abundance of each taxa, and changes within taxa are then measured over time. However, the power calculations reported here are based on detecting the overall change of microbial composition before and after p-inulin treatment. Once such association is established, we will then perform analysis at the genus level to identify those genera that demonstrate change in their abundance.

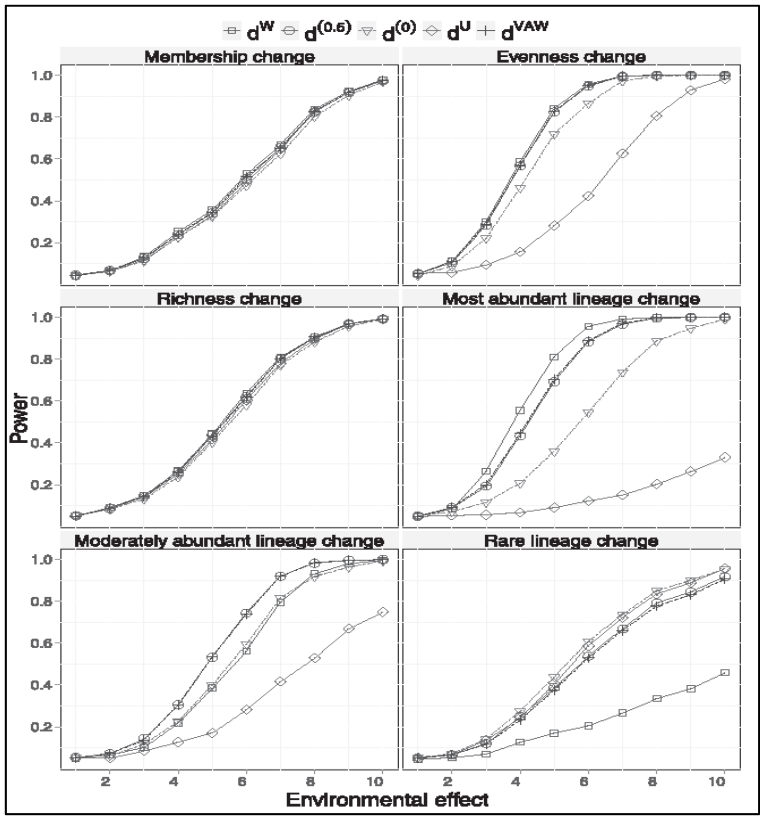


Figure 2. Power to Detect Composition Changes. The specific community difference caused by p-Inulin treatment is indicated in the panel title. The power curves are created by varying the degree of p-inulin effect.

d^U= unweighted UniFrac defined as the fraction of the branch length that leads to descendants from each single community.[83]
d^W= weighted UniFrac, weights each branch length by the abundance differences of the branch along the tree of the communities.[84]
$d^{(0)}$= relative difference in UniFrac.[85]
$d^{(0.5)}$= distance in the middle of the distance series.[85]
d^{VAW}= variance adjusted weighted UniFrac.[86]
d^{MAX}= combines d^W and d^U by taking the maximum of their pseudo-F statistics.[85]

Figure 2 shows the power for detecting changes in community membership, evenness, richness and lineages for various effect sizes based on distance-based MANOVA analysis using different distances based on a 2D circle simulation to detect changes in community membership, evenness, richness and certain clades of bacteria with different abundances, respectively. The number (richness) and distribution (evenness) of taxa expected within a single population are important estimates.[82] Alpha diversity captures both the organismal richness of a sample and the evenness of the organisms' abundance distribution. UniFrac can be used to test if the phylogenetic lineages between samples are significantly different, or to cluster samples using multivariate statistical techniques.[83] Several distances are used in these plots, where the generalized UniFrac distance seems to give better power in general. The proposed sample size of n=10 enables the detection of moderate to large effect sizes.

Figure 3 shows the power of change of taxa clusters of various abundances based on simulating data on a phylogenetic tree. For a sample size of 10, we have adequate power to detect moderate to large effect sizes. The plots in this Figure are based on several microbial distances. In general, we observed that the generalized UniFrac distances give better power.

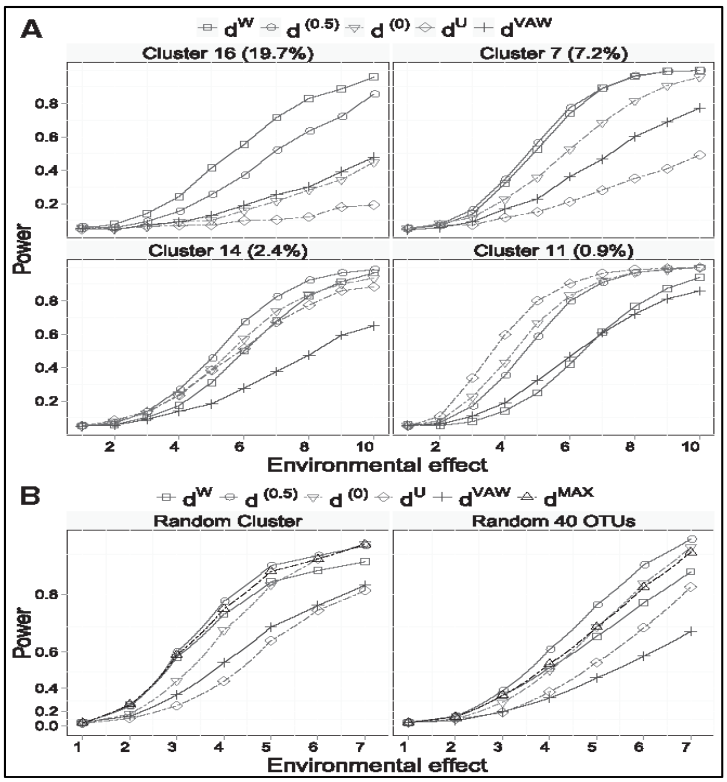


Figure 3. Power to Detect Changes of Taxa Clusters. Various distances (see Figure 2 legend) are used in PERMANOVA to detect change of microbial compositions, where the effect size is measured by fold-change of the abundances of specific bacterial clade.

We will use the paired t-test to identify the metabolites that change their abundances before and after the p-Inulin treatment. Based on a paired t-test, a sample size of 10 can detect an effect size of 1.7 or more of the change of metabolite due to p-inulin treatment, where effect size is the Cohen's d, defined as the difference between two means divided by a standard deviation for the data, assuming that 25 metabolites will be tested and that adjustment for multiple comparisons will be conducted within the framework outlined by Benjamini and Hochberg for controlling the false discovery rate (FDR).[83] The multicenter nature of the study will provide information about the feasibility of stool sample collection for future multicenter studies of the gut microbiome.

The repeated measurements of gut microbiome enable us to study the temporal variability of microbiomes after p-inulin treatment. Besides the paired comparisons, we will also perform analysis based on repeated measurements to identify the bacterial taxa that change their abundances over time. We expect high statistical power to detect these taxa when repeated measurement analysis is performed.

7.2 Missing Data

In general, missing data will not be imputed. Every effort will be made to use statistical methods that are robust to missingness, and the number of participants included with each analysis will be given with the results.

7.3 Statistical Methods

7.3.1 Analysis of Microbial Composition

Sequence data will be processed using QIIME, augmented by the R package QIIMER (http://cran.r-project.org/web/packages/qiimer). Taxonomy will be assigned to the sequences using Ribosomal Database Project (RDP) for 16S, augmented by analysis of specific sequences using BLAST. The 16S tag sequences will be collected into operational taxonomic units (OTUs) with 97% sequence identity and samples summarized as vectors of proportions. Stool samples will be compared longitudinally within individual subjects, and between groups. The analysis will be performed at the overall microbiome composition level by calculating the alpha and beta diversities using UniFrac distances. Principal coordinate analysis (PCA) based on the UniFrac distances will be performed and used to display the samples in PCA space. Alpha-diversity is used to describe the structure of the bacterial community based on the number of species, and the proportion in which each species is represented in the community. This can be defined using Shannon's index (H), Simpson's index (D) and Renyi entropy.[87] We then calculate the intra-class correlation coefficient using mixed models, with the individual as the cluster and multiple diversity observations as groups. We then assess the effects of p-inulin on gut microbiome compositions using repeated measures ANOVA with alpha-diversity as the outcome. This assesses whether p-Inulin has any effect on the overall gut microbial diversity. To measure inter-patient variability, we will calculate the beta diversity to measure the compositional dissimilarity between two individuals at a given time point. Some commonly used beta diversity indices include Bray-Curtis dissimilarity, percent similarity index and Jaccards index.[88] To account for the phylogenetic tree information of the bacterial taxa, we will use the UniFrac distances (both weighted, unweighted and generalized UniFrac) as the beta diversity measure.[83] We will then compare the within-individual distances and the between-individual distances and perform clustering analysis of all the samples using the UniFrac distances. A repeated measure distance-based MANOVA will be used to assess the changes of the overall microbial compositions over time and to test effect of p-inulin on overall microbial compositions using UniFrac distances.

Besides evaluating the intra-patient and inter-patient variability at the overall microbial compositional level, we will also evaluate such variability at the taxon level, e.g., at each genus level. From 16S data, we obtain the estimate of the relative proportion of each of the bacterial genera observed in the data. For each bacterial taxon, we will fit a zero enriched beta regression model with random effects to assess the intra-patient and inter-patient variability, both in terms of presence/absence and also the relative abundances. Similar analyses will be performed to compare the microbiome compositions between week 0 and study competition and between treatment stop and the end of study.

As a secondary analysis, we will also test microbiome differences between diabetic subjects and non-diabetic subjects prior to p-inulin treatment, taking into account week-to-week variability using mixed-effects model for alpha diversity, and zero-enriched regression analysis for each of the taxa. However, small sample sizes may limit the power to detect such differences.

7.3.2 Analysis of metabolomic profile and targeted metabolites

For each of the metabolites, we will apply standard mixed-effects models to assess the intra- and inter-individual variability. To account for possible heterogeneity in the intra-individual variance structure, we will fit nonlinear random effect model.[89] Standard repeated measures ANOVA and mixed-effect models will be applied to study change of metabolites before and after p-inulin treatment and to identify the metabolites that change after p-inulin treatment. Bonferonni adjustments will be used to adjust for multiple comparisons.

7.3.3 Association between microbiome composition and metabolites

We will explore association between microbiome and metabolites by correlation analysis and clustering analysis, focusing on the metabolites and microbial taxa that change over the study periods. We will fit repeated measures regression analysis of metabolite with time-varying covariates (microbiome diversity or microbial genera) to identify the microbial genera that are associated with changes of metabolites due to p-inulin treatment. Due to limited sample sizes, our analysis will focus on well-characterized metabolites and those that show large change in abundance over time during the p-inulin treatment.

7.3.4 Analysis of Safety

Standard descriptive statistics will be used to summarize adverse events during the three phases (pre-treatment, p-inulin treatment, and post-treatment) and graphical methods including stem-and-leaf diagrams will be used to examine distributions of adverse events. Frequencies will be compared between the treatment and non-treatment phases for the total number of events, number of participants experiencing events, events within body system categories (e.g., gastrointestinal system events), and events within severity categories.

8 Safety and Adverse Events

8.1 Definitions

Definitions are per the January 2007 Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Participants or Others and Adverse Events, Office on Human Research Protection (OHRP) Guidance. http://www.hhs.gov/ohrp/policy/advevntguid.html

8.1.1 Adverse Event

An *adverse event (AE)* is any untoward or unfavorable medical occurrence in a human study participant, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the participant's involvement in the research, whether or not considered related to the participant's participation in the research.

8.1.2 Serious Adverse Event

A *serious* adverse *event* (SAE*)* is any AE that is:

- fatal or results in death
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- results in congenital anomalies or birth defects
- an important medical event*

*Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance.

8.1.3 Unanticipated Problems Involving Risk to Participants or Others

An Unanticipated Problem is any incident, experience, or outcome that meets **all** of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given the research procedures that are described in the IRB-approved research protocol and informed consent document and the characteristics of the participant population being studied;
- Related or possibly related to participation in the research; possibly related means that there is a reasonable possibility that the incident, experience or outcome may have been caused by the procedures involved in the research; and
- Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) related to the research than was previously known or recognized.

8.1.4 Pre-Existing Condition

A preexisting condition is one that is present at the start of the study. A preexisting condition will be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

8.2 Adverse Event Reporting Period

The study period during which adverse events must be tracked and reported is defined as the period from the initiation of study procedures to study completion.

8.2.1 Post-study Adverse Event

All unresolved adverse events will be followed by the investigator until the events are resolved, the participant is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator will instruct each participant to report any subsequent event(s) that the participant, or the participant's personal physician, believes might reasonably be related to participation in this study. The investigator will notify the DCC of any death or adverse event occurring at any time after a participant has discontinued or terminated study participation that may reasonably be related to the study.

8.3 Recording of Adverse Events

At each contact with the participant, the investigator or site designee will seek information on adverse events by specific questioning and, as appropriate, by examination. Information on adverse events will be recorded in the source document, and also on the adverse event log case report form (CRF). All signs, symptoms, and abnormal diagnostic procedure results relating to the same event will be recorded under one diagnosis name.

8.3.1 Anticipated Adverse Events

The following adverse events are anticipated in the CKD population and will not be considered to be Unanticipated Problems. Note that the designation as "Anticipated" does not imply that the event is not an SAE but relates to the regulatory definition of Unanticipated Problems as provided in Section 8.1.3.

- Death
- Coronary Ischemia including:
    - Unstable angina
    - Acute MI

  - Coronary revascularization
- Heart failure hospitalization or exacerbation
- Cardiac arrest
- Cardiac arrhythmia (ventricular or atrial)
- Peripheral vascular revascularization
- Amputation
- Development of ESRD, initiation of dialysis
  - Surgery for placement of dialysis access
- Infections Including:
  - Pneumonia
  - Septicemia
  - Clostridium difficile infection
  - Diabetic foot infections in patients with diabetes

Non-Reportable Events

The CKD population is characterized by number of mineral and electrolyte abnormalities, changes in medications and fluctuation in volume status/blood pressure. Due to the unique nature of this population, the following events will not be considered to meet the criteria of SAE in this study except as noted:

- Anemia—will be reported only when hemoglobin <9.0 mg/dL
- Hyperphosphatemia-will be reported only when phosphorous >7.0 mg/dL
- Hypocalcemia-will be reported only when corrected serum calcium < 7.0 mg/dL
- Hypercalcemia-will be reported only when serum calcium >11.0 mg/dL
- Hyperparathyroidism-will be reported only when PTH>700 pg/mL
- Hypotension-will be reported only when requiring emergency room visit or hospitalization

Note that phosphorus, calcium, PTH, and blood pressure are not measured during the course of the study.

8.4 Reporting of Serious Adverse Events and Unanticipated Problems

Study sites are required to report SAEs to the DCC within 24 hours of first knowledge of the event. To report such events, an SAE form will be completed by the investigator and faxed to the DCC. The DCC will facilitate the timely medical review and reporting of the event and updates to the NIDDK, the DSMB, and the FDA in accordance with DSMB-approved study policies, regulatory requirements, and standard MedWatch guidelines.

The investigator will keep a copy of the SAE form on file at the study site. At the time of the initial report, the following information should be provided:

- Study identifier
- Study Center
- Participant number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

Within the following 7 days, the investigator will provide further information on the SAE or the unanticipated problem in the form of a written narrative. This should include a copy of the completed SAE form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing serious adverse events should be provided promptly to the DCC.

If a patient becomes pregnant while participating in the trial it will be reported as an adverse event and will trigger the collection of additional documentation about the pregnancy.

SAEs that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any SAE that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

8.4.1 Investigator Reporting to the IRB

Site investigators will report SAEs and Unanticipated problems to their IRB in accordance with the reporting requirements of the local IRB or with the Office of Human Research Protections (OHRP) guidelines, whichever is sooner. OHRP recommends that:

1) Unanticipated problems that are serious adverse events should be reported to the IRB within 1 week of the investigator becoming aware of the event; and

2) Any other unanticipated problem should be reported to the IRB within 2 weeks of the investigator becoming aware of the problem.

Reporting Process

Unanticipated problems posing risks to participants or others as noted above will be reported using the appropriate IRB-designated form or as a written report of the event (including a description of the event with information regarding its fulfillment of the above criteria, follow-up/resolution and need for revision to consent form and/or other study documentation).

Copies of each report and documentation of IRB notification and receipt will be maintained in the Clinical Center Investigator's study file.

Other Reportable events:

- Any adverse event that would cause the sponsor to modify the protocol or informed consent form, or would prompt other action by the IRB to assure protection of human participants.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency.
- Change in FDA safety labeling or withdrawal from marketing of a drug used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the participant to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.

- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of participants.

8.4.2 DCC Notification to Participating Investigators

The DCC will notify all Clinical Center principal investigators, in a written safety report, of any adverse event that meets the criteria of an unanticipated and related event as described in **Section 8.1.3**.

8.5 Medical Monitoring

Each Clinical Center Principal Investigator will be responsible for overseeing the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan. Medical monitoring will include a regular assessment of the number and type of serious adverse events.

8.5.1 Independent Data and Safety Monitoring Board (DSMB)

The information provided in this section of the protocol is a general description of the DSMB responsibilities and processes. A DSMB charter for the Pilot Clinical Trials in CKD Consortium will be prepared and will include additional detail. An example of an NIDDK DSMB charter is provided as an attachment in **Section 15.**

A DSMB has been established by the NIDDK and provides input to the Institute. The DSMB is comprised of individuals with expertise in clinical trials design and methodology, biostatistics, clinical nephrology and other relevant medical specialties. The DSMB members are not affiliated with the study and are appointed by the NIDDK. DSMB members will be free of conflicts of interest that could be affected by the outcomes of the study. During the study, DSMB members who develop real or perceived conflicts of interest that impact objectivity will disclose them to NIDDK project officers, who will arrange for replacement of the member, if indicated.

The DSMB will review the protocol prior to initiation of the study. After initial approval during the course of the study, the primary responsibilities of the DSMB will be to:

- Review safety data and provide input to protect the safety of the study participants;
- Provide input on major changes to the research protocol and plans for data and safety monitoring;
- Provide input on the progress of the study, including periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of the study sites, and other factors that may affect study outcomes;
- Consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the need for continuation of the study, safety of the participants or the ethics of the study;
- Provide input on modification of the study protocol or possible early termination of the study because of attainment of study objectives, safety concerns, or inadequate performance (such as enrollment and retention problems).

9 Data management

An internet-based registration system designed by the DCC will be used for all of the pilot and feasibility studies of the Pilot Clinical Trials in CKD Consortium in order to promote uniformity across studies. The central registration system will include a randomization module for each study that will confirm eligibility. Central participant registration will also allow the DCC to generate recruitment reports across concurrent studies.

An Oracle Clinical data management system (DMS) designed by the DCC will be used for the collection, storage and management of data. Site personnel will enter data directly using Oracle Clinical Remote Data Capture. Electronic case report forms (eCRFs) will incorporate range and logical edit checks, both within and across forms. Data entry will be followed daily with manual and programmed checks and edits for errors and omissions. An Oracle Clinical data management system designed by the DCC will be used for the collection, storage and management of data. Site personnel will enter data directly using Oracle Clinical Remote Data Capture. Electronic case report forms (eCRFs) will incorporate range and logical edit checks, both within and across forms. Data entry will be followed daily with manual and programmed checks and edits for errors and omissions.

9.1 Data Quality

The DCC will collaborate with the Clinical Center investigators to establish parameters for primary and secondary outcomes, safety, and descriptive values. The data management team will use a data validation plan, rule set specifications, and programming logic to implement data validation rules. The DCC staff will interact with Clinical Center study staff to verify queried data and track all queries to resolution.

9.1.1 Quality Control Activities

The Quality Control Committee and the DCC will develop a quality assurance and control plan that ensures that study data are as precise and reliable as possible.

Manual of Procedures (MOP) – The MOP will describe the sequence of study conduct and provide detailed instruction for the performance of screening, baseline, enrollment, treatment allocation and follow-up procedures. The MOP will provide instruction in case report form completion, use of the electronic data management system, and collection, documentation and transfer of specimens and tests to laboratories and reading centers.

Training and certification procedures – The DCC will conduct a training session before the study starts to train and certify personnel in the performance of study procedures.

Site visits – Site visits will be conducted as outlined in the Study Monitoring Plan. Findings from site visits will be used to resolve problems and develop corrective action plans.

External data sources – The DCC will monitor quality control of data received from study laboratories and reading centers.

Internal quality control procedures – A data validation plan, rule set specifications, and programming logic to implement data validation rules will be implemented.

9.1.2 Routine reports

The DCC will develop a set of standard enrollment, tracking, quality review, and safety monitoring reports. Adverse event reports, DSMB reports and reports for statistical analysis will be developed and produced on an appropriate schedule.

9.2 Data Security

The data management system will be designed to prevent unauthorized access to trial data and to prevent data loss due to equipment failure or catastrophic events. The procedures to do so encompass user account management, user privilege assignment, data loss prevention (database backup), and DMS change management. User access will be controlled by assignment of confidential usernames and passwords.

Study data collected at the Clinical Centers will be entered into Oracle Clinical. This data management system uses a secure connection between the client browser at the Clinical Center and the web server at the DCC. Data transmitted over this connection is authenticated by the use of digital certificates and is encrypted as it travels the Internet to the DCC.

Where applicable, electronic files containing data from hand held devices, central laboratories, or central reading centers will be transferred to the DCC using secure FTP technology. The DCC team will maintain a secure FTP server. The files transmitted using this method will be encrypted during the exchange.

9.2.1 Confidentiality

Information about study participants will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed participant authorization informing the participant of the following:

- What protected health information (PHI) will be collected from participants in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research participant to revoke their authorization for use of their PHI.

In the event that a participant revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of participant authorization. For participants that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the participant is alive) at the end of their scheduled study period.

9.3 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: outpatient records, hospital records, clinical and office charts, laboratory reports, memoranda, participant diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, participant files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3.1 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF will be recorded. All missing data will be explained. "N/D" will be used to indicate on the CRF that a procedure was not done or a question was not asked rather than leaving a space blank. "N/A" will be used to indicate that an item is not applicable to the individual case. All entries will be printed legibly in black ink. If any entry error has been made, to correct such an error, a single straight line will be drawn through the incorrect entry and the correct data will be entered above it. All such changes will be initialed and dated. Erasing or white-out will not be used for errors. For clarification of illegible or uncertain entries, the clarification will be printed above the item, and the clarification will be initialed and dated.

9.3.2 Maintaining Anonymity of Submitted Medical Records

Clinical site personnel will de-identify all medical records before sending them to the DCC by obliterating any Protected Health Information (PHI). Upon receipt, DCC personnel will review the records to ensure that no PHI is visible.

9.3.3 Data Sharing

Research results will be made available to the scientific community and public in a timely manner. The primary method by which data will be shared with the scientific community will be through peer-reviewed publications and presentation at scientific and professional society meetings. In addition, data and results will be submitted to the NIH in the annual progress reports required under the terms and conditions of the funding award. This study will also be registered with clinicaltrials.gov prior to initiation.

Data from the study will be submitted to the NIDDK Data Repository in accordance with the NIDDK Data Sharing policy. The policy requires that data sets be transferred no later than 2 years after study completion or 1 year after publication of the primary results, whichever comes first. Through the repository, the study data will be made available to external investigators.

9.3.4 Records Retention

The site investigators will retain study documents, including participant files and Regulatory Binders, for at least 5 years after the close of the study, or longer depending on site institutional requirements.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

A monitoring plan that includes formal visits to the Clinical Centers by members of the Consortium (DCC, Clinical Center investigators and study coordinators, and NIDDK representatives) will be developed by the Consortium Executive Committee. Clinical Center investigators will allocate adequate time for such monitoring activities. The Principal Investigator will also ensure that the monitor and other compliance or quality assurance reviewers are given access to study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and have adequate space to conduct the monitoring visit.

10.2 Auditing and Inspecting

The DCC and Clinical Center investigators will permit study-related monitoring, audits, and inspections by the EC/IRB, the NIH, government regulatory bodies, and University compliance and quality assurance groups

of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The DCC and Clinical Center investigators will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11 Ethical Considerations

This study will be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the Clinical Center investigator and a copy of this decision will be provided to the sponsor before commencement of the study at the site.

All study participants will be provided a consent form describing the study and providing sufficient information to make an informed decision about participating in the study. The consent form will be submitted with the protocol for review and approval by the EC/IRB. The formal consent of a participant, using the EC/IRB-approved consent form, must be obtained before that participant undergoes any study procedure. The consent form must be signed by the participant or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

12 Study Finances

12.1 Funding Source

This study is financed through grants from the National Institute of Diabetes and Digestive and Kidney Diseases of the U.S. National Institutes of Health.

12.2 Conflict of Interest

All investigators will follow the conflict of interest policies of the National Institutes of Health as well as their home institution. Any investigator who has a potential conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study.

12.3 Participant Stipends or Payments

Participants will be compensated for participating in the study. Compensation approaches will be determined by the Clinical Centers and approved by the local EC/IRB.

13 Publication Plan

Neither the complete, nor any part of, the results of the study carried out under this protocol, nor any of the information provided by the Pilot Clinical Trials in CKD Consortium for the purposes of performing the study, will be published or passed on to any third party without the consent of the Consortium Executive Committee. Any investigator involved with this study is obligated to provide the Data Coordinating Center with complete test results and all data derived from the study.

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15 Attachments

15.1 Study Procedures

	SCREENING		Observation with no treatment						TREATMENT PHASE WEEKS 9-20									Observation with no treatment				
Procedure	Pre screening	Screening/ Baseline†	Week 1	Weeks 2-3	Week 4	Weeks 5-6	Week 7	Week 8	Week 9	Weeks 10-11	Week 12	Weeks 13-15	Week 16	Week 17	Week 18	Week 19	Week 20	Weeks 21-23	Week 24	Weeks 25-26	Week 27	Week 28
Preliminary eligibility assess	X																					
Informed consent		X																				
Confirm eligibility		X	X																			
Demographic/ medical hx		X																				
Concomitant medications		X	X		X			X			X		X				X		X			X
Urine pregnancy test		X																				
Blood specimen collection			*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X
Stool sample collection			*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X
Urine sample collection			*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X	*X
24 hour urine collection								X									X					X
CBC (local measure)		X						X					X				X					X
CMP (central measure)		X						X					X				X					X
Cystatin C, phosphorus, magnesium, FGF23, PTH, uric acid (central measure)		X						X					X				X					X
Antibiotic use review (wkly)		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Hemoglobin review		X							X									X				
GI symptom assessment			X		X			X			X		X				X		X			X
Food frequency questionnaire			X					X									X					X
Review adverse events					X			X			X		X				X		X			X
Dispense study medication								X			X		X									
Reconcile study medication											X		X				X					
Dose adjustment (prn)									X													

†Screening and baseline activities can be performed at two separate visits or as one visit *Collect 1 time per week

15.2 Example of an NIDDK DSMB Charter

Data and Safety Monitoring Board (DSMB) Charter
[CKD Pilot Trials Consortium]

The Data and Safety Monitoring Board (DSMB) will act in an advisory capacity to the National Institute of Diabetes, Digestive and Kidney Diseases (NIDDK) to monitor patient safety and evaluate the efficacy of the interventions. Pilot Clinical Trials in CKD Consortium – Gut Microbiome and p-Inulin in CKD study is funded by the NIDDK.

DSMB RESPONSIBILITIES
The initial responsibility of the DSMB will be to review the study protocols, consent documents and plans for data safety monitoring, and approve the initiation of these clinical trials. After this approval, and at periodic intervals during the course of the trials, the DSMB responsibilities are to:

- review and approve major changes in the research protocol, informed consent documents and plans for data safety and monitoring, including all proposed revisions;
- evaluate the progress of the trial, including periodic assessments of data quality and timeliness, participant recruitment, accrual and retention, participant risk versus benefit, performance of the trial sites, and other factors that may affect study outcome;
- consider factors external to the study when relevant information becomes available, such as scientific or therapeutic developments that may have an impact on the safety of the participants or the ethics of the trial;
- protect the safety of the study participants;
- report on the safety and progress of the trial;
- make recommendations to the NIDDK, the Steering Committee and, if required, to the Food and Drug Administration (FDA) and the Institution Review Boards (IRBs) concerning continuation, termination or other modifications of the trial based on the observed beneficial or adverse effects of the treatment under study;
- if appropriate, conduct interim analysis of efficacy in accordance with stopping rules which are clearly defined in advance of data analysis and have the approval of the DSMB;
- ensure the confidentiality of the trial data and the results of monitoring;
- assist the NIDDK by commenting on any problems related to study conduct, enrollment, sample size, and/or data collection.

MEMBERSHIP

The DSMB consists of ten members. Six participating members will constitute a quorum. The members have been appointed by the NIDDK. Members of the DSMB shall have no financial, scientific, or other conflict of interest with the studies. Collaborators or associates of the investigators in this trial are not eligible to serve on the DSMB. Written documentation attesting to absence of conflict of interest is required.

Dr. David Warnock, MD of University of Alabama School of Medicine has been selected by the NIDDK to serve as the DSMB Chairperson for the remainder of the study. He is responsible for overseeing the meetings and developing the agenda in consultation with the NIDDK Program Directors, Dr. Michael Flessner and Dr. John Kusek. Dr. Flessner will serve as the DSMB Executive Secretary. The Chairperson is the contact person for the DSMB. Other NIDDK official (s) or NIDDK appointee (s) may serve as an ex-officio member (s)

of the DSMB. The DCC, at the Cleveland Clinic Foundation, shall provide the logistical management for the DSMB, in coordination with NIDDK (Dr. Yining Xie, as point of contact). Whenever possible, Dr. Robert Star, Director of the Division of Kidney, Urology and Hematology of NIDDK will also attend meetings.

BOARD PROCESS

The DSMB will meet a minimum of once a year at the call of the Chair, with advance approval of the NIDDK Program Director. An NIDDK representative will be present at every meeting.

Meetings shall be closed to the public because discussions may address confidential patient data. Meetings are attended, when appropriate, by the principal investigator and members of his/her staff. Meetings may be convened as conference calls/webinars as well as in person. An emergency meeting of the DSMB may be called at any time by the Chairperson or by the NIDDK Program Director should questions of patient safety arise. The DSMB Chairperson should contact the NIDDK Program Director prior to convening the meeting.

MEETING FORMAT

An appropriate format for DSMB meetings consists of an open, closed (if the DSMB is monitoring a study in which the investigators are masked in any way), and executive session. This format may be modified as needed.

Open Session

Members of the DSMB, the principal investigator, NIDDK representatives, and members of the steering committee, including the study biostatistician may attend the open session. Issues discussed will include the conduct and progress of the study, including patient recruitment, data quality, general adherence and toxicity issues, compliance with protocol, and any other logistical matters that may affect either the conduct or outcome of the study. Proposed protocol amendments will also be presented in this session. Patient-specific data and treatment group data may not be presented in the open session.

Closed Session

The closed session will be attended by voting DSMB members, representatives from the NIDDK, or its appointees, and the study biostatistician. **The discussion at the closed session is completely confidential.**

Analyses of outcome data are reviewed by masked intervention groups, including baseline characteristics, primary and secondary outcomes, adverse events, adherence and dropouts, and examination of any relevant subgroups. However, the DSMB may request unmasking of the data for either safety or efficacy concerns.

Executive Session

The executive session will be attended by DSMB members and NIDDK representatives only, who will discuss the information presented during the closed and open sessions and provide input on the continuation or termination of the study, protocol modification or other changes to the conduct of the study. The DSMB can be unmasked at any time if trends develop either for benefit or harm to the participants.

The DSMB will make a recommendation for either continuation or termination of the study. Termination may be suggested by the DSMB at any time. Reasons for early termination include:

- Serious adverse effects in entire intervention group or in a dominating subgroup;
- Greater than expected beneficial effects;
- A statistically significant difference by the end of the study is improbable;
- Logistical or data quality problems so severe that correction is not feasible. Sound rationale for either decision (continuation or termination of the study) should be presented.

REPORTS TO THE DSMB

Reports will be prepared by the unmasked biostatistician on a quarterly or semi-annual basis as decided by the investigator(s) and the DSMB. The reports will be distributed to the DSMB at least 10 days prior to a scheduled meeting. These reports shall be provided in sealed envelopes within an express mailing package, by secure email, or by access to a secure website, as the DSMB prefers.

Data reports for randomized clinical studies or any study in which the investigators are masked generally consist of two parts: an Open Report and a Closed Report.

Open Session Report:

The executive session will be attended by voting DSMB members, and the NIDDK Staff, or its appointees.
The DSMB will discuss information presented to it during the closed and open sessions and decide whether to recommend continuation or termination, protocol modification or other changes to the conduct of the study in the Executive Session.

Should the DSMB decide to issue a termination recommendation, a full vote of the DSMB will be required. In the event of a split vote, majority vote will rule and a minority report should be appended. Reasons for early termination may include:

- Serious adverse effects in the entire intervention group or in a dominating subgroup;
- Greater than expected beneficial effects;
- A statistically significant difference by the end of the study is improbable;
- Logistical or data quality problems so severe that correction is not feasible.
- Comparison of Target Enrollment to Actual Enrollment by Month;
- Comparison of Target Enrollment to Actual Enrollment by Site;
- Overall Subject Status by Site, including: Subjects Screened, Enrolled, Active, Completed and Terminated;
- Demographic and Key Baseline Characteristics by Group;
- Treatment Duration for Subjects who Discontinue Therapy;
- Adverse Events/Serious Adverse Events by Site and Subject.

Final Open Session (optional):

The final session may be attended by voting DSMB members, steering committee members, the study biostatistician or other study members, and the NIDDK staff. The Chairperson of the DSMB or the NIDDK Staff shall report on the recommendations of the DSMB regarding study continuation and concerns regarding the conduct of the study. Requests regarding data presentation for subsequent meetings will be made. Scheduling of the next DSMB meeting may be discussed.

REPORTS

Interim Reports

Interim reports will be prepared by the Data Coordinating Center, located at the Cleveland Clinic. The reports will be distributed to the DSMB and the NIDDK Program Director at least 7 days prior to a scheduled meeting. These interim reports are numbered and provided in sealed envelopes within an express mailing package or by secure email as the DSMB prefers. The contents of the report are determined by the DSMB. Additions and other modifications to these reports may be directed by the DSMB on a one-time or continuing basis. Interim data reports generally consist of two parts:

Part 1 (**Open Session Report**) provides information on study aspects such as accrual, baseline characteristics, and other general information on study status. This report is generally shared with all investigators involved with the clinical trial. The reports contained in this section may include:

- Comparison of Target Enrollment to Actual Enrollment by Month
- Comparison of Target Enrollment to Actual Enrollment by Site
- Overall Subject Status by Site, including: Subjects Screened, Enrolled, Active, Completed and Terminated
- Demographic and Key Baseline Characteristics by Group
- Treatment Duration for Subjects who Discontinue Therapy
- Adverse Events/Serious Adverse Events by Site and Subject

Part 2 (**Closed Session Report**) may contain data on study outcomes, including safety data, including serious adverse events or termination. Data will be presented by masked treatment groups; however, the DSMB may request that the treatment groups be unmasked to ensure that there are no untoward treatment effects. This report should not be viewed by any members of the clinical trial except the designated study statistician.

Reports from the DSMB
A formal report containing the recommendations for continuation or modifications of the study, prepared by the Executive Secretary with concurrence of the DSMB, will be sent to the Chair of the Steering Committee and the DCC PI. This report will also contain any recommendations of the NIDDK in reference to the DSMB recommendations. It is the responsibility of the DCC PI to distribute this report to all other PIs and to assure that copies are submitted to all the IRBs associated with the study.

Each report should conclude with a recommendation to continue or to terminate the study. This recommendation should be made by formal majority vote. A termination recommendation may be made by the DSMB at any time by majority vote. The NIDDK is responsible for notifying the Chair of the Steering Committee of a decision to terminate the study. In the event of a split vote in favor of continuation, a minority report should be contained within the regular DSMB report. The report should not include unblinded data, discussion of the unblinded data, or any other confidential data.

Mailings to the DSMB
On a scheduled basis, (as agreed upon by the DSMB) blinded safety data should be communicated to all DSMB members and the NIDDK Program Director. Any concerns noted by the DSMB should be brought to the attention of the NIDDK Program Director.

Access to Interim Data
Access to the accumulating outcome data should be limited to as small a group as possible. Limiting the access to interim data to the DSMB members relieves the investigator of the burden of deciding whether it is ethical to continue to randomize patients and helps protect the study from bias in patient entry and/or evaluation.

CONFIDENTIALITY
All materials, discussions and proceedings of the DSMB are completely confidential. Members and other participants in DSMB meetings are expected to maintain confidentiality.