THE EFFECTS OF ADDED FIBER TO THE DIETS OF CHRONIC KIDNEY DISEASE PATIENTS ON QUALITY OF LIFE, CLINICAL MARKERS AND, GASTROINTESTINAL AND KIDNEY FUNCTION

By

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To my wife, Maryam, my son, Zaid, my daughter, Jenna, and my mother and family
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THE EFFECTS OF ADDED FIBER TO THE DIETS OF CHRONIC KIDNEY DISEASE PATIENTS ON QUALITY OF LIFE, CLINICAL MARKERS AND, GASTROINTESTINAL AND KIDNEY FUNCTION

By

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Chair: Wendy Dahl
Major: Food Science and Human Nutrition

Chronic kidney disease (CKD) patients may consume lower than recommended amounts of dietary fiber due to typical dietary habits, dietary restrictions, and uremic symptoms. Progressive decline in kidney function causes an accumulation of uremic molecules that contribute to further progression of the disease and reduced quality of life. The objective was to conduct clinical trials to determine the effects of added fiber in the diet of CKD patients on uremic molecules, kidney function, uremia, and quality of life.

Two single blind, intervention clinical trials with patients with moderate to severe decline in kidney function (eGFR of ≤ 50 mL/min/1.73 m²) were conducted. The first study was 6 weeks in duration; 2 weeks of control and 4 weeks intervention with 23 g/d of mixed fiber sources. The second study was 12 weeks in duration; 2 weeks control, followed by 4 weeks intervention with 10 g/d of pea hull fiber, followed by 6 weeks of an additional 13.5 g/d of fiber from inulin.

Study 1: Provision of 23 g/d of added fiber lowered serum creatinine from 2.44±0.30 mg/dL (mean±SE) at baseline to 2.21±0.26 mg/dL after 4 weeks of
intervention (p<0.05). Blood urea nitrogen (BUN) decreased from 41.9±5.9 during control to 33.5±4.9 mg/dL after 4 weeks of fiber intervention (p<0.05). The decline in serum creatinine corresponded with an increase in eGFR\textsubscript{MDRD} from 29.6±3.5 mL/min/1.73m\textsuperscript{2} at baseline to 32.5±3.6 mL/min/1.73m\textsuperscript{2} after 4 weeks of intervention (p<0.05). Functional Physical Health Related Quality of Life increased from 30±2 at baseline to 35±3 (p<0.05) post intervention. Study 2: Foods fortified with 10 g/d of pea hull fiber did not improve uremic profile or eGFR\textsubscript{(creatinine-cystatin C)}. Inulin supplement at 3 weeks improved eGFR (47.6±5.6 mL/min/1.73 m\textsuperscript{2}) compared to control period (42.4±5 mL/min/1.73 m\textsuperscript{2}), but not baseline (43.9±4.8 mL/min/1.73 m\textsuperscript{2}). However, this improvement diminished after 6 weeks of inulin supplementation when supplement compliance dropped from 90% to 77%. Plasma \textit{p}-cresol decreased by 20% from baseline at study end.

Supplementing the diet of CKD patients with insoluble and fermentable fibers may reduce uremic molecules and thus improve renal function and improve some aspects of quality of life.
CHAPTER 1
INTRODUCTION

Dietary fiber intakes in the US fall short of the recommendations. Moreover, chronic kidney disease (CKD) patients may be more likely to consume lesser amounts of dietary fiber in the diet due to typical dietary habits, dietary restrictions, and symptoms. Reduced kidney function leads to accumulation of nitrogenous waste and uremic molecules that can alter normal physiological state and thus introduce uremic symptoms in this population. Uremic molecules originate from exogenous sources and/or are generated endogenously through various metabolic and bacterial processes. Uremic syndrome or uremia is a broad term to describe symptoms associated with the disease but “cannot be explained by derangements in extracellular volume, inorganic ion concentrations or known renal synthetic products.” (1) It commonly develops with the later stages of CKD and is believed to be due to accumulation of uremic molecules above physiological tolerance.

Uremic molecules such as urea and creatinine are excreted primarily via the urinary route. However, kidney disease causes partial or near complete loss of the ability to excrete these uremic molecules. Thus, the loss of filtration capacity increases the uremic burden caused by the accumulation of these molecules in the blood. In a study where kidney patients were supplemented with mixed fiber (insoluble and fermentable), nitrogen excretion was shifted away from the urinary route toward the fecal route (2). The increase in fecal nitrogen and decrease in urinary nitrogen was coupled by a significant decline in blood urea nitrogen (BUN), suggesting an approach to reduce uremic molecules through fiber intervention and reducing the filtration burden on the remaining nephrons. By lowering uremic molecules, potential improvements in
symptoms and thus, quality of life can be speculated. A similar approach to lower another uremic molecule by using fiber was carried out in both healthy and hemodialysis (HD) patients. In a study of healthy volunteers, decreased, urinary $\mu$-cresol excretion was observed by ingestion of a 50/50 v/v mixture of inulin and fructooligosaccharides (FOS) (3). A 4-week trial in HD patients using a total of 20 g/d of the inulin and FOS confirmed that $\mu$-cresol generation and $\mu$-cresol sulfate concentrations were lowered in these patients (4).

Dietary therapy that may provide means to reduce urea, phenols and potentially other molecules may be effective to reduce overall uremic load which may impact GFR by reducing the need for the remaining nephrons to hyperfiltrate, a process in which nephrons adapt higher filtration to compensate for the loss of nephrons mass. Several uremic molecules are toxic and can induce oxidative stress and inflammatory response in various parts of the nephron. Uremic toxic molecules propagates disease progression by inducing fibrosis related genes and oxidative stress causing tubulointerstitial fibrosis and glomerular sclerosis (4-13). Dietary intervention that can reduce the generation, uptake, and improve excretion of these molecules may provide additional protective benefits to preserve the remaining nephrons and slow their loss.

In addition, lowering of uremic molecules may contribute to improved health and wellbeing by reducing uremic symptoms that are typically caused by increased uremic molecules concentration in the blood due to declining clearance capacity. Supplementing the diet of CKD patients with mixed sources of fiber is potentially a safe and practical way to increase fiber content in the diet while providing the various benefits attributed to the various types of fibers. An increase of insoluble fiber may
contribute to improvement in bulking and bowel frequency, while increasing intake of soluble fermentable fibers may enhance utilization of carbohydrates as a source of energy while depressing protein putrefaction in the large gut. Enhancing the role of the large intestine to capture and eliminate uremic molecules may provide a viable approach to reducing uremic load, which may impact kidney health and function. Ultimately such impacts on uremic molecules generation and elimination may result in improved uremic symptoms and quality of life.

Increasing fiber intake is associated with many health benefits and is shown to have many protective effects. There are a limited number of studies investigating the effect of fiber on uremic molecules and eGFR. These studies focused on late stage CKD and dialysis patients. Omission of stage 3 patients may have hindered the potential effect that fiber may play in altering uremic load, serum creatinine, and health related quality of life. It is likely that some benefits maybe more measurable at more advanced stage of the disease, but it also likely that other factors are less affected with such decline in kidney function, making any benefits from dietary therapy more subtle. Additionally, studies with fiber as nutritional therapy to impact uremic molecules in late stage CKD and HD patients have not investigated health-related quality of life and uremic symptoms. The potential of a nutritional intervention to restore fiber in the diet of renal patients, to maintain healthy gut function and help decrease uremic load, has great merits to the area of applied nutrition and dietetics. A study to determine the effect of early dietary intervention in the course of the disease and the impact on various parameters including symptoms and quality of life is essential to the area of medical nutrition therapy.
The major aim of this research was to determine the impact of increased fiber intake on uremic molecules, kidney function, quality of life and symptoms in CKD patients who have experienced over 50% loss of their kidney function. While previous studies focused on maximizing fiber intervention (40-50 g/d), the aim was to use practical and achievable fiber intake levels. In addition, the focus was to explore the potential effects in patients with moderate to severe decline in kidney function compared with previous trials. The first study was to investigate the effects of 23 g/d of fiber from pea hull, resistant corn dextrin and inulin on BUN, serum creatinine, and eGFR; uremic symptoms; and quality of life. The second study was carried out to further assess the impact of pea hull fiber alone and in combination with supplemental inulin/FOS on quality of life, bowel movement frequency, and uremic molecules.
CHAPTER 2
REVIEW OF THE LITERATURE

Chronic diseases are worldwide problems and are leading causes of death in
developed countries. In the United States, chronic diseases account for 70% of all
deaths (14). There are several contributing factors to chronic diseases including
smoking, sedentary life style, and diet. Early interventions are critical to reduce risk,
severity and mortality of chronic diseases. For example, interventions that focus on
smoking cessation result in reduced risk of cardiovascular disease (CVD) and certain
cancers, while increasing physical activity may help reduce the risk of many diseases
such CVD and diabetes (15). Nutritional interventions may also help with reducing risk
of various diseases and improve management. It is established that lowering trans-fat
intake and cholesterol may help reduce the risk of CVD while fiber has been proposed
to be protective against chronic diseases such as CVD (16).

Lack of fiber in the diet has been implicated in many of the chronic diseases seen
today in modern societies lending the assumption that fiber, although not currently
defined as a nutrient, is of importance comparable to nutrients (17). Chronic diseases
contribute significantly to the financial burdens experienced both by individuals and
economies as a whole. In the past, nutrition research was mainly focused on prevention
of nutritional deficiencies and malnourishment. In the early 1900s, researchers began to
understand the link between nutrients deficiencies and illnesses. In the first half of the
20th century, the focus was to eradicate nutritional deficiencies in the United States.
Iodine fortification started in the 1922, vitamin D fortification in 1932, iron, niacin,
riboflavin, and thiamin were added to foods in 1941 and the RDA was developed in
1943 “to serve as a guide for planning an adequate diet for every normal person.” (18)
Later, and once most deficiencies became less prevalent and the health status of the population improved; the focus shifted toward promotion of optimal health. Once the link between nutrients, intakes, and chronic disease risk was elucidated, dietary recommendations were set to address these links from nutritional standpoint. Diets that emphasize better health and reduced risk of chronic diseases are promoted for specific populations. Nutritional and governmental agencies aim to reduce financial burdens associated with such diseases and improve the quality of life for people by publishing and promoting these dietary recommendations.

One particular disease of high cost is CKD with high direct burden costs, and indirect burden costs related to its co-morbidities. Diabetes and hypertension are the leading causes of CKD (19, 20). Chronic kidney disease patients are at very high risk for developing CVD (21). The stage and severity of kidney disease are determined by kidney function and/or damage of the kidneys. Patients with lower kidney function have a higher stage disease and health care costs associated with each stage increases significantly ranging anywhere from as low as $16,000 per year for total costs in stage 2 to as high as $44,500 per year in stage 4 (22). One of the most costly co-morbidities of diabetes and hypertension, in terms of health care expenditures and patient quality of life, is that of CKD. Health care costs are significantly higher in CKD patients that have other co-morbidities compared with those only diagnosed with CKD (22). The decline in kidney function is typically slow and take years before approaches kidney failure. Any potential therapy to reduce or slow the decline in kidney function, manage co-morbidities, alleviate symptoms will contribute significantly to reduced total health care costs and improved quality of life.
It is estimated that 26 million adults are affected by a decline in kidney function in the United States (23). The Kidney Disease Quality Outcome Initiative (KDQOI) guidelines define CKD as either kidney damage or a decreased kidney glomerular filtration rate (GFR) of less than 60 mL/min/1.73 m$^2$ for three or more months irrespective of etiology (24). The kidney’s primary functions are to filter blood and maintain electrolyte balance. When kidney function declines, molecules that are usually filtered efficiently accumulate in the blood and tissues. Molecules that accumulate and “interact negatively with biologic functions” are referred to as uremic retention solutes, uremic toxins, or uremic molecules (25). It is suggested that the progressive accumulation of these uremic molecules or toxins causes a decline in health and increase uremic symptoms severity (25).

Uremia is a broad term to describe symptoms experienced by renal patients that “cannot be explained by derangements in extracellular volume, inorganic ion concentrations, or lack of known renal synthetic products.” (1) It commonly develops with CKD in the later stages of the disease. Accumulation of organic waste products or uremic molecules have been identified as contributors of uremia. An estimated 90 retention uremic molecules identified so far that may explain uremia and contribute to the uremic load (25). Uremic molecules can originate from endogenous sources such as amino acids and protein catabolism, can be ingested, or generated from proteins bacterial fermentation (26).

Uremic molecules have various properties that contribute to their diverse physiological effect in the body including water solubility, dimensions, charge distribution, concentration, molecular mass, and protein binding (26). Declining renal
function and thus, clearance capacity can result in elevated concentrations of such molecules in blood and tissues, some of which can contribute to uremic symptoms and derangement in health and wellbeing. Uremic toxic molecules may accelerate glomeruli and tubular structural damage and stimulate disease progression by stimulating renal expression of fibrosis-related genes such as transforming growth factor β (TGFβ1) (8), induction of renal oxidative stress and free radicals production (13, 27), (5), and inhibition of cellular proliferation (28). Creatinine is a byproduct of the breakdown of creatine phosphate and is not viewed as a harmful molecule, but its elevation indicates an increase in muscle breakdown or decline in renal function. Ammonia is a very toxic molecule and blood levels need to stay very low. Ammonia forms by removal of an amino group from amino acids by deamination, via glutamic acid deamination by glutamate dehydrogenase, or from ammonia generation by bacteria in the large intestine. As it forms, the body keeps ammonia levels from elevation by converting it to urea, which is relatively non-toxic and biologically inactive. Urea is the primary end product of nitrogen metabolism that is produced via the urea cycle and is the largest pool of uremic nitrogenous molecules in the blood. Urea is normally excreted to maintain a narrow range but can become very elevated with declining kidney function. About 70% of the urea produced in healthy adults is eliminated in the urine, while 30% is hydrolyzed and a portion of that is retained in the body (29). This occurs because urea in circulation can diffuse through the colonic wall due to its small molecular mass of 60 Da. It is suggested that hydrolyzed urea in the large intestine ranges from 20-25% to as high as 40% of total urea production (30). In patients with reduced kidney function, urea accumulates in the blood contributing to the rise in pool of uremic molecules.
Uremic molecules and toxins become elevated in the blood with inadequate renal clearance and are associated with uremia (31-33). These uremic molecules can affect biochemical, biological and physiological functions which may explain the broad uremic symptoms profile. \(p\)-Cresol for example is a microbiota product that diminishes oxygen uptake by brain cells, disrupts endothelial progenitor cell function, is related to growth retardation in the weanling pig, and causes alteration of cell membrane permeability (34, 35). Symptoms of uremia include fatigue, intense itching, anorexia, cognitive dysfunction and constipation (1). As the kidneys’ ability to filter the blood and excrete urine decline, nitrogenous compounds become elevated. It is possible that by inducing bacterial demand for nitrogen in the large intestine by providing the preferred substrate to support bacterial proliferation, and subsequently depress the return of nitrogen to circulation, that the uremic load can be mitigated. In addition, interfering with bacterial generation of uremic molecules such as \(p\)-cresol would also provide a viable approach to reduce the uremic load. Eventually, by targeting certain uremic molecules and reducing their retention and/or generation, the uremic load can be reduced assuming no increase in other uremic molecules. Such reduction may lead to improved renal hemodynamics and excretion due to reduced concentration of inflammatory molecules and/or by reductions in volume of molecules to be filtered through the renal tubules.

It is therefore very valuable to have a nutritional intervention that targets the colon and enhances its role in reducing uremic molecules generation and uptake. Reduction of such molecules may provide valuable treatment for uremia and the burden associated with such symptoms. Fiber may provide such potential therapy due to its fermentation.
Fermentable fibers stimulate bacterial proliferation by increasing carbohydrate substrate availability, thus leading to increase nitrogen demand for bacterial protein synthesis. Saccharolytic fermentation, as opposed to proteolytic, results in an increase in the production of short chain fatty acids (SCFA), which is thought to provide benefits to the host. Butyrate for example, is shown to be a potent trophic factor that stimulates cell wall hypertrophy and maintenance (36, 37). Induction of fermentation utilizing fermentable carbohydrate sources in animal models show significant hypertrophy of the cecal cell wall, which increases blood flow into the cecum (38-40). Increasing blood flow into the cecum, along with increased nitrogen demand, will lead to an enhanced entry and retention of nitrogenous molecules such as urea and ammonia in the colon (39-41). Animal studies show that addition of fermentable fiber to the diet of animals leads to nitrogen shift from urinary to fecal excretion (41-46). The addition of inulin to the rat diet causes cecum enlargement with declining urinary nitrogen and increased urea flow into the cecum with significant increase in acid production (40, 41, 47). Increased acidity creates a unfavorable environment for a number of pathogenic bacterial species (48), which typically contribute to the uremic load by generating uremic molecules such as amines and phenols.

Although scarce, reports from human studies involving renal patients are also suggestive of a more interesting role of the colon in removing, capturing and/or reducing uremic molecules appearance in the blood (2, 7, 49-51). In a study where CKD patients were supplemented with soluble fibers (30 g/d arabinogalactan or 7 g/d ispaghula) for 6-8 weeks, BUN decreased by 11%-19% (50). Fifty gram per day of gum arabic was shown to increase fecal nitrogen excretion by 41% while decreasing BUN levels in 16
CKD patients (51). Younes et al. demonstrated that five weeks of feeding 40 g/d of mixed fiber sources (4.5 g inulin, 10.5 g resistant starch, and 25 g of fiber from wholemeal bread) resulted in a 51% increase in fecal N excretion and 12% reduction in urinary nitrogen excretion in 9 uremic CKD patients along with a significant reduction in BUN levels (-23%) (2). There was a 1.7 fold increase in stool content of nitrogen with bacterial fraction of feces accounting for (59%) of this increase suggesting with the observed decrease in BUN that the colonic bacteria are largely responsible for the decrease in serum urea.

Fermentable substrates can alter the luminal pH, and selectively enhance the growth of bacterial species, which can alter uremic molecules generation. Several uremic molecules are primarily generated by gut bacteria and contribute to the uremic load. \( p \)-Cresol is generated by tyrosine fermenting bacteria and can be elevated in uremic patients (52). Similar to BUN, lowering of \( p \)-cresol, can be achieved via fiber supplementation and may contribute to lowering the uremic load. In a 4-week study, inulin and FOS supplementation resulted in lower concentrations of \( p \)-cresol and its conjugated form \( p \)-cresol sulfate in HD patients (7). However, lowering uremic and toxic molecules in CKD may be dependent on several factors including fiber dose and type, stage of kidney disease, colonic bacterial make up, fiber intake, and transit time.

Parillo et al. investigated the metabolic effect of feeding high and low fiber diets to CKD patients (49). One group received a high fiber diet (65 g/d) (legumes, vegetables and fruit) with moderate protein intake (69 g/d), and the other group received a low fiber (22 g) diet with lower protein content (50 g/d or 9%). The high-fiber diet induced a significant improvement in blood glucose control and a significant decrease in serum
cholesterol. While some beneficial metabolic outcomes were observed, no changes in serum creatinine, BUN, or eGFR were observed. There was, however, a significant increase in fecal nitrogen losses with no changes in urinary nitrogen excretion. The study by Younes et al. showed a significant impact of fiber on BUN but not serum creatinine with an increase in fecal nitrogen loss and a parallel decline in urinary nitrogen loss (2), unlike the observation of Parillo et al.

These results suggest a potential impact of the use of fiber as a therapeutic nutritional component to reduce various uremic molecules and impact various markers depending on the fiber source and dosage. This potential positive effect may impact kidney function, uremic symptoms, and subsequently, health-related quality of life. Because fiber intake is likely to be low in this population, restoration of adequate fiber intake may also provide additional health benefits such as improved lipid profile, improved glycemic control, and improved bowel habits.

Dietary fibers such as wheat and oat brans, coarse and wheat flours have been used for centuries as a laxative and bulking agents. In the past few decades, the role fiber plays in health maintenance and disease prevention have been expanded to include risk reduction of CVD, stroke, hypertension, diabetes, and certain gastrointestinal disorders, along with improvements in lipid profile (53). Moreover, the effect of fiber on gut health was expanded to include symbiosis by establishing the importance of certain fibers as prebiotic substances. A prebiotic is “non-digestible food ingredient that beneficially affect the host by selectively stimulating the growth and/or activity of one of more limited number of bacteria in the colon and thus improve host health” (54). More recently, a prebiotic has been defined as “selectively fermented
ingredient that allows specific changes, both in the composition and/or activity in the gastrointestinal microbiota that confers benefits upon host well-being and health.” (55) Examples of prebiotic fibers include: resistant starch (56-58), inulin, lactulose, lactosucrose, xylo-oligosaccharides, soybean oligosaccharides, lacto-oligosaccharides among others (59).

Several experimental studies and some human trials have shown potential benefits of prebiotics in inflammatory bowel disease, mineral bioavailability and constipation. In addition, human studies have shown that prebiotic fibers reduce some serum uremic molecules and fecal nitrogenous byproducts (2, 7, 40, 41, 60). It is therefore important to determine the potential beneficial role that fiber may play in the diet of CKD patients, particularly those with uremia, beyond that usually ascribed to healthy individuals. This is particularly important when considering that over half of CKD patients are diagnosed as stage 3 CKD patients and thus, any beneficial therapy can have significant impact.

Fiber intakes of CKD populations have not been documented. Current estimation of fiber intake in the general population is about half of the adequate intake (AI) recommendation of 31-38 g/d for men and 21-25 g/d for women (16). Although there are no published estimates of fiber intakes for CKD patients’ population, it is logical to predict that individuals with advanced CKD may consume less fiber than the general public due to diet restrictions and uremic symptoms such as anorexia (61).

Restricted sodium, restricted phosphorus and high calcium intake are required for the management of late stages of CKD, since with the loss of kidney function, electrolyte, mineral and fluid balance are disrupted. High phosphorus foods may be
restricted. These include primarily dairy products, beans, bran cereals and whole grains. Limitations on potassium intake for some patients necessitate the exclusion of beans and milk, as well as nuts and many fruits and vegetables. The restriction of beans, whole grains and many fruits and vegetables may leave a diet seriously depleted of dietary fiber, and may lead to constipation and inadequate fermentable substrate for colonic and general health. Although it is recommended that CKD patients consume diets high in fiber, no kidney disease diet recommendations provide guidelines as to how to achieve this recommendation.

There is evidence suggesting reduced risk of CVD, improved management of diabetes and constipation with higher fiber intakes, but no specific benefits have been established between increased fiber intake and the health of CKD patients. Emerging evidence suggest that fiber may provide benefits for CKD patients beyond that of reducing CVD risk, specifically reducing uremic molecules which are implicated in uremia. However, there is very limited research on the benefits fiber may play in the treatment of uremia in kidney disease population and the effect on quality of life.

**Fiber Definition**

In the United States, fiber is typically categorized as soluble and insoluble. The categorization is derived based on the Association of Official Analytical Chemists (AOAC) method, typically the AOAC 985.29 (16). The Institute of Medicine (IOM) suggests that the solubility-based definitions are limiting and should be replaced with dietary fiber, functional fiber, and total fiber when defining types of fiber (16). The IOM defines dietary fiber as the “non-digestible carbohydrates and lignin that are intrinsic and intact in plants”, functional fiber is the “isolated, non-digestible carbohydrates that have beneficial physiological effects in humans”, and total fiber is “the sum of dietary
fiber and functional fiber.” (16). Dietary fibers are of plant origin and are composed of non-digestible polysaccharides and lignin while their matrix is largely intact. These fibers maintain plant cells’ three-dimensional interrelationships that are typically responsible for some of the physicochemical properties attributed to dietary fiber (16). Added fibers may be functional fibers. As defined by the IOM, they are functional fibers if they are non-digestible carbohydrates that are isolated and extracted and are shown to have beneficial physiological effects in human (16). They can be made available by modification of plant sources, or can be synthetically made by enzymatic reactions such as oligosaccharides and resistant starches (16).

Whether dietary or functional, fiber must be a carbohydrate that is non-digestible by the human intestinal enzymes. The different properties of fibers such as solubility, viscosity, bulking and fermentability among others determine the way fiber behaves in the small and large intestines, and the physiological role it may play during its passage throughout the digestive tract. Fiber according to the IOM classification must be 1) part of an edible plant cell; 2) carbohydrates (both oligosaccharides and polysaccharides); 3) resistance to hydrolysis by human digestive enzymes; 4) resistance to absorption in the small intestine (62). While dietary fiber is the intact part of the plant, functional fibers are added to products after being isolated, extracted, or synthesized. In order to bare the definition functional, however, they must be “non-digestible carbohydrates that have beneficial physiological effects in human.” (16).

**Pea Hull Fiber**

Plant seeds are an important agricultural product that are consumed as a whole, or used as food ingredients. Generally, the outer layer of a seed is composed of insoluble polysaccharides, pectin, and lignin while the inner polysaccharides
components varies in their solubility and are mainly composed of non-starch polysaccharides. Peas belong to the Leguminosae family. The main tissues in a pea seed are the seed coat (hull) and the cotyledons (inner plant storage tissue). The two tissues are distinctively different. The outer layer is relatively higher in cellulose, hemicellulose and lignin. The cellulosic content of the hull fibers is comparable to fibers isolated from cereal bran (63). Pea hull fiber is light in color, has a smooth mouth feel and yields products neutral in flavor and odor with moderate water-holding capacity characteristics. It is composed of 89% dietary fiber, 82% of which is insoluble with the primary insoluble fiber being hemicellulose and only 7% soluble fiber (63, 64).

**Inulin and FOS Fiber**

Fructans are predominantly linear chains of fructose units joined by β (2-1) linkage. Fructans can be found in a variety of plants such as garlic, asparagus, wheat, rye, onions, chicory roots, and Jerusalem artichoke with later ones having the highest content. Inulin is a generic term that covers all β (2-1) linear fructans with mostly one terminal glucose-unit, and with a degree of polymerization (DP) ranging from 2-60 (average of 10-12) (16, 59, 65). Inulin is typically produced commercially by extraction from chicory roots (Cichirium intybus) using hot-water extraction process; the extracted native inulin is then purified into a white powder. Partial enzymatic hydrolysis of chicory inulin using endo-inulinase yields oligofructoses (66). In addition, FOS fructans are produced from sucrose using enzymatic transfer of fructosyl groups. Oligofructose have a DP ranging from 2-8 while FOS have DP ranging from 2-4 (16). Unlike inulin, oligofructose and FOS may or may not have a terminal glucose unit.
Resistant Corn Dextrin Fiber

Dextrins are hydrolysates obtained from the breakdown of starch by hydrolysis. The treatment of these low molecular weight simple carbohydrates with heat followed by enzymatic treatment results in resistant maltodextrins. On average, resistant maltodextrins have a molecular weight of about 2 kDa consisting of glucose polymers with α (1-4) and α (1-6) glucosidic bonds and 1-2 and 1-3 linkages (16). Specifically, treatment of cornstarch by heat/acid and amylase treatment produce resistant corn dextrin.

Fiber Recommendations and Current Intakes in General Population

In the United States, fiber recommendations are established as Adequate Intakes (AI). The recommendations derived as AI because protective evidence against coronary heart disease (CHD) is found across continuous ranges of intakes and therefore an Estimated Average Requirement (EAR) was not possible to establish (16). The current AI recommendations for adults younger than 50 years of age are 25 g/d for women and 38 g/d for men, while for those 50 and older will need to consume 21 g/d and 31 g/d for women and men respectively, or 14 g per 1000 kcal (16). Current fiber intakes in the United States fall short of these recommendations. From the 1994-1996, 1998 Continuing Survey Food Intakes by Individuals (CSFII), median fiber intakes ranged from 16.5 to 17.9 g/d and 12.1 to 13.8 g/d for men and women, respectively, suggesting Americans consume about half the recommendations (67).

Dietary Reference Intakes (DRIs) for Fiber and Current Intakes in CKD Population

Current dietary fiber recommendations for kidney disease population are not different from that for healthy individuals. There are no specific recommendations for consuming high fiber intakes in this population. The recommendations are similar to
those for the general population in which consuming high fiber is protective against heart disease. Fiber intake, however, falls dramatically short of the amount recommended for good health. The majority of Americans consume an average fiber intake that is half of the recommendations (68).

Fiber intake in populations with CKD has not been established, but is expected to be lower than the current intakes in the healthy population. This is because CKD patients, especially those with advanced stages of the disease, may need to restrict foods that are typically high in fiber such as whole grains, legumes and certain fruits and vegetables due to their content of phosphorous and/or potassium. These minerals are usually restricted in later stages of CKD. Uremic symptoms also may lead to loss of appetite and anorexia which results in lower food intakes and subsequently lower fiber intakes.

**Chronic Kidney Disease Prevalence Overview**

The Kidney Disease Quality Outcome Initiative defines CKD as “either kidney damage or a decreased kidney GFR of less than 60 mL/min/1.73 m$^2$ for 3 or more months irrespective of etiology” (24). Fourteen percent of U.S. women ages 20 and older have CKD compared to 11% of males (69). Fourteen percent of white have CKD compared to 12% African Americans and 8% Mexican Americans (69). Whites make up 72.6% of CKD population while African Americans represent 10.5% (70). The leading risk factors for kidney disease are hypertension (19, 20) and diabetes (71). About 7% of the CKD population has diabetes and 27% are hypertensive (70). In 2006, 7 out of 10 people who had developed renal failure had diabetes or hypertension (69). Diabetes is the leading cause of kidney failure (69). It is estimated that the prevalence of CKD in the United States as of 2008 was 13.1% (72). Prevalence of CKD by stages according to
the 2004 Third National Health and Examination Survey (NHANES III), using eGFR as a
benchmark, is as follows: stage 1 (1.8%), stage 2 (3.2%), stage 3 (7.7%), stage 4 (0.35%) (70), and 570,000 patients on transplant functioning kidney or dialysis as of
2011 (72). Health care costs associated with each stage increases significantly and can
range anywhere from as low as $16,000 for total costs in stage 2 to as high as $44,500
per year in stage 4 (22). One of the most costly co-morbidities of diabetes and
hypertension, in terms of health care expenditures and patient quality of life, is that of
CKD. Health care costs are significantly higher in CKD patients that have other co-
morbidities compared with those only diagnosed with CKD (22).

**Stages of Chronic Kidney Disease**

The Kidney Disease Quality Outcome Initiative guidelines define CKD as
persisting structural or functional abnormalities for over three months as determined by
kidney damage or GFR of less than 60 mL/min/1.73m². Damage can be structural as
determined by imaging tests or functional as determined by abnormal blood, or urine
tests (e.g. proteinuria). The calculated GFR is typically based on the Modification of Diet
in Renal Disease (MDRD) study equation, which uses serum creatinine, age, gender
and race (African American or other). Based on the above KDQOI criteria, CKD is
classified in 5 stages: stage 1: GFR ≥ 90 mL/min/1.73m² with evidence of kidney
damage; stage 2 (Mild): GFR of 60 to 89 mL/min/1.73m² with evidence of kidney
damage; stage 3 (Moderate): GFR of 30 to 59 mL/min/1.73m²; stage 4 (Severe): GFR of
15 to 29 mL/min/1.73m²; and stage 5 (Kidney failure): GFR <15 mL/min/1.73m². Without
any structural or functional abnormalities, CKD is only diagnosed as such when GFR is
<60 mL/min/1.73m². The threshold of GFR is established because it is less than half the
adult level of GFR, and lower levels are associated with increasing complications of
CKD and adverse outcomes such as cardiovascular disease morbidity and mortality. In addition, this level can be detected with current estimating equations for GFR based on serum creatinine, and is substantially above level associated with kidney failure for prevention therapy to take place in a timely manner (24).

The Kidneys

Kidneys are important organs that provide homeostatic roles in addition to being the primary site of filtration in the body. They are the essential components of the urinary system and homeostatic regulation of electrolytes, acid-base balance, and regulation of blood pressure. The primary function of the kidney is the removal of cellular metabolites, toxins and waste products.

Removal of uremic molecules is via urine production in the kidneys, which is produced by the process of filtration, reabsorption, and tubular secretion. The basic functioning urine-producing unit of the kidney is the nephron. There are about two million nephrons in both kidneys. The renal artery branches to form several other smaller arteries that eventually give rise to the glomerular capillaries where the renal corpuscles (Bowman’s capsule and the glomerulus) forms filtrate. Briefly, each nephron consists of renal corpuscle and a renal tubule. The renal corpuscle consists of glomerulus (cluster of capillaries) surrounded by Bowman’s capsule. The renal tubule is divided into several segments, the proximal tubule (convoluted and proximal straight tubule), the loop of Henle (proximal straight tubule, thin limb, and thick ascending limb), and the distal convoluted tubule. The connecting tubule will converge the distal convoluted tubule with the next segment at the cortical collecting duct where several nephrons drain. “[T]he entire renal tubule and collecting duct system consists of a single layer of epithelial cells surrounding fluid in the tubule or duct lumen.” (73)
Due to pressure gradients that exist between blood in the capillaries and fluid in the Bowman’s capsule, solutes are filtered and fluids and soluble materials are forced out into the urinary space of the Bowman’s capsule in a process of ultrafiltration. Filtrate from the glomerulus collected in the urinary Bowman’s capsule space flows downstream from the tubule lumen where the composition and volume are altered by tubular activity (73). Tubular reabsorption (active or passive) allows substances to be transported out of tubular filtrate (out of urine), where they are returned to the peritubular capillaries surrounding the tubules. These reabsorbed substances include: ions, water, glucose, amino acids, and uremic molecules such as urea, creatinine and uric acid. Tubular secretions (active or passive) allow the disposal of unfiltered substances, passively reabsorbed substances such as urea and uric acid, and excessive potassium ions, in addition to maintenance of blood pH.

Unlike glomerular filtration, which is non-selective, tubular transport is selective. In the glomerulus, all molecules are filtered via the same and only mechanism, while in the tubules various mechanisms are involved in transportation of the molecules and substances. The transport process can involve absorption, secretion or both, and it can be passive or active depending on the particular substance and conditions. In the proximal tubule, organic anions (mainly protonated carboxylic and sulfonic acids) are actively secreted in the proximal tubule and become saturated at high plasma concentrations, which leads to organic anions secretion competition (73). Organic cations (mainly amine and ammonium compounds) are also secreted via active transport mechanism. The broad substrate specificity of these anions and cations
transporters allow for secretion of a variety of chemically diverse compounds. Organic acids can also be passively transported by accepting/releasing hydrogen ions.

Ammonia is a lipid-soluble base and its excretion to form ammonium is favored to increase acid losses, as unbound hydrogen ions are not well excreted. This occurs when blood pH decreases, which signals the tubules to increase secretion of hydrogen ions along with increasing the retention of bicarbonate and potassium ions to allow the urine to drain more acids. If blood pH rises, more chloride anions are reabsorbed and bicarbonate is eliminated in the urine while ammonium is reabsorbed to recycle the hydrogen ions to further furnish the bicarbonate formation.

Post proximal convoluted tubule, urea travels through the thick ascending limb, distal convoluted tubule, connecting tubule, cortical collecting duct, and outer medullary-collecting duct with very limited absorption action due to impermeability of these segments to urea (73). Because a large volume of water through these segments has been reabsorbed, urea concentration rises in the inner medulla segment of the nephron. Concentrated urea is then transported via urea transporter into the interstitial fluid of the inner medulla (73). Urea can also re-enter the loop of Henle to be recycled into the inner medulla or can be added to the inner medulla by diffusion from urine (73). Secretion and absorption mechanisms can vary in various parts of the nephron and collecting duct segments.

Filtered blood travels out the renal vein while urine collects in the collecting tubule. Approximately, 1200 mL of blood flows through the kidneys each minute generating 180 L of filtrate a day and approximately 2 L of urine.
Filtration

Glomerular filtration rate is defined as the volume of plasma that can be completely cleared of a particular substance by the kidneys in a unit of time (74). The kidneys produce approximately 180 L of ultrafiltrate per day or 125 mL/min. The adult normal GFR range is 120-130 mL/min/1.73m². It is similar in men and women after adjusting for body surface area.

The functional state of the kidneys is routinely evaluated by estimating GFR. It is done indirectly by measuring the flow rate of filtered fluid from glomerular capillaries into the Bowman’s capsule per minute. Glomerular filtration rate provides an excellent index of functioning renal mass or filtering capacity (75). Measuring GFR can only be done indirectly using a substance that is “physiologically inert, freely filtered at the glomerulus, and neither, secreted, reabsorbed, synthesized, nor metabolized by the kidney, the amount of that substance filtered at the glomerulus is equal to the amount excreted in the urine.” (21) The fructose polysaccharide, inulin, has been considered the ideal substance to estimate GFR by injecting the substance into the blood and collecting it in the urine using timed urine collections. Inulin meets all the above requirements as a substance and thus its clearance rate equals GFR. However, the inherent problem of intravenous infusion and timed collections make this method very impractical, costly and cumbersome, especially in a clinical setting and for screening purposes.

Because inulin use to estimate GFR is impractical, serum creatinine is widely used to estimate GFR. Both urinary creatinine clearance and plasma creatinine can be used to estimate GFR. Once measured, estimation equations such as the MDRD or the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation are used to calculate GFR.
Briefly, the MDRD equation is a 4-variable equation (serum creatinine, age, gender and race) developed in 1999 using a large data set from over 1600 CKD patients with GFR ranging from 5-90 mL/min/1.73 m². It was re-expressed in 2005 using standardized serum creatinine assay. In 2009, the CKD-EPI equation was developed to estimate GFR using serum creatinine, age, sex and race (76). It is more accurate than MDRD in predicting GFR in patients with function greater than 60 mL/min/1.73 m², but is not different when estimating lower functions (77).

Although not widely used, cystatin C also can be used to estimate GFR using CKD-EPI cystatin C based equation (78). Equations with a single marker, however, underestimate the measured GFR and lack precision. Two recent meta-analysis studies suggest that using both serum creatinine and cystatin C with age, sex, and race would be better than equations that use only one of these serum markers (79, 80), and that the combined equation is more precise to estimate higher GFRs (76). Recently, Inker et al. confirmed that the combined creatinine and cystatin C equation performs better than equations with a single marker (78).

**Serum Creatinine**

Creatinine is small organic molecule (113 Da) that is not bound to plasma proteins. Creatinine is the end product generated in muscle from conversion of creatine and creatine phosphate. About 98% of creatine pool is in muscle, and of that amount, about 1.6%–1.7% is converted to creatinine per day (81). Creatinine breakdown rate is fairly stable and depends on muscle mass. Serum creatinine is used as an index of kidney function. Under conditions of normal renal function, 90%-95% of creatinine is filtered by glomerular filtration with 5%-10% tubular secretions and reabsorption with the latter being less of a factor (82). Serum creatinine is the acceptable and preferable method
used in clinical settings to index kidney function to date with the use of estimating
equations. However, several factors can affect serum creatinine independent of actual
filtration. For adults’ age 18 and older, normal serum creatinine levels range from 0.50-
1.53 mg/dL (83) depending on gender, age and race. Dietary changes such as an
increased protein intake can induce a transient rise in serum creatinine levels and
urinary excretion as tubular secretions are induced (81). Similarly, urea generation is
induced with such dietary changes. Thus, a decrease in serum creatinine that is not
related to improved kidney function has clinical implications for the interpretation of
serum creatinine that will lead to false positive results.

In healthy individuals, almost all creatinine elimination happens via the urinary
route. However, with severe renal function impairment such as in CKD stage 5, limited
evidence suggests that elevated creatinine pool may induce higher losses from external
routes (84, 85). Degradation of creatinine by bacterial creatininase in the colon is
thought to be responsible for the removal of creatinine (86, 87). Burnett and Jones
suggested that creatinine, similar to urea and uric acid, can enter the gut from the blood
when elevated in the blood where it can be recycled through the gut or utilized by gut
flora (84). Rats fed creatinine had higher colonic creatininase activities compared to
controls (87), and earlier work with rats showed that creatinine fed to rats was
recovered in the feces (88). A more recent study suggested that retained creatinine
induces creatininase activity throughout the bowel and causes creatinine degradation by
gut flora (86). In the study, creatinine degradation was determined by measuring
creatininase activity in stool isolates in advanced renal patients (stage 5) compared to
normal. In patients with serum creatinine above 6 mg/dL, higher stool creatinine
degradation was seen compared to controls and to patients with creatinine levels lower than 6 mg/dL who did not use antibiotics for a period of three months or more (86). Very early work by Goldman, however, did not show creatinine in the feces (89). While the evidence is inconclusive at best, there is no evidence that in CKD patients with moderately elevated creatinine levels, creatininase activities will be induced. Furthermore, studies supplementing CKD patients with fiber have not shown any significant reduction in serum creatinine suggesting that it is unlikely that significant quantities of serum creatinine can enter the colon and become substrate for microbiota.

**Serum Cystatin C**

Cystatin C is a non-glycosylated protein, 120-residue polypeptide chain (90), that belongs to the cystatin super-family of cysteine protease inhibitors and damage to cysteine proteases is regulated by these proteins (91). Nucleated cells produce cystatin C at a constant rate where it is concentrated in the fluid rich environments of the body such as seminal fluid, cerebrospinal fluid and synovial fluid (91). Elimination of cystatin C is through the glomerular filtration with no extra-renal elimination. The normal reference range is 0.5-1.0 mg/L (92). Filtered cystatin C is completely reabsorbed by the tubule cells and rapidly degraded (93), thus it does not re-enter circulation, nor does it appears in the urine. Cystatin C has a low molecular mass of 13 kDa with positive charge at physiological pH leading to free filtration by the glomerulus (91). Its correlation with GFR is not influenced by gender, muscle mass, age, or protein intake (93, 94). Therefore, the use of cystatin C as an additional indexing molecule is becoming more relevant.
**Uremic Load, Progressive Hyperfiltration, Uremic Toxins and Disease Progression**

Several factors can contribute to the progression of CKD although the exact reasons are not known. Because the rate of glomerular filtration is dependent on the number of nephrons and the single-nephron GFR, when nephrons decline in number, the load on the remaining nephrons will be higher. Filtration is measured as the flow of filtrate from the glomerular capillaries (glomerulus) out into the Bowman’s capsule space per minute. In CKD, the number of residual nephrons decline progressively leading to reduced volume of filtrate formed per minute. As such, the adaptive response of nephrons to hyperfiltrate to compensate for the loss of nephrons and maintain optimal filtration capacity progressively increases. Hyperfiltration is characterized by reduced afferent arteriolar resistance, which increases glomerular capillary plasma flow rate. This allow greater fraction of the systemic blood pressure to be transmitted into the glomerular capillary network therapy raising the glomerular capillary hydraulic pressure without affecting perfusion pressure. While this can lead to temporarily improved filtration capacity, chronically, it causes permanent glomerular damage (95, 96). This is because the distention of the glomeruli capillary lumen leads to the stretching of the mesangial cells associated with the glomerular capillaries that stimulates the release of fibrosis related genes such as TGFβ1 that accelerates glomerular sclerosis (95, 97, 98). In addition, a declining number of nephrons means less tubular surface area available for tubular secretions and absorption as well as increase competition for the available transporters. Plasma becomes saturated with uremic toxins that are otherwise filtered easily leading to build up of these toxins that can induce chronic inflammation and damage the tubular cells.
Changes in dietary therapy are likely to affect hyperfiltration by means of altering uremic molecules in the blood. It has been shown that dietary protein restriction limits the adaptive increase in single-nephron GFR and glomerular capillary hydraulic pressure that leads to amelioration of glomerular damage and loss of function (96, 99). Animal studies show that the addition of uremic toxic molecules leads to accelerated disease progression and decreased filtration capacity while removal of such toxins is nephron-protective. Animal treated with these uremic toxic molecules score significantly higher on the glomerular sclerosis index and exhibit a more advanced interstitial fibrosis with increased glomerular dysfunction (lower GFR) compared to controls (11, 12). Uremic toxins can accumulate in tubular cells and induce oxidative stress (13, 27) and inhibit cellular proliferation (28). It is thought that chronic exposure to oxidative uremic molecules induces the production of vasoactive and inflammatory substances that increase expression of TGFβ1, tissue inhibitor of metalloproteinase-1 (TIMP-1), and pro-α (I) collagen in proximal tubule cells (8, 11, 95) leading to accelerated interstitial fibrosis, glomerular sclerosis and glomerular dysfunction. Therefore an “early intervention to reduce uremic toxins maybe effective to delay the progression of renal dysfunction.” (11)

**C-Reactive Protein**

C-reactive protein (CRP) is produced by the liver (100), and typically measured to assess general inflammation with no specificity to the location of infection or inflammation. Values of circulating CRP have been found to correlate closely with other markers of inflammation (100). Normal values are <0.80 mg/dL (101). CRP is a predictor of type 2 diabetes and coronary events in the general population (100). Inflammation plays a significant role in the progression of CKD (102). Systemically,
elevated CRP levels are associated with hyperfiltration, glomerulosclerosis, and cortex scarring (102, 103). The inflammatory process induces the release of inflammatory mediators that can accumulate in tissues without clearance. Such processes lead to maladaptation and sustained inflammation (104).

**Fermentation**

The human large intestine is approximately 150 cm long and is slightly acidic with a pH range of 6.0-7.0. The resident microbial community is complex, diverse, and biochemically very active with a bacterial population count of \(10^{11} - 10^{12}\) per g content within the colon (26). The principal role of the colon is water and solute retention and waste disposal. Other roles include immunity, and energy and nitrogen salvaging from carbohydrates and proteins. On average, 0.3–4.1g of nitrogen, primarily proteins (50%) and peptides (20–30%), enters the colon daily (105).

The bacterial mass and diversity in the colon are dependent on several factors, one of which is substrate availability. Typical fermentation substrates are sloughed cells and mucous, unabsorbed sugars/starch and fiber. Protein fermentation occurs in the large intestine and increases when carbohydrate substrate is lacking. Fermentation is largely dependent on substrate availability, substrate physical properties, and colonic conditions such as pH and transit time. Carbohydrate fermentation by saccharolytic activity results in short chain fatty acids (SCFAs), \(\text{CO}_2\), and \(\text{H}_2\) production while protein and amino acid fermentation by proteolytic activity yields \(\text{CO}_2\), \(\text{CH}_4\), and \(\text{H}_2\), phenols, amines and branched SCFAs, but comparatively fewer SCFAs (106, 107).

Protein bacterial fermentation in the colon can result in the generation of tumorigenic and uremic molecules (26, 108). It was first proposed by Niwa et al, that uremic metabolites originating from bacterial protein fermentation in the colon have key
effect in the progression of CKD (9, 10). It is shown that a high protein diet can increase uremic molecules and is associated with accelerated glomerulosclerosis while low-protein diet ameliorate hyperfiltration (109). Along the length of the colon, the ratio of available fermentable substrate to nitrogen progressively decline which impacts bacterial composition and metabolism (110). This decline of fermentable substrate, allows the fermentation of proteins to be more significant. Saccharolytic and proteolytic activity predominate in the right colon and left colon, respectively (26). Reduced transit time can cause a reduction in the carbohydrate substrate reaching distal parts of the colon and as a result proteolytic activity leading to increase generation of toxins (26).

Increasing intake of fermentable fiber substrate can lead to interference with microbial metabolites generation by selectively increasing saccharolytic and reducing proteolytic bacteria activities and metabolites (26). Bacterial generated uremic toxins such as amines, phenols, and indoles are increasingly recognized as contributors of uremia (26). These microbial metabolites believed to be increased in the blood under two conditions, reduced renal clearance and increased colonic generation and absorption (26).

Fermentable fibers alter uremic toxins positively by depressing the colonic protein fermentation as a result of improved carbohydrate availability (108). Factors that are thought to promote uremic toxins “generation and absorption include an increased ratio of dietary protein to carbohydrate due to insufficient intake of fiber and/or reduced intestinal protein assimilation, as well as prolonged colonic transit time.” (26) While prebiotic fibers can provide the preferred substrate to promote the proliferation of saccharolytic bacteria, insoluble fibers can promote regularity, and increase bowel
movement frequency thus allowing rapid removal of nitrogenous molecules, while potentially expanding the availability of fermentable substrates distally.

**Uremic Molecule “Toxins”**

**Description**

Loss of kidney function leads to accumulation of uremic molecules above physiological levels that result in uremic symptoms or “uremia”. There is no single uremic molecule that can be singled out as a cause of uremia because retained solutes have variable characteristics such as “water solubility, dimensions, charge distribution, concentration, molecular mass, and protein binding” (25), which result in various symptoms among patients. There are 90 retention molecules that are identified as uremic as they become elevated above normal ranges (25). Elevated concentration and potential accumulation of uremic molecules and toxins in the interstitial fluids, inner medulla, collecting duct or any part of the renal cortex may lead to ischemia, inflammation or interstitial fibrosis due to potential toxic effect of some of these uremic toxic molecules on the glomerulus and tubules cells (5, 6, 8, 11, 13, 27, 28).

**Urea**

Urea is an organic compound with molecular mass of 60 Da. Urea makes up the largest proportion of nitrogenous waste in urine of humans. Blood urea nitrogen is a measure of the amount of nitrogen in the blood in the form of urea used as a measurement of kidney function. Normal levels for adults are between 7-25 mg/dL. Elevation of BUN greater than 60 mg/dL can be interpreted as moderate to severe kidney disease. However, BUN is not sensitive enough because it can be elevated independent of kidney disease, but is mostly used as an indicator of uremic molecules levels in the blood.
**p-Cresol**

*p*-Cresol is volatile phenol with a low molecular mass of 108.1 Da. It is partially lipophilic and strongly binds to plasma protein under normal conditions (111). *p*-Cresol is generated from partial breakdown of tyrosine and to a lesser extent phenylalanine mainly by aerobic *enterobacteria* and the anaerobic *clostridium perfringens* bacterial species (26, 52, 112). They are eliminated primarily in the urine as conjugates (113) with the main circulating form *p*-cresol sulfate (114, 115). *p*-Cresol is metabolized through inorganic sulfate via conjugation (sulfation) pathway and to a lesser extent to glucuronic acid (glucuronization) (113), (116, 117). However the removal of the unconjugated *p*-cresol is via the kidneys (111).

*p*-Cresol is associated with uremia (33), and along with its sulfate and glucuronide conjugates, is associated with CVD in HD patients, and is implicated in uremic immunodeficiency and endothelial dysfunction (118). Serum *p*-cresol levels are increasingly suggested as a cardiovascular risk marker (119), and cardiovascular death is the leading cause among renal patients. While these correlations point toward a relationship, it is not clear how modifiable is *p*-cresol as a risk factor for cardiovascular disease in CKD patients. In a prospective observational study in 499 CKD patients, *p*-cresol was a predictor of cardiovascular events independent of GFR with higher baseline concentrations of free *p*-cresol directly associated with cardiovascular events (120). *p*-Cresol and its main conjugated derivative, *p*-cresol sulfate, have been shown to contribute to endothelial dysfunction in HD patients (119), have pro-inflammatory effect on unstimulated leucocytes and activates leucocyte free radical production (121), and caused a significant increase in cellular inflammation in cultured mouse proximal renal tubular cells (5). Median total *p*-cresol for controls was 1.58 mg/L compared to
20.10 mg/L for HD patients (114). In another study, serum concentration averaged 1.14 mg/L for healthy individuals (122) while uremic outpatients had serum p-cresol levels of 6.70±2.11 mg/L (31).

Reduction of p-cresol generation and excretion has been demonstrated using fermentable fibers. In a study of healthy volunteers, decreased, urinary p-cresol excretion was observed by ingestion of a 50/50 v/v mixture of inulin and FOS (3). A 4-week trial in HD patients using a total of 20 g daily (10 g the first week) of the inulin and FOS reduced p-cresol sulfate concentrations in HD patients by 17% (7).

**Uremic Symptoms “Uremia”**

Uremic molecules such urea and p-cresols accumulation lead to uremic symptoms (1, 123). As such, uremic symptoms can range in both type and severity in individuals and is not necessarily present similarly among individuals. Symptoms of uremia include fatigue, intense itching, slowed thinking, anorexia (loss of appetite), fishy taste, constipation, impaired sleep, and restless leg syndrome (1, 61, 124-126). Excessive buildup of metabolic wastes causes acidosis induced anemia, damaged nerves and muscle cells, shortness of breath, nausea, vomiting, and malnutrition (61). Uremic symptoms are usually not detected until at least half of the kidney function capacity is lost compared to normal healthy individuals (1). Patients with 50% decline in kidney function are said to have a moderate decline in kidney function as classified by KDQOI with uremic symptoms being more moderate as compared to more severe stages. A review of the existing evidence by the KDQOI panel shows that eGFR of less than 60 mL/min/1.73m² is associated with reduced wellbeing (21), and cognitive impairments (127). Current treatment of uremia has poor outcomes compared to intervention of
other complications associated with chronic diseases such as diabetes because uremia symptoms cannot be traced to single molecule (1).

In the MDRD study, subjects with eGFR less than 55 mL/min/1.73m² had a correlated fatigue and reduced stamina (128), which may be attributed to muscle energy failure and neural defects (129). Simple nitrogen-containing uremic molecules such as amines may be responsible for impaired brain function as seen in human and animal studies (130-132), while indoles and phenols may interfere with central nervous system function due to structural similarity with neurotransmitters (1).

**Health-Related Quality of Life and Symptoms**

Health-related quality of life is a self-reported multidimensional measure usually of physical and mental health and how the disease interferes with day-to-day activities (133). Typically, patients suffering from chronic diseases see a reduction in quality of life as the disease progresses. In CKD, patients may experience a reduction in quality of life due to the number of neural, muscular, endocrine or metabolic symptoms. A valid qualitative tool to assess quality of life is the Kidney Disease Quality of Life (KDQOL-36™) (Appendix A). The self-assessment questionnaire is a kidney disease-specific measure and is divided into five categories. The first two are the *Physical Component Summary* subscale and *Mental Component Summary* subscale spanning the first 12 questions. These items cover general health, activity limits, ability to accomplish desired tasks, depression and anxiety, energy levels, and social activities. *Burden of Kidney Disease* subscale (questions 13-16) contains questions about how much kidney disease causes frustration, or makes the respondent feel like a burden, interferes with daily life, or takes up time. The *Symptoms/Problems List* subscale (17-28th questions) contains items about how bothered a respondent feels by various uremic symptoms. These
symptoms are faintness/dizziness, lack of appetite, sore muscles, itchy or dry skin, chest pain, cramps, or shortness of breath, feeling wash out or drained, numbness in the hands or feet, and nausea. The final subscale items (questions 29-36) refer to the Effects of Kidney Disease on daily life. They contain questions about how bothered respondent feels being dependent on doctors and other medical staff, by fluid limits, diet restrictions, stress or worries, ability to work around the house or travel, sex life, and personal appearance.

Chronic kidney disease patients in stage 4 or 5 score significantly lower on the Physical Component Summary subscale of the KDQOL-36™ compared to patients with hypertension, diabetes mellitus, asthma, chronic obstructive lung disease, or liver failure suggesting a significantly reduced quality of life (23-25). Chronic kidney disease patients with creatinine clearance <60 mL/min/1.73 m² reports lower physical function independent of age, sex, and other confounding factors (134). In dialysis patients, both the Physical and Mental Components were consistent predictors of hospitalizations and mortality rates with each point increase in either component reducing mortality relative risk by 2% and hospitalization relative risk by 2% and 1%, respectively (135).

Gorodetskaya et al. reported that each one mL/min/1.73 m² decline in eGFR per year was associated with 5.0 points changes in Burden of Kidney Disease subscale score in stage 4 and 5 CKD patients (136).

**Gastrointestinal Health and Well-being**

Gut health may be measured as the absence or presence of gut disorders or disease and severity. Complex factors including neural, and chemical, nutritional can alter the wellbeing perceptions originating from the gut. The area of wellbeing and functional gastrointestinal health is not well understood, and there is remarkably little
known about it. The autonomic nervous system governs gut function and thus regulatory reflexes and digestion processes are not consciously perceived. However, there is evidence that under certain circumstances sensory dysfunction of the gut may induce conscious perception (symptoms) (137). Therefore, it is possible that one’s wellbeing is affected by gastrointestinal changes.

One of the symptoms of uremia is constipation (138). Constipation can be particularly bothersome. The prolonged time between evacuations and difficulty emptying and associated straining can introduce a feeling of anxiety or un-wellness. Increased transit time in itself can be detrimental as proteins and amino acids can be exposed to increased bacterial activities that are thought to negatively impact the host cell due to the increased production of uremic molecules. Improved gut transit time may therefore produce desirable effects and may contribute to enhanced quality of life. Lower fiber intakes in the diet leads to reduced bulking. Bulking is necessary for regular defecation. Thus, reduced bowel frequency may be attributed to lower fiber intake in CKD. In addition, decreased movement of wastes along the colon will increase the time in which substances come in contact with epithelial cells and/or bacteria that may lead to increased protein fermentation when carbohydrate substrates are depleted in the colon, particularly in the distal colon. This will lead to increased generation and absorption of molecules that may otherwise be excreted in feces adding to the uremic load. Elevated levels of uremic toxins in the blood can impair neural and muscular functions (1), which in turn may affect transit time as well. The self-administrated version of the validated Gastrointestinal Symptom Rating Scale (GSRS) (Appendix B) can be used to assess gastrointestinal symptoms presence and severity in patients with
gastrointestinal disorders and patients who suffer from gastrointestinal related side effect secondary to non-gastrointestinal complaint (139, 140). The questionnaire includes 15 items representing five syndromes and uses a seven-point grade Likert-like scale defined by descriptive anchors where “1” represents no discomfort and “7” represents severe discomfort (maximum). The reflux syndrome (heartburn and acid regurgitation), abdominal pain syndrome (abdominal pain, hunger pains, and nausea), indigestion syndrome (rumbling, bloating, burping gas, and passing gas), diarrhea syndrome (diarrhea, loose stools, and urgent need for defecation), and constipation syndrome (constipation, hard stools, and feeling of incomplete evacuation) (141, 142).

**Appetite and Sleepiness**

Anorexia and weight loss are also symptoms of uremia. The simplified nutritional appetite questionnaire (SNAQ) (Appendix C) assessment tool is a short 4-item, validated clinical tool that objectively quantifies appetite in people (≥20 and older) at risk for weight loss (143). It is a reliable and efficient tool with singular construct of appetite with a view to prevent weight loss. A score of <14 may identify a person with anorexia and significant risk of weight loss.

The Epworth Sleepiness Scale (ESS) questionnaire is used to assess participants likelihood of daytime sleepiness (144). The test measures the average sleep propensity in the day time situations such as sitting and reading, sitting inactive in public environments, as a passenger in a car for an hour without a break, in a car while stopped for a few minutes in the traffic, and watching TV among others (Appendix D). Each situation can be given a scale option from “Would never doze” to “High chance of dozing”, 4-point scale. These options rank from 0 for the lowest scale option to 3 for the
“High chance of dozing”. The maximum possible score is 24 and the lowest possible is 0. Scores from 0-10 are within the normal range, 10-12 Borderline, 12-24 is abnormal.
CHAPTER 3
MATERIALS AND METHODS

Study 1: Overview

This study was designed as a pilot study to provide information on the effect of adequate fiber intake on blood markers, quality of life and tolerance in patients with CKD. Future directions would be to conduct studies with larger sample size, longer duration, and additional blood markers. The following section provides a description of the design and methods used in this pilot study. The Institutional Review Board 1 (IRB 1) at the University of Florida approved the study. Clinical visits were arranged at the Food Science and Human Nutrition Department (FSHN), University of Florida.

The Division of Nephrology, Hypertension, and Renal Transplantation within the Department of Medicine at the University of Florida in Gainesville, FL carried out the initial screening of patient medical records for patient referrals.

Study Design

A single blind, rolling admission intervention study was carried out for six weeks. Seventeen CKD patients were consented, 16 enrolled, and 15 completed the study (Figure 3-1). Participants were in various stages of the disease ranging from stage 3 to 5, but not on dialysis. Participants started a control period, which lasted two weeks. During this period, participants consumed control foods containing a small amount of fiber (1.6 g/day). After two weeks, participants started the second period in which they consumed closely matched foods with higher fiber content (23 g/d). Participants were seen in the clinical lab at the FSHN Department on days 1, 14, 28 and 42 (Figure 3-2). Two blood draws were collected during the control period. The intervention period lasted four weeks and two blood draws were made during that period. Visits consisted
of collecting blood samples and questionnaires data. Participants also received coaching about how to record dietary information using food records. During each visit participants picked up study related questionnaires, food records and study foods.

**Screening, Recruitment and Obtaining Consent**

Eligible participants were screened by clinical nurses from the Division of Nephrology, Hypertension, and Renal Transplantation within the Department of Medicine at the University of Florida in Gainesville, FL. Initial screening by the clinical nurses was done according to the following criteria: i) age 18 and older ii) eGFR of ≤ 50 mL/min/1.73 m² (mid stage 3, 4 and 5 but not on dialysis) iii) not diagnosed with acute kidney injury, glomerulonephritis, or lupus disease iii) not prescribed immunosuppressant medications) able to understand, verbalize and sign the informed consent in English. The clinical nurses of the nephrology department carried out the screening process and provided potential participants’ contact information after obtaining initial verbal consent as per IRB protocol number (16-2010).

Participants meeting the initial screening criteria and expressing interest in the study were contacted by the study coordinator by phone to further assess eligibility. Upon calling, a telephone script was used to provide potential participants with more details about the study and check for inclusion/exclusion criteria (Appendix E). Participants self-excluded if they had a history of kidney transplant, liver disease, or diagnosed with active gastrointestinal bleeding, were breastfeeding, or scheduled to be on dialysis or undergo transplantation within three months of study initiation, had changes in medication over the past four weeks prior to study initiation, were taking probiotic supplement and refused to discontinue, or were on immunosuppressant or
steroid medications. Potential participants who indicated that they met all inclusion/exclusion criteria according to protocol, and expressed willingness to participate were scheduled to come to the clinical lab in the FSHN building to review the informed consent. The informed consent was signed in person after allowing participants the chance to reflect on it.

**Study Foods**

The study protocol required participants to consume control foods for 14 days (control period) followed by intervention foods for 28 days (intervention period) according to study flow. For the control period, individually packaged, commercially-available Publix® chocolate chip cookies (1 serving/day), Kellogg's® Special K® bars (1 serving/day) and Kellogg's® Corn Pops with no fiber (2 servings/day) were provided to participants. Control foods were consumed from day 1 to day 14 providing a total of 1.6 g of dietary fiber a day (Table 3-1). For the intervention foods, individually packaged Weight Watchers® chocolate chip cookies (1 serving/day), Fiber One® bars (1 serving/day) and Kellogg's® Corn Pops with fiber (2 servings/day) were provided unchanged. Total fiber contents from these foods was 23 g/d mostly of fermentable fiber sources (chicory root fiber and resistant corn dextrin) and smaller amount of insoluble fiber source (pea hull) (Table 3-1). Unused foods were collected during clinic visits to determine fiber consumption and compliance to the protocol.
Study Food Packaging

Portions of breakfast cereal were weighed using electronic precision scales (Adam Equipment® PGW 1502e - Precision Balance). After weighing, portions of breakfast cereal were sealed in airtight vacuum-sealed bags. Cookies and bars were removed from their original wrapping and were sealed in airtight vacuum-sealed bags. All packaged foods were placed in large zip-lock bags labeled with participant study number and dated according to study period.

Clinic Visits

Consented participants visited the clinical lab four times throughout the study to collect blood samples, fill out questionnaires, check for adherence, and collect daily dairy and food records. Prior to each visit, participants were asked to fast overnight. Once participants arrived, a licensed phlebotomist drew a 10 mL blood sample in the blood draw room of the FSHN clinical lab. Participants’ weights and heights were measured using conventional methods described below. After completing the KDQOL-36™, the SNAQ, GSRS and the ESS questionnaires and after questions and concerns were addressed, each participant was given a two-week supply of the study foods according to the period of the study. In addition, a food journal was given during visits 1, and 3 and the GSRS questionnaire was given on visits 2 and 3 in a dated envelop to be filled out the following week.

Qualitative Questionnaires

Gastrointestinal Symptoms and Daily Diary

A self-administrated tool to assess changes in gastrointestinal symptoms was administered to participants every week of the study. The GSRS was administered during clinic visits at days 1, 14, 28, and 42. Additionally, participants were given the
GSRS questionnaire in dated envelops during clinic visits 2 and 3 to be filled out at home the following week (weeks 3, and 5). A daily diary workbook (Appendix F) was given to participants during the first visit. The daily diary contained 42 tear-out pages for each day of the study in which participants were asked to bring back at each visit. During visits, dated pages were removed from the workbook.

**Quality of Life, Appetite and Risk of Weight Loss**

Patients' Health-Related Quality of Life was assessed using the self-assessment KDQOL-36™ questionnaire. The questionnaire was administered to participants every two weeks during scheduled clinic visits. Higher scores for the KDQOL-36™ indicates better results. Appetite and risk of weight loss were assessed during clinic visits using the SNAQ.

**Anthropometric Measurements**

Patients' weights and heights were measured using conventional methods with shoes removed. Height was measured the first visit using Ayrton® 226 Hite-Rite stadiometer, and weight was monitored during each clinical visit using Seca® 874 portable flat platform scale. Both devices were calibrated prior to use.

**Food Intake Assessment**

Participants were given a food record (Appendix G) to log intakes of foods and beverages for three days during each period. Food journal workbooks (Appendix H) containing a sample page of food record, tips to follow for a consistent and successful logging of food entries, and visual examples of food servings were also provided. The first 3-day food record was collected for the second week of the control period. The second 3-day food journal was collected for the last week of the intervention period. To ensure compliance and consistency, participants were briefly coached about the use of
the food journal, and how to log related information. In addition, measuring bowls (Measure Up Bowl™, Measure Up Bowel LLC, Brooklyn, NY) were given to participants to use throughout the study for consistency in reporting portion quantities. The coaching sessions were administered during clinic visits 1 and 3.

**Nutrient Assessment**

Three-day food records were analyzed using (Food Processor® version 10.4.0.0 ESHA Research Inc., Salem, OR) for nutrient and energy intake. Foods equivalent or similar to those eaten by the participants during the study period were chosen from the database. For unique foods, such as combination of salads and desserts individual ingredients were entered into the database.

**Blood Samples Collection**

Blood samples were collected in two 5 mL tubes and were placed in upright position unstirred for 30 minutes to allow specimen clotting. All samples were centrifuged and then placed in stoppered containers with ice at 2-8 °C. All samples were processed within 12 hours of centrifugation. Aurora Diagnostics Clinical Services, a Florida-based clinical laboratory, provided blood assay services.

**Assays**

All Assays were performed using SIEMENS ADVIA® Clinical Chemistry System

**Blood Urea Nitrogen**

Blood urea nitrogen was assayed using the Urea Nitrogen Concentrated Reagent method based on Roch-Ramel enzymatic reaction using urease and glutamate dehydrogenase (145). Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide. The ammonia reacts with α-oxoglutarate in the
presence of glutamate dehydrogenase and NADH. The oxidation of NADH to NAD is measured as an inverse rate reaction at 340/410 nm.

**Serum Creatinine**

Serum creatinine reacts with picric acid in an alkaline medium to produce a red-colored creatinine-picrate complex. The rate of complex formation is measured at 505/571 nm and is proportional to the creatinine concentration. Serum creatinine is analyzed by modification of the Jaffé method (146). The method is modified by using rate blanking and intercepts correction to minimize bilirubin interference and serum measurements are corrected by subtracting 0.3 mg/dl (26.5 μmol/L) from each result to correct for the non-specific serum protein interactions with reagent which produces a positive bias of 0.3 mg/dl (26.5 μmol/L).

**Serum Triglycerides (TG)**

The triglycerides are hydrolyzed to glycerol and free fatty acids by lipase. The glycerol was then converted to glycerol-3-phosphate by glycerol kinase followed by its conversion by glycerol-3-phosphate-oxidase to hydrogen peroxide and dihydroxyacetone phosphate. A colored complex is formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase. The absorbance of the complex is measured as an endpoint reaction at 505/694 nm.
**Total Serum Cholesterol**

Cholesterol esters are hydrolyzed by cholesterol esterase to cholesterol and free fatty acids. The cholesterol is converted to choletenone by cholesterol oxidase in the presence of oxygen to form hydrogen peroxide. A colored complex is formed from hydrogen peroxide, 4-aminoantipyrine and phenol under the catalytic influence of peroxidase. The absorbance of the complex is measured as an endpoint reaction at 505/694 nm.

**High Density Lipoprotein (HDL)**

Cholesterol esters in serum were hydrolyzed by cholesterol esterase. The free cholesterol produced is oxidized by cholesterol oxidase to choletenone with the simultaneous production of hydrogen peroxide, which oxidatively couples with 4-aminoantipyrine and phenol in the presence of peroxidase to yield a chromophore. The red quinoneimine dye formed is measured spectrophotometrically at 596 nm as an increase in absorbance.

**Low Density Lipoprotein (LDL)**

LDL cholesterol is assayed in two steps. Step 1: Release of cholesterol from non-LDL particles followed by degradation of the cholesterol by cholesterol esterase and cholesterol oxidase and elimination by catalase of the resulting hydrogen peroxide.

Step 2: Release of cholesterol from LDL-cholesterol by a surfactant in reagent 2, followed by degradation of the cholesterol by cholesterol esterase and cholesterol oxidase in the same manner as in the first step, except that the catalase in reagent 1 is inhibited by sodium azid in reagent 2. The intensity of the quinoneimine dye produced in the reaction is directly proportional to the LDL-cholesterol concentration when measured at 596 nm.
Glucose

Glucose is phosphorylated by adenosine triphosphate in the presence of hexokinase. The glucose-6-phosphate that forms is oxidized in the presence of glucose-6-phosphate dehydrogenase causing the reduction of NAD to NADH. The absorbance of NADH is measured as an endpoint reaction at 340-410 nm.

eGFR Calculation

Estimated Glomerular Filtration Rate was calculated based on the non-standardized original MDRD study equation (147) as follows:

\[ \text{eGFR} = 186 \times \text{SCr}^{-1.154} \times \text{age}^{-0.203} \times 1.212 \text{ (if African American)} \times 0.742 \text{ (if female)} \]

General Statistical Approach

All data are presented as mean ± SE (standard error). Correlations were determined using Pearson’s test. Paired t-test was used for all comparisons of parameters for each test between control and intervention periods. Significance was concluded when \( p<0.05 \).

Study 2: Overview

This study was designed to provide further information about the effect of adequate fiber intake on uremic blood markers, eGFR, symptoms and quality of life. The study was conducted to determine the effect of insoluble fiber alone and in combination with inulin/FOS supplement on uremic blood markers, eGFR and quality of life. This section provides a description of the design and methods used. The IRB 1 at the University of Florida approved the study (IRB protocol 580-2011). The Division of Nephrology, Hypertension, and Renal Transplantation within the Department of
Medicine at the University of Florida in Gainesville, FL carried out the initial screening of patient medical records for patients’ referrals.

**Study Design**

A twelve-week, single blind, intervention study with escalating fiber regimen. Study was carried out with CKD patients’ stages 3 to 5, non-dialysis. The study required participants to consume control muffins and 5.5 g of sucrose for two weeks (control period) followed by consumption of pea hull fiber containing muffins and sucrose for four weeks (pea hull fiber period), and finally, pea hull fiber containing muffins and Frutafit® HD inulin supplement for six weeks (pea hull and inulin fiber period) (Figure 3-3). The 5.5 g of sucrose was chosen as a control for inulin and the amount was selected to match inulin energy content.

Two blood samples were collected during each period. During the control period at 0 and 2 weeks. During the pea hull fiber period blood samples were collected at 4 and 6 weeks. For the pea hull and inulin fiber period, blood samples were collected at 9 and 12 weeks. Additionally, two single blood samples were collected during the study for p-cresol analysis. One sample was collected during first week of the control period and another sample was collected at week 12. Questionnaires and 3-day food records were delivered (dropped off or mailed) to participants prior to the day in which they were required. Participants were asked to bring any leftover bags of sucrose and inulin at the end of the study.

**Screening and Recruitments and Obtaining Consent**

Eligible participants were screened for by clinical nurses from the Division of Nephrology, Hypertension, and Renal Transplantation within the Department of Medicine at the University of Florida in Gainesville, FL (Figure 3-4). Initial screening by
the clinical nurses was done according to the following criteria: i) age 18 and older ii) have an eGFR of $\leq 50 \text{ mL/min/1.73 m}^2$ (stage 3, 4 and 5, but not on dialysis) iii) not diagnosed with acute kidney injury, glomerulonephritis or lupus disease i) not prescribed immunosuppressant medications iii) able to understand, verbalize and sign the informed consent in English. Participants meeting the initial screening criteria and expressing interest in the study were contacted by the study coordinator by phone to further assess eligibility (Appendix I).

Participants were excluded if they had a history of kidney transplant, liver disease, active gastrointestinal bleeding, lactating, or scheduled to be on dialysis or undergo transplantation during study duration, had celiac disease, food allergies or were not willing/could not discontinue the use of fiber/prebiotic/probiotics containing products for two weeks prior to first blood draw, or were scheduled to have a major surgical procedure during the duration of the study. Potential participants who were eligible and expressed willingness to participate were then scheduled to come to the clinical lab in the FSHN building to review the informed consent. Potential participants who were unable to come to the lab were offered the option to be visited at home for consenting according to IRB protocol. The informed consent signed in person after allowing participants the chance to reflect on it. Consented participants received all study supply in mail or in person according to study calendar.

**Study Food**

During the control period participants consumed control muffins with 1 g fiber (Orange Blueberry Muffin with No Pea Fiber, AC125-72-1b, Penford Ingredients, Centennial, CO. USA) (Appendix J) and approximately 5.5 g sucrose. Immediately after,
participants started the pea hull fiber period that lasted four weeks. During this period, participants consumed muffins containing 10 g/d of pea hull fiber (Orange Blueberry Muffin with Pea Fiber, AC125-72-2d, Penford Ingredients, Centennial, CO. USA) (Appendix J) and approximately 5.5 g/d of sucrose to serve as a control. After six weeks of the control and pea hull fiber periods, participants started the pea hull and inulin fiber period which lasted six weeks consuming a total of 23.5 g/d of fiber everyday (10 g/d of pea hull in muffins and 13.5 g/d of fiber from a supplement (inulin/FOS) (see product sheet Appendix K) (Frutafit®HD, SENSUS, The Netherlands) (Nutrient Composition Table 3-2). Muffins were delivered every two weeks by overnight UPS® carrier or dropped off by study coordinator. Sucrose and inulin were delivered by mail or dropped off to participants prior to the day in which they were needed. Penford Ingredients (Centennial, CO) formulated control and fiber-containing muffins.

Study Food Packaging

Prior to packaging, sucrose and inulin were measured using the precision scales (Adam Equipment® PGW 1502e - Precision Balance). Packaged products were placed in corresponding bins during packaging periods to avoid mixing. Control products were weighed and packaged on separate days. Once all control products were prepared, intervention products were prepared in a similar fashion. Muffins were placed in airtight vacuum-sealed bags, while sucrose and inulin were placed in small 2x2 inch clear, double sealed bags.

Demographic and Baseline Data Collection

After the informed consent was obtained, participants provided information about age, race, medical history, medications, antibiotics use, supplement use, nutritional foods and beverages using the baseline questionnaire (Appendix L).
Food Intake and Nutrient Assessment

Three-day food records (Appendix M) were mailed to participants in pre-paid envelopes for each period of the study. Food records were explained during consenting process. Food intake and nutrient assessment analysis was conducted using (Food Processor®, version 10.6.0.0 ESHA Research Inc., Salem, OR). Foods equivalent or similar to those eaten by the participants during the study period were chosen from the database. For unique foods, such as combination salads and desserts individual ingredients were entered into the database.

Qualitative Questionnaires

The self-administered KDQOL-36™ questionnaire and a bowel frequency questionnaire (Appendix N) were administered to participants three times during the study. Questionnaires were mailed for each period. Mailed envelopes contained returning envelop with pre-paid postages.

Clinic Visits

Participants visited the FSHN clinical lab during the first and the last week of the study to provide anthropometric measurements, and additional blood samples to determine changes in p-cresol concentrations. Patients' weights and heights were measured using conventional methods with shoes removed. Weight was measured using portable flat platform scale (Seca® 874). For height, portable stadiometer (Seca® 213) was used. A licensed phlebotomist drew a maximum of 10 mL blood during each visit using 10mL plasma EDTA tubes in which they were centrifuged. Samples were then transferred into two 3 mL vials and stored within 45 minutes in -80 °C until analysis of total plasma p-cresol.
Quest Diagnostics

Quest Diagnostics Inc. provides the diagnostic testing and services with several facilities around the state of Florida. Quest Diagnostics Inc. was contracted to provide blood draw and assays services according to protocol. Blood draw requisitions were mailed to participants prior to the day in which they were required. Participants were requested to visit nearby Quest Diagnostics facilities during the specified dates according to study calendar for total of six visits.

Assays

BUN and Serum Creatinine

Serum urea was determined by a kinetic method using urease and glutamate dehydrogenase (145). Serum creatinine was analysed by a kinetic method based on the Jaffe’s reaction (146), both methods were described earlier. Assays were performed using Olympus AU5400® Chemistry System.

Ammonia

Enzymatic method, with glutamate dehydrogenase was applied to derive and measure ammonia (148, 149). Glutamate dehydrogenase catalyzes the reductive amination of 2-oxoglutarate with NH₄⁺ and NADPH to form glutamate and NADP⁺. The concentration of the NADP⁺ formed is directly proportional to the ammonia concentration. It is determined by measuring the decrease in absorbance at 340 nm. Assay was performed using COBAS INTEGRA® 800.

CRP

Particle enhanced turbidimetric assay was used to measure CRP (150, 151). Human CRP agglutinates with latex particles coated with monoclonal anti-CRP
antibodies. The precipitate is determined turbidimetrically at 552 nm. Assay was performed using COBAS INTEGRA® 800.

**Cystatin C**

Cystatin C was measured by the **N Latex cystatinC assay on the SIEMENS BN™ II nephelometer system using a particle-enhanced immunonephelometric assay**. In this assay, polystyrene beads coated with rabbit antibodies to cystatin C agglutinate when mixed with samples containing cystatin C. The intensity of the scattered light in the nephelometer depends on the concentration of cystatin C (antigen) in the sample. This concentration is determined by comparison with dilutions of a calibrator (152).

**GC-MS Analysis of Total p-Cresol in Plasma**

Concentrations of total p-cresol in plasma were measured using a gas chromatography-mass spectrometry (GC-MS) method on plasma samples stored at -80 °C prior to analysis. After combined acid and heat deproteinization and deconjugation (hydrolysis of conjugates), p-cresol was extracted in ethyl acetate and injected into the Thermo Finnigan Trace DSQ Single Quadrupole GC-MS instrument. For use as standard, p-cresol was purchased from Acros Organics (part of Thermo Fisher Scientific). The p-cresol-d₇ was obtained from C/D/N ISOTOPES and used as internal standard. For the heat-acid intervention of the plasma samples and subsequent cresol extraction, a previously described method (114) was used, with minor modifications.

**eGFR Calculations**

Estimated Glomerular Filtration Rate was calculated based on the CKI-EPI creatinine equation adjusted for age, sex, and race (76):

\[
eGFR = 141 \times \min(\text{Scr}/k, 1)^{a} \times \max(\text{Scr}/k, 1)^{-1.209} \times 0.993^{\text{age}} \\
\times [x\ 1.018\ \text{if female}]\ [x\ 1.159\ \text{if black}]
\]

Scr is serum creatinine, k is 0.7 for females and 0.9 for males.
a is -0.329 for females and -0.411 for males

The CKD-EPI cystatin C equation by Inker et al. (78):

\[
eGFR = 133 \times \min(\text{Scys}/0.8, 1) - 0.499 \times \max(\text{Scys}/0.8, 1) - 1.328 \times 0.996 \times \text{age} \\
\text{[ x 0.932 if female ]} \\
\text{Scys is serum cystatin C.}
\]

The CKD-EPI creatinine-cystatin C equation adjusted for age, sex, and race by Inker et al. (78):

\[
eGFR = 135 \times \min(\text{Scr}/k, 1) - a \times \max(\text{Scr}/k, 1) - 0.601 \times \min(\text{Scys}/0.8, 1) - 0.375 \times \\
\max(\text{Scys}/0.8, 1) - 0.711 \times 0.995 \times \text{age} \text{[ x 0.969 if female ]} \text{[ x 1.08 if black ]} \\
\text{Scr is serum creatinine and Scys is serum cystatin C. k is 0.7 for females and 0.9 for males. a is -0.248 for females and -0.207 for males}
\]

**General Statistical Approach**

All data are presented as mean ± SE (standard error). To determine significance, ANOVA was used to compare each mean and overall model significance was determined when p<0.05. For bowel movement frequency, the KDQOL-36™ subscales, and the individual symptoms list in the *Symptoms/Problems List* subscale of the KDQOL-36™, multiple paired t-tests were calculated independent of the ANOVA significance. P-value of <0.05 is considered for unadjusted significance level. For significant after adjustment, Bonferonni correction was used and significance was set at 0.01667. The natural logarithmic transformation was applied to meet the assumptions of normality when comparing the two transformed means of total p-cresol.
Table 3-1. Nutrient composition of foods provided in the control and fiber intervention periods

<table>
<thead>
<tr>
<th></th>
<th>Weight (g)</th>
<th>Quantities</th>
<th>Energy (kcal)</th>
<th>Fiber (g)</th>
<th>Fat (g)</th>
<th>Protein (g)</th>
<th>Carbohydrates (g)</th>
<th>Source of Added Fiber</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control foods</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kellogg’s® Corn Pops</td>
<td>58</td>
<td>2 oz</td>
<td>220</td>
<td>&lt;1</td>
<td>0</td>
<td>2</td>
<td>52</td>
<td>None</td>
</tr>
<tr>
<td>(Battle Creek, MI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Publix® Chocolate Chip</td>
<td>24</td>
<td>2 cookies</td>
<td>120</td>
<td>&lt;1</td>
<td>6</td>
<td>1</td>
<td>15</td>
<td>None</td>
</tr>
<tr>
<td>Cookies (Lakeland, FL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kellogg’s Special K Bar</td>
<td>23</td>
<td>1 bar</td>
<td>90</td>
<td>&lt;1</td>
<td>1.5</td>
<td>1</td>
<td>17</td>
<td>None</td>
</tr>
<tr>
<td>(Battle Creek, MI)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Fiber intervention</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kellogg’s® Corn Pops with Fiber (Battle Creek, MI)</td>
<td>64</td>
<td>2 oz</td>
<td>240</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>58</td>
<td>Resistant corn dextrin</td>
</tr>
<tr>
<td>Weight Watchers® Chocolate Chip Cookies (Jerico, NY)</td>
<td>50</td>
<td>2 cookies</td>
<td>180</td>
<td>8</td>
<td>5</td>
<td>2</td>
<td>36</td>
<td>Inulin, pea hull fiber</td>
</tr>
<tr>
<td>General Mills FiberOne® Bar (Minneapolis, MN)</td>
<td>40</td>
<td>1 bar</td>
<td>140</td>
<td>9</td>
<td>4</td>
<td>2</td>
<td>29</td>
<td>Chicory root fiber (inulin)</td>
</tr>
</tbody>
</table>
Table 3-2. Nutrient composition of foods and supplements provided

<table>
<thead>
<tr>
<th></th>
<th>Muffins (Control)</th>
<th>Muffins (Intervention)</th>
<th>Frutafit® HD</th>
<th>Sucrose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fiber g</td>
<td>1</td>
<td>10</td>
<td>13.5</td>
<td>0</td>
</tr>
<tr>
<td>Energy kcal</td>
<td>180</td>
<td>170</td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>Carb g</td>
<td>30</td>
<td>26</td>
<td>0</td>
<td>5.5</td>
</tr>
<tr>
<td>Fat g</td>
<td>4.5</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Protein g</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Figure 3-1. Renal Study 1: screening and recruitment
Figure 3-2. Renal Study 1: study design

Figure 3-3. Renal Study 2: study design
Figure 3-4. Renal Study 2: screening and recruitment
CHAPTER 4
RESULTS STUDY 1

Demographics Data

Sixteen participants were enrolled in Study 1. Average age was 66 ± 4 years (mean ± SE). Nine females and six males completed the study with eight participants diagnosed with type 2 diabetes. Eleven participants were white, and four were African American. Four patents were categorize with stage 3 CKD, ten with stage 4, and one with stage 5.

Weight

BMI was calculated based on recorded measurements of height and weight. Weight was 89±6 during control and 90±6 during intervention, which was not significantly different.

Nutrient and Fiber Intake

There was no difference in energy, protein, potassium or phosphorous intakes during the control and the intervention period (Table 4-1). Fat intake decreased from 57±6 g/d to 47±6 g/d (p<0.05). Mean total fiber intake increased from 10.7±0.8 g/d (9.3 g usual intake and 1.4 g from control foods) during the control period to 26.5±2.2 g/d (9.7 g usual intake and 16.8 g from intervention foods) during the intervention period (p<0.0001).

Compliance Data

During the control period, 4 servings of study foods provided 1.6 g of fiber a day. Compliance was determined by amount of food consumed compared to food distributed. Study foods that were returned were weighed and counted after each period. Study compliance was consistent throughout the study. During the
control period, participants were 85% compliant with the control foods and the mean fiber intake was 1.4±0.1 g/d from these foods. Compliance during the 28 days of the intervention period was 73% with an estimated fiber intake of 16.8±1.5 g/d from intervention study foods (12.5 of which came from fermentable fibers (8.5 g inulin, 4 g resistant corn dextrin) and 4 g/d from pea hull fiber. Compliance was not significantly different between control and intervention periods. Compliance was highest with cereal bars and cookies (82% and 81% respectively) and lowest for cereal (68%).

**Bowel Movement Frequency**

A daily journal was used to assess bowel movement frequency and changes in patients' medications. Figure 4-1 represents the average number of bowel movements before and with fiber intervention. Daily bowel movement frequency increased from 1.3±0.2 to 1.6±0.2, p<0.05. Participants did not report any changes in medication use. Two participants reported antibiotic use during the study.

**Lipid and Blood Glucose**

There were no changes in fasting blood glucose, HDL or triglycerides between control and intervention periods. Total cholesterol was significantly lower between the two periods declining from 175±12 mg/dL to 167±11 mg/dL (p<0.05) (Table 4-2). LDL decreased from 100±8 mg/dL during control to 93±7 mg/dL at intervention (p=0.053). Total cholesterol/HDL ratio declined significantly from 4.0±0.3 during control to 3.7±0.2 during intervention (p<0.05).
Total Cholesterol, Dietary Fat and Fiber Correlations

Total Cholesterol correlated with total fat intake during control (r=0.62, p<0.05) and intervention (r=0.6, p<0.05) but not total fiber during these two periods.

BUN, Serum Creatinine and eGFR

All pair-wise comparisons between each day for BUN, serum creatinine, and eGFR are shown in (Table 4–2). BUN decreased by 10.5% from 38±6 mg/dL during control to 34±5 mg/dL during intervention period (p=0.058).

Serum creatinine decreased by 8% from 2.40±0.29 mg/dL during control to 2.20±0.26 mg/dL (p<0.005).

eGFR was significantly higher increasing by 10% from 30±3 mL/min/1.73m² during control to 33±4 mL/min/1.73m² during intervention (p<0.005).

Qualitative Results

SNAQ

Participants reported an average score of 15±1 points during control SNAQ questionnaire. A score of ≤14 indicates poor appetite and significant risk of at least 5% weight loss within six months. There were no significant changes in the overall score mean during intervention (14±1).

Epworth Sleepiness Scale (ESS)

Mean scores for the sleepiness scale decreased from 10±1 during control to 8±1 during intervention (p<0.05). There were five participants with scores of 10 or higher, 33% of the sample during control, compared to three participants during intervention or 20% of the sample. The scale score range of 10-12
indicates borderline risk of day time sleep propensity while a score range of 12-24 indicates an abnormal tendency.

**GSRS**

Mean GSRS scores did not change between the two periods with a mean of 23±1 during control compared to a score of 22±1 during intervention. There were no differences in the sub-scales for each of the syndromes.

**Health-Related Quality of Life**

There were no changes in KDQOL-36™ overall mean score. In addition, there were no significant differences in the *Symptom/Problem List, Effects of Kidney Disease, and Burden of Kidney Disease* subscales mean scores between the periods. However, *Physical Component Summary* subscale mean score was significantly higher increasing from 31±2 during control to 35±3 during intervention (p<0.05). Mean score for the *Mental Component Summary* subscale decreased significantly from 53±2 during control to 48±2 during intervention (p<0.05) Table 4-2.
Table 4-1. Energy, fiber, and macronutrients intakes

<table>
<thead>
<tr>
<th></th>
<th>Control Period</th>
<th>Intervention Period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy Kcal</td>
<td>1665±71</td>
<td>1576±92</td>
</tr>
<tr>
<td>Carbohydrate g</td>
<td>214±10</td>
<td>227±13</td>
</tr>
<tr>
<td>Total Fiber g</td>
<td>10.7±0.8</td>
<td>26.5±2.2&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Usual Fiber Intake g</td>
<td>9.3</td>
<td>9.7</td>
</tr>
<tr>
<td>Control Foods Fiber g</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Intervention Foods Fiber g</td>
<td></td>
<td>16.8</td>
</tr>
<tr>
<td>Protein g</td>
<td>59±7</td>
<td>56±6</td>
</tr>
<tr>
<td>Fat g</td>
<td>57±6</td>
<td>47±6&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are means±SE, n=12. Three participants did not complete/provide the daily records.

<sup>a</sup> significance indicated at p<0.0001.

<sup>b</sup> significance indicated at p<0.05.
<table>
<thead>
<tr>
<th></th>
<th>Control Period (no fiber added)</th>
<th>Intervention Period with added fiber</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (kg)</td>
<td>89±6</td>
<td>90±6</td>
<td>NS</td>
</tr>
<tr>
<td>Clinical Markers (reference range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BUN mg/dL (7-25 mg/dL)</td>
<td>38±6</td>
<td>34±5</td>
<td>P=0.058</td>
</tr>
<tr>
<td>Serum Creatinine mg/dL (0.60-1.30 mg/dL)</td>
<td>2.40±0.29</td>
<td>2.20±0.26</td>
<td>P&lt;0.005</td>
</tr>
<tr>
<td>eGFR mL/min/1.73m² (≥60 mL/min/1.73m²)</td>
<td>30±3</td>
<td>33±4</td>
<td>P&lt;0.005</td>
</tr>
<tr>
<td>Glucose mg/dL (65-106 mg/dL)</td>
<td>136±29</td>
<td>146±25</td>
<td>NS</td>
</tr>
<tr>
<td>LDL mg/dL (&lt;130 mg/dL)</td>
<td>100±8</td>
<td>93±7</td>
<td>P=0.053</td>
</tr>
<tr>
<td>HDL mg/dL (40-50 mg/dL)</td>
<td>47±4</td>
<td>47±4</td>
<td>NS</td>
</tr>
<tr>
<td>Total CHOL mg/dL (0-200 mg/dL)</td>
<td>175±12</td>
<td>167±11</td>
<td>P=0.015</td>
</tr>
<tr>
<td>TG mg/dL (30-150 mg/dL)</td>
<td>165±21</td>
<td>154±21</td>
<td>NS</td>
</tr>
<tr>
<td>CHOL/HDL Ratio</td>
<td>4.0±0.3</td>
<td>3.7±0.2</td>
<td>P=0.02</td>
</tr>
<tr>
<td>KDQOL-36™</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptom/Problem List (17-28)</td>
<td>78±3</td>
<td>80±3</td>
<td>NS</td>
</tr>
<tr>
<td>Effects of Kidney Disease (29-36)</td>
<td>84±4</td>
<td>87±3</td>
<td>NS</td>
</tr>
<tr>
<td>Burden of Kidney Disease (13-16)</td>
<td>67±6</td>
<td>73±6</td>
<td>NS</td>
</tr>
<tr>
<td>Physical Component Summary (1-12)</td>
<td>31±2</td>
<td>35±3</td>
<td>P=0.02</td>
</tr>
<tr>
<td>Mental Component Summary (1-12)</td>
<td>53±2</td>
<td>48±2</td>
<td>P=0.01</td>
</tr>
<tr>
<td>Overall Mean Scores</td>
<td>63±3</td>
<td>64±3</td>
<td>NS</td>
</tr>
<tr>
<td>Symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SNAQ</td>
<td>14±1</td>
<td>14±1</td>
<td>NS</td>
</tr>
<tr>
<td>GSRS</td>
<td>23±1</td>
<td>22±1</td>
<td>NS</td>
</tr>
<tr>
<td>ESS</td>
<td>10±1</td>
<td>8±1</td>
<td>P=0.04</td>
</tr>
</tbody>
</table>

Data are mean±SE. Means are compared using paired t-test with significance set at p<0.05, n=15. * Means are reported for n =13.
Figure 4-1. Average bowel movement per day.
Data presented as mean ± SE, n=12, p < 0.05.
CHAPTER 5
RESULTS STUDY 2

Demographic Characteristics

Thirteen participants were enrolled in Renal Study 2 with a mean age of 65±3 years (mean±SE). Seven females and six males completed the study. Most participants had hypertension (12, 92%), while only 3 (23%) had type 2 diabetes. There was no changes in body weight (Table 5-1).

Nutrient and Fiber Intake

Mean fiber intake increased from 16.6±1.7 (15.6 g from usual intake and 1 g from control muffins) to 26.5±2.4 g/d (17.3 g from background diet and 9.2 g from intervention muffins) (p<0.0001) in the pea hull fiber period, and 34.5±2.2 g/d (14.9 g from background diet, 9.2 g from intervention muffins and 10.4 g from inulin/FOS supplement) during the pea hull and inulin fiber period compared to control (p<0.0001) and pea hull fiber period (p=0.003) (Table 5-2). No changes in energy, fat, protein, or carbohydrate, intakes were observed.

Compliance

Compliance was 97% for muffins during control period. For intervention muffins, compliance was 92% providing 9.2±0.2 g/day from pea hull fiber during the intervention periods. Compliance was 91% for sucrose supplement during the first six weeks of the study and before inulin intervention. Inulin compliant was at 84% (57% - 100%) or 11.3±0.6 g/day (7.7 g/d – 13.5 g/d).

Reported Symptoms

There were no significant side effects reported prior to inulin supplement introduction. Upon supplement introduction, participants complained of flatulence (mild
n=5) moderate (n=1) severe (n=2), feeling bloated (n=5), frequent bowel movement, watery stool or lose stool (n=3), rumbling (n=1), nausea (n=1). Three participants did not report any symptoms throughout the study. After being advised to divide the supplement into smaller portions during the day, symptoms subsided or improved significantly (n=8). Two participants reduced their supplement intake by 25 to 50% and reported improved symptoms. Other complaints were related to taste and inability to dissolve inulin in cold beverages. No adverse events were reported during the study.

**Bowel Movement Frequency**

A 5-day bowel movement frequency journal was used during first week of control period (control muffin and sucrose), last week of the pea hull fiber period, and last week of the pea hull and inulin fiber period. Mean bowel movement frequency increased from 1.4±0.2 to 1.9±0.3 (n=13, p=0.026) during pea hull fiber period and remained significantly higher during the pea hull and inulin fiber period (1.9±0.3, p=0.044, Figure 5-1).

**BUN, Ammonia, Serum Creatinine, Cystatin C, CRP and eGFR**

BUN, ammonia, creatinine, cystatin C, CRP, and eGFR were not significantly different between periods (Table 5-3).

**p-Cresol**

The mean difference for total plasma p-cresol between control and post intervention measure was -20% changing from 7.25±1.74 mg/L (LN 1.73) to 5.82±1.72 (N 1.27) mg/L (significance based on natural log mean difference, p<0.05) Table 5-3. Percentage change averaged -24% (+54% to -83%, median -49%) Figure 5-2. When excluding non-compliant patients (defined by < 70% consumption of inulin), mean difference for total plasma p-cresol between control and post intervention measure was
-37% (n=10) changing from 6.71±1.98 (LN 1.63) mg/L to 4.22±1.16 (1.04) mg/L (significance based on natural log mean difference, p<0.05). Figure 5-2. Results are GC-MS output adjusted for method of extraction by adding 10% to the GC-MS output to account for the difference between the 90% efficiency of the extraction method and the absolute total in the blood (114).

**KDQOL-36™**

Higher KDQOL-36™ scores indicate better results. Overall mean score for KDQOL-36™ questionnaire was not significantly different between periods. There were no changes in the *Symptom/Problem List, Effects of Kidney Disease*, and *Mental Component Summary* subscale scores between periods. However, *Burden of Kidney Disease* subscale mean score increased from 65±8 at baseline to 77±7 (p= 0.014) during the pea hull fiber period (Table 5-3). Mean score for the *Physical Component Summary* subscale increased from 37±3 at baseline to 41±3 during the pea hull fiber period (p=0.046) (Table 5-3).

Using the *Symptom/Problem List* (Questions 17-27) of the KDQOL-36™ to assess a host of uremic symptoms. During the pea hull and inulin fiber period, participants reported a scores of 86±9 for the dry skin compared to control 69±11 (P=0.04). During the pea hull and inulin fiber period participants also reported a score of 83±6 for the numbness in hand or feet compared to control 65±9 (P=0.02) (Table 5-4). Higher scores indicate that participants were less bothered by these reported symptoms. Responses to how bothered by other symptoms did not differ between periods.
Table 5-1. Participants’ characteristics

<table>
<thead>
<tr>
<th>Category</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M/F)</td>
<td>6/7 (47%/53%)</td>
</tr>
<tr>
<td>CKD by stage</td>
<td></td>
</tr>
<tr>
<td>-Stage 3</td>
<td>n=10</td>
</tr>
<tr>
<td>-Stage 4</td>
<td>n=1</td>
</tr>
<tr>
<td>-Stage 5</td>
<td>n=2</td>
</tr>
<tr>
<td>Age y</td>
<td>65±3</td>
</tr>
<tr>
<td>Race (White/Black/Other)</td>
<td>7/5/1 (54%/38%/8%)</td>
</tr>
<tr>
<td>Pre-weight kg</td>
<td>86±4</td>
</tr>
<tr>
<td>Post-weight kg</td>
<td>86±4</td>
</tr>
<tr>
<td>Type 2 diabetes</td>
<td>3 (23%)</td>
</tr>
<tr>
<td>Hypertension</td>
<td>12 (92%)</td>
</tr>
</tbody>
</table>

Data presented as percentage and mean ± SE, n=13.

Table 5-2. Macronutrients, energy and fiber intakes compared between each period

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Pea hull fiber period</th>
<th>Pea hull and inulin fiber period</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories Kcal</td>
<td>1743±123</td>
<td>1733±145</td>
<td>1523±122</td>
</tr>
<tr>
<td>Carbohydrate g</td>
<td>225±19</td>
<td>213±20</td>
<td>192±20</td>
</tr>
<tr>
<td>Protein g</td>
<td>71±7</td>
<td>68±60</td>
<td>66±6</td>
</tr>
<tr>
<td>Fat g</td>
<td>65±6</td>
<td>69±6</td>
<td>55±5</td>
</tr>
<tr>
<td>Total Fiber g</td>
<td>16.6±1.7</td>
<td>26.5±2.4</td>
<td>34.5±2.2</td>
</tr>
<tr>
<td>Background Fiber Intake g</td>
<td>15.6</td>
<td>17.3</td>
<td>14.9</td>
</tr>
<tr>
<td>Control Muffins Fiber g</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intervention Muffins Fiber g</td>
<td>9.2</td>
<td>9.2</td>
<td></td>
</tr>
<tr>
<td>Inulin/FOS g</td>
<td>10.4</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Macronutrients, fiber and energy intakes are compared between each period and are reported as mean±SE, n=13.
ANOVA model p value < 0.05 for total fiber.

a significantly higher than control (p<0.0001).
b significantly lower than the pea hull and inulin fiber period (p=0.003).
c significantly higher than the control (p<0.0001).
Table 5-3. Participants’ clinical markers and quality of life scores

<table>
<thead>
<tr>
<th>Clinical Markers (reference ranges)</th>
<th>Control</th>
<th>Pea hull fiber period</th>
<th>Pea hull and inulin fiber period</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BUN mg/dL (7-25 mg/dL)</td>
<td>30±4</td>
<td>30±4</td>
<td>29±4</td>
<td>NS</td>
</tr>
<tr>
<td>Ammonia µmol/L (≤47 µmol/L)</td>
<td>43±4</td>
<td>43±3</td>
<td>50±5</td>
<td>NS</td>
</tr>
<tr>
<td>Cystatin C mg/L (0.5-1.0 mg/L)</td>
<td>1.61±0.24</td>
<td>1.68±0.26</td>
<td>1.62±0.23</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine mg/dL (0.5-1.52 mg/dL)</td>
<td>2.14±0.38</td>
<td>2.17±0.39</td>
<td>2.15±0.40</td>
<td>NS</td>
</tr>
<tr>
<td>CRP mg/dL (&lt;0.80 mg/dL)</td>
<td>0.33±0.13</td>
<td>0.39±0.12</td>
<td>0.45±0.21</td>
<td>NS</td>
</tr>
<tr>
<td>Total plasma p-cresol mg/L</td>
<td>7.25±1.74</td>
<td>--</td>
<td>5.82±1.72 (P=0.039)</td>
<td></td>
</tr>
<tr>
<td>Kidney Function Estimating Equations</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>eGFR mL/min/1.73 m² (creatinine)</td>
<td>37±4</td>
<td>36±4</td>
<td>37±4</td>
<td>NS</td>
</tr>
<tr>
<td>eGFR mL/min/1.73 m² (creatinine-cystatin C)</td>
<td>43±5</td>
<td>43±5</td>
<td>43±5</td>
<td>NS</td>
</tr>
<tr>
<td>eGFR mL/min/1.73 m² (cystatin C)</td>
<td>51±6</td>
<td>50±7</td>
<td>50±6</td>
<td>NS</td>
</tr>
<tr>
<td>KDQOL-36™</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Symptom/problem list (17-28)</td>
<td>77±5</td>
<td>80±4</td>
<td>81±3</td>
<td>NS</td>
</tr>
<tr>
<td>Effects of Kidney Disease (29-36)</td>
<td>81±5</td>
<td>81±5</td>
<td>86±5</td>
<td>NS</td>
</tr>
<tr>
<td>Burden of Kidney Disease (13-16)</td>
<td>65±8</td>
<td>77±7</td>
<td>72±7</td>
<td>P=0.014α</td>
</tr>
<tr>
<td>Physical Component Summary (1-12)</td>
<td>37±3</td>
<td>41±3</td>
<td>40±3</td>
<td>P=0.046α</td>
</tr>
<tr>
<td>Mental Component Summary (1-12)</td>
<td>54±3</td>
<td>55±2</td>
<td>53±3</td>
<td>NS</td>
</tr>
<tr>
<td>Overall Mean Score</td>
<td>63±4</td>
<td>67±3</td>
<td>66±3</td>
<td>NS</td>
</tr>
</tbody>
</table>

Overall ANOVA model p-value > 0.05 (NS), values are mean±SE, n=13. α P-value is for paired t-test comparisons between control and pea hull fiber period. β Significance is based on transformed mean for total plasma p-cresol from control period (LN 1.73) 7.25±1.74 mg/L to pea hull and inulin fiber period (LN 1.27) 5.82±1.72 mg/L, n=12.
Table 5-4. Individual responses for uremic symptoms listed in the Symptom/Problem List subscale of the KDQOL-36™

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Control Mean ± SE</th>
<th>Pea hull fiber period Mean ± SE</th>
<th>Pea hull and inulin fiber period Mean ± SE</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soreness in your muscle</td>
<td>65±8</td>
<td>69±5</td>
<td>67±6</td>
<td>NS</td>
</tr>
<tr>
<td>Chest pain</td>
<td>88±5</td>
<td>94±3</td>
<td>90±6</td>
<td>NS</td>
</tr>
<tr>
<td>Cramps</td>
<td>63±10</td>
<td>67±9</td>
<td>63±9</td>
<td>NS</td>
</tr>
<tr>
<td>Itchy skin</td>
<td>81±6</td>
<td>73±8</td>
<td>79±7</td>
<td>NS</td>
</tr>
<tr>
<td>Dry Skin</td>
<td>69±11</td>
<td>69±11(^b)</td>
<td>88±6</td>
<td>P=0.04(^a)</td>
</tr>
<tr>
<td>Shortness of breath</td>
<td>79±6</td>
<td>85±5</td>
<td>83±7</td>
<td>NS</td>
</tr>
<tr>
<td>Faintness or dizziness</td>
<td>85±6</td>
<td>81±8</td>
<td>85±6</td>
<td>NS</td>
</tr>
<tr>
<td>Lack of appetite</td>
<td>94±4</td>
<td>98±2</td>
<td>88±5</td>
<td>NS</td>
</tr>
<tr>
<td>Washed out or drained</td>
<td>79±7</td>
<td>73±7</td>
<td>79±6</td>
<td>NS</td>
</tr>
<tr>
<td>Numbness hand/feet</td>
<td>65±9</td>
<td>79±8</td>
<td>83±6</td>
<td>P=0.02(^a)</td>
</tr>
<tr>
<td>Nausea or upset stomach</td>
<td>83±7</td>
<td>88±5</td>
<td>81±6</td>
<td>NS</td>
</tr>
</tbody>
</table>

Overall ANOVA model p-value > 0.05 (NS), values are mean±SE, n=13. \(^a\) P-value is for paired t-test comparisons between control and pea hull and inulin fiber period. \(^b\) One value is missing, n=12.
Figure 5-1. Average bowel movement frequencies. Bars are reported as means±SE, n=13. Overall ANOVA p-value > 0.05 (NS). Paired t-test is used to compare means. \textsuperscript{a} p=0.026 compared to control. \textsuperscript{b} p=0.044 compared to control.

Figure 5-2. Total \textit{p}-cresol comparisons before and after intervention. Data presented as mean ± SE (natural log mean). \textsuperscript{a} 7.2 ±1.74 mg/L (LN 1.73) to 5.82±1.71 (LN 1.27) (p<0.05), n=12, percentage change averaged -24% (54% to – 83%, median -49%). \textsuperscript{b} 6.71±1.98 (LN 1.63) mg/L to 4.22 ± 1.16 (1.04) mg/L (p<0.05), n=10, percentage change averaged -35% (24% - 83%, median -62%).
CHAPTER 6
DISCUSSION AND CONCLUSIONS

There are limited studies of patients diagnosed with CKD showing changes in BUN after supplementing the diet of these patients with various fiber sources (2, 7, 50, 51). These studies had been carried out in patients with stage 5 CKD only, both HD and not on HD. Further, investigators have not assessed the impact of fiber on symptoms of uremia or the potential impact on quality of life. In addition, these studies have not demonstrated changes in serum creatinine and thus, corresponding eGFR. To address some of these limitations, and to determine whether consumption of added fiber can impact uremic molecules beyond BUN in patients with more moderate decline in kidney function, we conducted two clinical studies with insoluble and fermentable fibers. Furthermore, the objective was to assess the changes that may result from pea hull fiber (less fermentable) alone and in combination with inulin (fermentable) on these changes and the potential impact on quality of life and a host of uremic symptoms.

Quality of Life and Symptoms

Quality of life was assessed by utilizing the KDQOL-36™ questionnaire. The subscales varies in their correlation with eGFR in CKD patients’ stage 4 and 5. The Physical Component Summary and Burden of Kidney Disease correlate with eGFR while the Mental Component Summary does not (136). In Study 1, the Physical Component Summary increased by 4 points after intervention compared to control (P=0.02) while eGFR increased by about 10% (P=0.005). Lowrie et al. reported that for each point improvement in this scale, a 2% reduction in the rate of hospitalization and mortality was observed in HD patients (135). Improved functional health leads to
improved engagement in daily activities and independence, which ultimately can
improve the quality of life. In addition, potential reduction in the rate of hospitalization
due to improved physical health has great financial savings impact. The study was short
to assess any impact and the total scores for the overall changes in quality of life were
not different. This is mainly because unexpectedly the Mental Component Summary
scores were significantly lower (P=0.01) leading to unchanged overall quality of life
scores. The lower Mental Component Summary scores is unlikely to be due to improved
eGFR or treatment effect. Out of the fifteen participants, seven reported lower scores
five of which were non responsive to treatment.

It is reported that quality of life is significantly impacted by constipation (153),
improved stool frequency may thus partially contribute to feeling well. In Study 1, stool
frequency was higher after intervention compared to control (Figure 4-1). It is unclear
how much the improvement in stool frequency impacted this observed improvement in
the reported improvement in physical health.

In Study 2, results for all ANOVA tests were not significant and thus results are
highly open to criticism when using multiple pairwise comparisons. We used t-test
comparisons for the qualitative data and stool frequency as a way to cautiously explore
the data to derive hypothesis for future studies because of lack of power to carry out
ANOVA on the qualitative data. Participants reported a score of 41±3 for physical
health during the pea hull fiber period (P=0.046) compared to control 37±3 (Table 5-3).
Since stool frequency during control was 1.4±0.2 compared to pea hull fiber period
(1.9±0.3, P=0.026) this may explain this improvement in the perceived physical health.
However, although the stool frequency increased during the pea hull and inulin fiber
period (1.9±0.3) compared to control (P=0.044), the Physical Component Summary was not statistically different. The score during the pea hull and inulin fiber period was (40±3) during which participants (n=8) experienced undesirable effects such as flatulence and bloating which may have impacted gut health and ultimately the reported scores. This is because some of the reported side effects (i.e. flatulence) impacts socialization significantly. In Study 1, stool frequency was higher along with eGFR which may lead to the observed improvement in reported scores for the physical health aspect of quality of life. If gut-related function such as bowel movements can affect perceived health this may explain the higher scores (better results, reduced burden) for the Burden of Kidney Disease during the pea hull fiber period (77±7, P=0.014) compared to the reported score during control (65±8). As discussed, increase flatulence and reported symptoms from fiber supplementation may have impacted the reported score during the pea hull and inulin fiber period (72±7).

**Bowel Movement Frequency and Fiber Intake Impact and Recommendations**

Intake of insoluble fibers are known to increase bulking and reduce symptoms of constipation. In study 1, there was a significant improvement in bowel frequency when participants consumed an additional 4 g/d of supplemental insoluble fiber, similar to previously reported finding in elderly people using similar pea hull fiber (154), along with 12.5 g/d of supplemental soluble fermentable fibers (p<0.05). It is unlikely that the fermentable fibers were the primary contributors to such improvement but likely to be due to the known effect of insoluble fibers on bulking and transit time. In Study 2, bowel frequency improved significantly when 10 g/d of pea hull fiber was added to the diet for four weeks resulting increased total fiber intake from 16.6 g/d to 26.5 g/d. The addition
of 13.5 g/d (average six weeks intake 11.3 g/d) of inulin/FOS did not have any added effect on bowel movement frequency (Figure 5-1), although total fiber intake increased significantly to 34.5 g/d (Table 5-2). Supplementing the diet of CKD patients with insoluble fiber is likely to help with management of constipation by increasing fecal output and/or reducing transit time. Accelerated transit time alone is not likely to impact protein fermentation significantly (155, 156). However, when sufficient FOS and/or inulin fibers are consumed, the relative proportion of saccharolytic to proteolytic bacterial species (157, 158) is likely to explain the observed suppression of proteolytic enzymes by fermentable fibers reported previously (110, 159). Such desirable effects will impact uremic load and toxins generation significantly, which may benefit uremic patients (especially when considering that uremic patients suffer from increased protein mal-digestion). However the diversity of the fermentable substrate is likely to determine the diversity of the impact on both negative and positive bacterial species and their byproducts.

Constipation is a serious symptom of uremia. Chronic kidney isease patients are likely to consume less than recommended fiber intakes, and intakes are likely to be even lower as disease worsens. We hypothesized that patients in advanced stages of the kidney disease are likely to consume less fiber than is recommended for healthy individuals and that intake is likely to be lower as disease progress. This was supported by our finding that total fiber intake in Study 1 during control period was 10.7 g/d when eGFR was 29.6 mL/min/1.73 m² which is lower than the average intake for healthy individuals. All participants fill short of the IOM recommendation during the control period during which low fiber foods replaced a portion of the daily food intake. It is
important to note that low fiber study foods replaced a portion of the participants’ daily food intake, which may have negatively impacted the usual fiber intake. Total fiber intake of the participants in Study 2, which had higher eGFR, was 16.6 g/d during the control period, which is similar to that of the healthy population (17 g/d). Both of these levels however fall short of the IOM recommendations (16). The higher than anticipated total fiber intake during the control period for participants in Study 2 may suggest that participants were less uremic and had lower dietary restrictions than those in Study 1.

These findings also provide additional framework to what an optimal nutritional therapy should target in terms of fiber composition. Younes et al. supplemented the diet of CKD participants with 40 g/d of fiber (25 g/d insoluble fiber, 15g/d soluble and fermentable fibers) (2). Bliss et al. provided 50 g/d of soluble and fermentable fiber (51). Amounts of 40 and 50 g/d of additional functional and dietary fibers are impractical and compliance is difficult in the long term. Additionally, fermentable and less fermentable fibers have distinctive effects on health parameters. These findings suggest a total supplemental added fiber of about 16.5 g/d, 4 g/d of which insoluble, and 12.5 g/d of fermentable fibers will provide benefits to treat constipation as well as impact uremic molecules, which may help preserve kidney function. This total of 16.5 g/d should provide, in addition to participants’ usual fiber intake, a total fiber intake that is in line with the IOM recommendations (16), while providing means to increase the important fermentable and soluble fibers that are typically low in the diet of CKD patients.

**Uremic Symptoms**

There is a diverse characterization of uremic symptoms and no single uremic molecule is responsible for their onset. Therefore, it is not likely that a single therapy
can improve all symptoms. Uremic patients suffer greatly from constipation, and rates in HD patients are as high as 64% (160) compared with 15% in the overall population (153). Improvement in bowel movement frequency in less constipated patients such as in these two studies is likely to indicate a greater potential in more constipated patients.

Uremic symptoms include sleep disturbances, fatigue and restless leg syndrome, which may result in an increased tendency to fall asleep in unusual daytime situations. Increasing fiber intake by an additional 16.8 g (total 26.5 g) was helpful to reduce the tendency to fall asleep during daytime (p<0.05). The Epworth Sleepiness Scale score mean was significantly lower after intervention (8±1, P=0.04) compared to control (10±1) indicating a reduced risk of daytime sleeping. This drop in the scale score overlapped with about 10% improvement in eGFR. Higher eGFR indicates higher rate of uremic molecules removal from the blood, which can lead to reduced accumulation of molecules that can cause fatigue, restless leg syndrome both of which can affect sleep cycle, as well as those that can hamper alertness and impact the central nervous system.

In Study 2, sub-analysis of the responses to the Symptom/Problem List (Questions 17-27) show a significant 19 point improvement in dry skin symptom, and a significant 18 point improvement in the numbness in hand or feet during the pea hull and inulin fiber period (Table 5-4) compared to control. Uremia is a broad term to describe the symptoms associated with increased uremic molecules accumulation in the blood with no specific underlying molecule or mechanism to explain these symptoms. Improvement in these two symptoms overlapped with a significant reduction in p-cresol. This preliminary finding may point to a potential link between these two symptoms and p-
cresol. Changes in total $p$-cresol correlated inversely with changes the dry skin score (higher dry skin score means less bothered by the symptom).

**Impact of Higher Fiber Intakes**

Total fiber intake from foods during the control period in Study 2 averaged 16.6 g/d while in Study 1 participants consumed on average 10.7 g/d of total fiber during the control period (Table 4-1). No negative impact on energy was seen in Study 1 or 2 after fiber supplementation up to a total daily fiber intake of 34.5 g in Study 2, although absolute caloric intake appear lower with this level of intake.

Participants reported bothersome symptoms of being bloated, loose stools, and flatulence after the introduction of inulin supplements in Study 2. The severity and frequency was highest during the first 3 weeks of the inulin period, and although some symptoms remained, the severity and frequency became less bothersome to participants during the last three weeks of the inulin supplementation period. The severity and frequency of these symptoms is likely related to the fact that most participants were consuming the inulin supplement at once. Participants were advised to separate the dosage into two or three portions to try and reduce symptoms occurrence, which may help explain the observed improvement. These symptoms are common and are reported with dosages of 10 and 20 g/d (161). It is widely accepted that consumption of fermentable prebiotic supplements should be in smaller increments during the day to avoid most major side effects. In addition, the composition of inulin can play a role in mitigating the symptoms. The supplement used in the present study contained 90% inulin and only 10% FOS, so to reduce gas production the proportion of
FOS to inulin in the supplement should be increased which was shown to be effective in reducing gas production (162).

**Cardiovascular Lipid Panel Markers**

CKD patients are at great risk of developing cardiovascular disease, and suffer from diabetes and hypertension in most cases. Low fiber intakes are linked to increased risk of cardiovascular disease, and fiber is shown to improve cardiovascular profile, and improve glycemic control. However, CKD patients, especially with advanced stage of the disease, consume less fiber in the diet than the IOM recommendations. Increasing fiber intake in the diet of CKD patients may have significant importance to cardiovascular health. Because foods rich in fiber are also often rich sources of potassium, and phosphorous, two nutrients that are usually restricted in the management of late stages of CKD, increasing fiber by supplement and/or fiber-fortified foods should be considered an effective way of achieving adequate fiber intakes and should be promoted more widely as an essential part of the Medical Nutrition Therapy for CKD. In Study 1, participants consumed about 11 g/d of fiber during control, and the addition of fiber to the diet brought total fiber intake to levels that are typically associated with better outcomes.

In Study 1 with this level of intake, may have resulted in improved total cholesterol/HDL ratio (Table 4-2). These findings warrant an emphasis on fiber recommendations for CKD patients that stress the importance of fermentable fiber for the management of disease co-morbidities. Increasing intake with inulin fiber can stimulate the proliferation of colonic bacteria, which then can lead to increased conversion of cholesterol to coprostanol leading to increase losses of cholesterol in the
stools (163), ultimately leading to less bile acid re-cycling and lower blood cholesterol levels. However it is possible that foods provided replaced the usual intake of fat in the diet as participants reported lower total fat intake compared to control (Table 4-2).

**Uremic Molecules**

Lowering of uremic molecules may help protect the kidneys by reducing the uremic load and thus lessening the extent of hyperfiltration as well as lowering the uremic toxic molecules (i.e. p-cresol) that can trigger oxidative damage and cause glomerular and tubular damage (5, 121). Thus, reducing the uremic load and the toxic oxidative molecules that need to be filtered by the glomerulus and the tubules may eventually lead to improved glomerulus and tubular hemodynamics and filtration. When patients consumed 26.5 g/d of total fiber (16.8 g/d from added fiber), BUN decreased by 10.5% after intervention compared to control (P=0.058). Serum creatinine decreased from 2.40±0.29 mg/L during control to 2.20±0.26 mg/dL after intervention (P<0.005). The decline in serum creatinine resulted in a significant improvement in kidney function eGFR by 10% increasing from 30±3 mL/min/1.73m² to 33±4 mL/min/1.73m² after intervention (P<0.005).

Reduction in the uremic load, represented here only by these two molecules may be explained by improved filtration of these molecules and/or increased losses from other routes. The reduction in urea can be explained by improved filtration and/or increased urea capture and utilization in the large intestine. It is most likely that the lowering of BUN was due to increased utilization by incorporation into bacterial nitrogen leading to increase nitrogen losses in the stools. Several animal studies demonstrate a
decline in BUN due to increased fecal losses (39-41, 44, 46). These animal studies show that feeding fermentable fibers increase urea entry into the colon (39, 40), which increases the nitrogen loses in the stools (40, 44) with concurrent reduction in urinary losses (41, 44). Human studies also demonstrate that the decrease in BUN is coupled with an increase in fecal nitrogen losses (2, 50, 51) sometimes coupled with a concurrent reduction in urinary output (2).

Such an alteration in urea’s fate leads to reduction in the contribution to the uremic load by urea, and potentially several other nitrogenous molecules, which may benefit the remaining nephrons. Reduction in the contribution to the uremic load by serum creatinine was also significant as serum creatinine decreased by 9.4% at the end of the study compared to baseline. This reduction in serum creatinine is unlikely to be due to increased influx into the large intestine but rather due to improved filtration. The view that during kidney failure significant losses of serum creatinine from other routes particularly the colonic route, has not been substantiated. It is well established that no significant quantity of creatinine can be recovered in the stool. In one study, only patients with serum creatinine above 6 mg/dL had an increased gut bacterial creatininsase activity in the stool (86). Study 1 participants had an average serum creatinine of 2.44 mg/dL, thus it is unlikely that significant quantities of serum creatinine entered the large intestine and induced creatininsase enzymes in the present study. In addition, previous human studies feeding various fermentable substrates have failed to show changes in serum creatinine in advanced stages of the disease where serum creatinine levels are more elevated than that in the present study. Therefore, it is likely that the reduction in serum creatinine observed in this study was due to improved
filtration. It is unlikely the results are due to changes in creatinine metabolism, as, both protein intake (Table 4-1) and body weights did not change during the study.

**Plasma p-Cresol**

*p*-Cresol sulfate concentrations, indirectly quantified as *p*-cresol, are independently associated with overall mortality and are an independent predictor of CVD incident in HD patients (118, 120). *p*-Cresol and its conjugated form *p*-cresol sulfate can induce formation of free radicals which can induce damage to tubular and glomerular cells (5, 6, 121). There was a 20% reduction in total *p*-cresol at the end of the study decreasing from 7.26 mg/dL to 5.82 mg/dL (p<0.05).

Two plausible mechanisms that are likely to work together and may explain the observed changes in *p*-cresol are bulking and reduction of protein bacterial fermentation. This is because slower colonic transit time may induce expansion of proteolytic bacterial species in the colon (26) which may lead to the increased production and/or retention of protein fermentation byproducts especially in carbohydrate-deprived environment. Such delays in colonic contents passage and longer transit times correlate with higher urinary phenols excretion (164). Insoluble fiber can provide bulking, which can stimulate faster passage of colonic contents.

By providing more fermentable fibers, the availability of carbohydrate substrates to the microbiota will lead to an increased saccharolytic bacterial proliferation while inhibiting proteolytic activity (3) leading to less byproducts of protein fermentation. The lack of a *p*-cresol blood sample during the pea hull fiber only intervention leaves the question of how much bulking alone may affect *p*-cresol concentration. Meijer’s *et al.* reported that feeding of 20 g/d of inulin without insoluble fiber resulted in a 17%
reduction in \( p \)-cresol sulfate levels in HD patients. High \( p \)-cresol levels can induce inflammatory oxidative stress response and expression of fibrosis related genes such as TGF\( \beta \)1 (5, 6) that may induce tubulointerstitial fibrosis and glomerular sclerosis (8, 95, 97, 98), and thus reduction of this oxidative metabolite and its conjugate is likely to help lessens the progression of renal inflammation and oxidative damage.

It is important to consider the dosage at which inulin is more effective when considering inulin compliance as a factor. When comparing the mean for patients who consumed \( \geq 70\% \) (10 g/d) or more of inulin, total plasma \( p \)-cresol was lower by 37\% compared to baseline, which may indicate a dose effect.

**Framework**

When patients consumed on average 12.5 g/d of additional fermentable fibers in Study 1 with total fiber reaching 26.5 g/d, BUN decreased by 10.5\% (P=0.058). As explained earlier, this decline in BUN is likely to be due to the increase utilization of urea in the large intestine (2, 40-44, 46). It is likely that the lower (normal and near normal) BUN levels are, the less likely reduction via a dietary therapy will be detectable. As the mean is normal or near normal, the magnitude of change in those with higher eGFR and lower BUN remains small compared to elevated levels. The normal and near normal levels are typical of patients whose eGFR is close to 50 mL/min/1.73m\(^2\) compared with those with more advanced decline in eGFR. Younes *et al.* reported a 23\% decline in BUN from 73 mg/dL to 56 mg/dL, while Bliss *et al.* reported a change of -12\% from 50 mg/dL to 44 mg/dL. This may explain why no changes in uremic molecules profile was seen in Study 2 (Table 5-3). Participants in Study 2 were “healthier” by comparisons to participants in previous studies and even by comparisons to Study 1.
For example, in Study 1, ten participants were diagnosed with stage 4 CKD, while in Study 2 had ten participants diagnosed with stage 3, thus participants had much more residual nephrons and higher tubular secretion capacity. While the inclusion of a “healthier” CKD sample may result in lack of detecting significance due to higher residual nephron as seen in Study 2, focusing on CKD stage 5 only may mask any potential benefit. This is likely why Younes et al. and Bliss et al. did not observe an effect on serum creatinine after supplementing the diet of advanced CKD patients with fiber even though uremic BUN decreased significantly. In these studies, patients had a significantly more advanced decline in eGFR. Serum creatinine levels are very stable even after the onset of kidney disease and until about 50% of the kidney function is lost (165) after which serum creatinine starts rising due to the loss of the adaptive mechanism and secretory tubules are overwhelmed. For example, serum creatinine for an average male patient in stage 3 is around 2 mg/dL, but for a CKD patient (stage 5), serum creatinine can be significantly higher (i.e. serum creatinine for male 50 year old with eGFR of 8 mL/min is 8 mg/d). Hyperfiltration allows more of the uremic molecules and creatinine to be filtered thus lowering serum creatinine concentrations. In addition, the proximal tubular secretion of creatinine maintains lower serum creatinine concentrations when the concentration is low. However, as the disease progresses the adaptive hyperfiltration response becomes inadequate to bring down serum creatinine, and the concentration rises significantly, the proximal tubular secretion is overwhelmed leading to an accelerated increase in serum creatinine levels. Therefore, it is unlikely that a dietary intervention provides distinguishable relief at such late stage of the disease when measured by concentration of serum creatinine as the range of serum
creatinine rise increase significantly. This is especially important considering that unlike urea, the decline in serum creatinine is likely to be due to improvements in filtration capacity and thus improvement is likely to be limited depending on the remaining functional nephrons.

The lack of any improvement in eGFR in these studies therefore is due to the proportion in which serum creatinine rises at later stages of the disease in the blood and the relative concentration. Serum creatinine levels for a male adult age 50 that rises from 1 mg/dL to 2 mg/dL represents a significant decline in eGFR from about 87 mL/min/1.73m² to 38 mL/min/1.73m², a decline of 49 mL/min (-55%). However, a rising serum creatinine for the same person from 4 mg/dL to 5 mg/dL for example, changes eGFR by only 4 mL/min from 16 to 12 mL/min. Younes et al. reported a non-significant decline in serum creatinine after feeding (40 g/d fiber, 15 g/d of which was fermentable), from 4.0 mg/dl to 3.8 mg/dl, but that decrease was not statistically significant and was not enough to improve eGFR, although the absolute decline in serum creatinine was comparable to the findings presented in Study 1. Furthermore, Rampton et al. reported that fiber intervention reduced the slope of rising serum creatinine to zero after 8 weeks of fiber intervention (50). In his study, patients had a higher baseline serum creatinine levels than that reported by Younes et al. While our second study with most patients in stage 3 did not show any impact on BUN or creatinine, the previous studies with patients in stage 5 did not show any impact on creatinine. When considered in light of the findings from Study 1 in which most patients were diagnosed with stage 4 CKD, it is possible to suggest that patients in stage 4 maybe the ideal target population to observe significant changes.
Conclusions

Increasing the fiber intake in the diet of CKD patients is achievable with minimum side effects using both fortified foods and supplementation. The addition of insoluble and soluble fibers to the diet had positive effects on quality of life, uremic symptoms, and blood parameters including kidney function markers. The current Medical Nutrition Therapy to manage the underlying pathophysiology of CKD is focused on the late stages of the disease with limited recommendations regarding the impact of fiber. The recommendations regarding fiber mainly pertain to the role fiber plays in protecting against CHD. Moreover, recommendations are unclear on how to obtain the maximum fiber intake without risking undesirable increases in other nutrients. As demonstrated in this preliminary work, increasing the fiber content of the diet without significantly impacting protein, phosphorus or potassium intakes can be successfully achieved. Supplements and fortified products that are low in these nutrients and high in fiber are well tolerated. In addition, this approach can significantly enhance the fiber intake in this population to meet the IOM recommendations.

Moreover, current medical nutrition therapy recommendations are unclear on the benefits various fibers can provide for renal disease patients. No distinction between fermentable and non-fermentable fibers has been emphasized regarding these recommendations. These studies provide preliminary framework for future studies. The consumption of 4 to 9 g/d of insoluble fibers resulted in significant improvement in bowel movement frequency and impacted perceived physical health and burden of kidney disease positively. Furthermore, supplementing the diet with additional fermentable fibers resulted in several desirable benefits for stage 4 CKD patients. While no benefit
was observed by adding fiber on kidney function for patients in stage 3, the reduction of 
$p$-cresol in this subset of the CKD population is an important findings. This confirms 
earlier results in HD but for the first time is demonstrated in patients in stage 3 of the 
disease. These findings pave the way to suggest that early fiber interventions may be 
useful to affect disease progression by targeting uremic molecules and reducing 
oxidative toxic molecules that otherwise stimulate disease progression. These findings 
also provide a new insight in the need to focus on interventions with stage 4 of CKD 
when considering fiber intervention to study the impact on eGFR.

These recommendations should include 12-13 g/d of added fermentable fiber 
sources (inulin, FOS, and resistant starch) while providing at least 4 g/d in the form of 
insoluble fiber beside usual intake. Future studies should aim to clearly distinguish 
between classes of fiber and the potential benefits that may be gained with each class 
to enhance future recommendations while finding the maximum practical potential 
benefit with the minimum effective dose. Long-term studies with large sample sizes can 
provide better understanding of the extent of increasing intake of mixed fibers on 
changes in the uremic profile, renal function markers and quality of life and symptoms.
Your Health
—and—
Well-Being

Kidney Disease and Quality of Life (KDQOL™-36)

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.

Thank you for completing these questions!
Study of Quality of Life
For Patients on Dialysis

What is the purpose of the study?
This study is being carried out in cooperation with physicians and their patients. The purpose is to assess the quality of life of patients with kidney disease.

What will I be asked to do?
For this study, we want you to complete a survey today about your health, how you feel and your background.

Confidentiality of information?
We do not ask for your name. Your answers will be combined with those of other participants in reporting the findings of the study. Any information that would permit identification of you will be regarded as strictly confidential. In addition, all information collected will be used only for purposes of the study, and will not be disclosed or released for any other purpose without your prior consent.

How will participation benefit me?
The information you provide will tell us how you feel about your care and further understanding about the effects of medical care on the health of patients. This information will help to evaluate the care delivered.

Do I have to take part?
You do not have to fill out the survey and you can refuse to answer any question. Your decision to participate will not affect your opportunity to receive care.
Your Health

This survey includes a wide variety of questions about your health and your life. We are interested in how you feel about each of these issues.

1. In general, would you say your health is: [Mark an ☐ in the one box that best describes your answer.]

<table>
<thead>
<tr>
<th>Excellent</th>
<th>Very good</th>
<th>Good</th>
<th>Fair</th>
<th>Poor</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>

2. Compared to one year ago, how would you rate your health in general now?

<table>
<thead>
<tr>
<th>Much better now than one year ago</th>
<th>Somewhat better now than one year ago</th>
<th>About the same as one year ago</th>
<th>Somewhat worse now than one year ago</th>
<th>Much worse now than one year ago</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2</td>
<td>3</td>
<td>4</td>
<td>5</td>
</tr>
</tbody>
</table>
During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of your physical health**?

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>4. Accomplished less than you would like..........</td>
<td>□ 1</td>
<td>□ 2</td>
</tr>
<tr>
<td>5. Were limited in the kind of work or other activities ........................................</td>
<td>□ 1</td>
<td>□ 2</td>
</tr>
</tbody>
</table>

During the **past 4 weeks**, have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems** (such as feeling depressed or anxious)?

<table>
<thead>
<tr>
<th></th>
<th>Yes</th>
<th>No</th>
</tr>
</thead>
<tbody>
<tr>
<td>6. Accomplished less than you would like..........</td>
<td>□ 1</td>
<td>□ 2</td>
</tr>
<tr>
<td>7. Didn’t do work or other activities as carefully as usual ........................................</td>
<td>□ 1</td>
<td>□ 2</td>
</tr>
</tbody>
</table>

8. During the **past 4 weeks**, how much did **pain** interfere with your normal work (including both work outside the home and housework)?

<table>
<thead>
<tr>
<th></th>
<th>Not at all</th>
<th>A little bit</th>
<th>Moderately</th>
<th>Quite a bit</th>
<th>Extremely</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>▼ 1</td>
<td>▼ 2</td>
<td>▼ 3</td>
<td>▼ 4</td>
<td>▼ 5</td>
</tr>
</tbody>
</table>
These questions are about how you feel and how things have been with you **during the past 4 weeks**. For each question, please give the one answer that comes closest to the way you have been feeling.

**How much of the time during the past 4 weeks…**

<table>
<thead>
<tr>
<th>All of the time</th>
<th>Most of the time</th>
<th>A good bit of the time</th>
<th>Some of the time</th>
<th>A little of the time</th>
<th>None of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
</tr>
</tbody>
</table>

9. Have you felt calm and peaceful? .......................... □ 1 ...... □ 2 ...... □ 3 ...... □ 4 ...... □ 5 ...... □ 6

10. Did you have a lot of energy? .............................. □ 1 ...... □ 2 ...... □ 3 ...... □ 4 ...... □ 5 ...... □ 6

11. Have you felt downhearted and blue? ............... □ 1 ...... □ 2 ...... □ 3 ...... □ 4 ...... □ 5 ...... □ 6

12. **During the past 4 weeks**, how much of the time has your **physical health or emotional problems** interfered with your social activities (like visiting with friends, relatives, etc.)?

<table>
<thead>
<tr>
<th>All of the time</th>
<th>Most of the time</th>
<th>Some of the time</th>
<th>A little of the time</th>
<th>None of the time</th>
</tr>
</thead>
<tbody>
<tr>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
</tr>
<tr>
<td>□ 1</td>
<td>□ 2</td>
<td>□ 3</td>
<td>□ 4</td>
<td>□ 5</td>
</tr>
</tbody>
</table>

Page 3
Your Kidney Disease

How true or false is each of the following statements for you?

<table>
<thead>
<tr>
<th>Definitely true</th>
<th>Mostly true</th>
<th>Don’t know</th>
<th>Mostly false</th>
<th>Definitely false</th>
</tr>
</thead>
</table>

13. My kidney disease interferes too much with my life .................

☐ 1 .......... ☐ 2 .......... ☐ 3 .......... ☐ 4 .......... ☐ 5

14. Too much of my time is spent dealing with my kidney disease .......

☐ 1 .......... ☐ 2 .......... ☐ 3 .......... ☐ 4 .......... ☐ 5

15. I feel frustrated dealing with my kidney disease .......

☐ 1 .......... ☐ 2 .......... ☐ 3 .......... ☐ 4 .......... ☐ 5

16. I feel like a burden on my family ........

☐ 1 .......... ☐ 2 .......... ☐ 3 .......... ☐ 4 .......... ☐ 5

Page 4
<table>
<thead>
<tr>
<th>Question</th>
<th>Not at all bothered</th>
<th>Somewhat bothered</th>
<th>Moderately bothered</th>
<th>Very much bothered</th>
<th>Extremely bothered</th>
</tr>
</thead>
<tbody>
<tr>
<td>17. Soreness in your muscles?</td>
<td>□ 1 □ 2 □ 3 □ 4 □ 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18. Chest pain?</td>
<td>□ 1 □ 2 □ 3 □ 4 □ 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19. Cramps?</td>
<td>□ 1 □ 2 □ 3 □ 4 □ 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20. Itchy skin?</td>
<td>□ 1 □ 2 □ 3 □ 4 □ 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21. Dry skin?</td>
<td>□ 1 □ 2 □ 3 □ 4 □ 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22. Shortness of breath?</td>
<td>□ 1 □ 2 □ 3 □ 4 □ 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>23. Faintness or dizziness?</td>
<td>□ 1 □ 2 □ 3 □ 4 □ 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>24. Lack of appetite?</td>
<td>□ 1 □ 2 □ 3 □ 4 □ 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25. Washed out or drained?</td>
<td>□ 1 □ 2 □ 3 □ 4 □ 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>26. Numbness in hands or feet?</td>
<td>□ 1 □ 2 □ 3 □ 4 □ 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>27. Nausea or upset stomach?</td>
<td>□ 1 □ 2 □ 3 □ 4 □ 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28a. (Hemodialysis patient only)</td>
<td>□ 1 □ 2 □ 3 □ 4 □ 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Problems with your access site?</td>
<td>□ 1 □ 2 □ 3 □ 4 □ 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>28b. (Peritoneal dialysis patient only)</td>
<td>□ 1 □ 2 □ 3 □ 4 □ 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Problems with your catheter site?</td>
<td>□ 1 □ 2 □ 3 □ 4 □ 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Page 5
Effects of Kidney Disease on Your Daily Life

Some people are bothered by the effects of kidney disease on their daily life, while others are not. How much does kidney disease bother you in each of the following areas?

<table>
<thead>
<tr>
<th>Not at all bothered</th>
<th>Somewhat bothered</th>
<th>Moderately bothered</th>
<th>Very much bothered</th>
<th>Extremely bothered</th>
</tr>
</thead>
<tbody>
<tr>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
<td>▼</td>
</tr>
</tbody>
</table>

29. Fluid restriction? ....

30. Dietary restriction? ....

31. Your ability to work around the house? .................

32. Your ability to travel? .................

33. Being dependent on doctors and other medical staff? .................

34. Stress or worries caused by kidney disease? .................

35. Your sex life? ....

36. Your personal appearance? ....

Thank you for completing these questions!
APPENDIX B
GASTROINTESTINAL RATING SCALE

Subject ID: ___________________ Date: ___________________

Response Scale:
(1) No discomfort at all.
(2) Slight discomfort.
(3) Mild discomfort.
(4) Moderate discomfort.
(5) Moderately severe discomfort.
(6) Severe discomfort.
(7) Very severe discomfort.

GSRS items:
(1) Have you been bothered by stomach ache or pain during the past week? (Stomach ache refers to all kinds of aches or pains in your stomach or belly.)

(2) Have you been bothered by heartburn during the past week? (By heartburn we mean burning pain or discomfort behind the breastbone in your chest.)

(3) Have you been bothered by acid reflux during the past week? (By acid reflux we mean regurgitation or flow of sour or bitter fluid into your mouth.)

(4) Have you been bothered by hunger pains in the stomach or belly during the past week? (This hollow feeling in the stomach is associated with the need to eat between meals.)

(5) Have you been bothered by nausea during the past week? (By nausea we mean a feeling of wanting to be sick.)

(6) Have you been bothered by rumbling in your stomach or belly during the past week? (Rumbling refers to vibrations or noise in the stomach.)

(7) Has your stomach felt bloated during the past week? (Feeling bloated refers to swelling in the stomach or belly.)

(8) Have you been bothered by burping during the past week? (Burping refers to bringing up air or gas through the mouth.)

(9) Have you been bothered by passing gas or flatus during the past week? (Passing gas or flatus refers to the release of air or gas from the bowel.)
(10) Have you been bothered by constipating during the past week? (Constipation refers to a reduced ability to empty the bowels.)
(11) Have you been bothered by diarrhea during the past week? (Diarrhea refers to frequent loose of watery stools.)
(12) Have you been bothered by loose stools during the past week? (If your stools have been alternately hard and loose. This question only refers to the extent you have been bothered by the stool being loose.)
(13) Have you been bothered by hard stools during the past week? (If your stool has been alternately hard and loose, this question only refers to the extent you have been bothered by the stools being hard.)
(14) Have you been bothered by an urgent need to have a bowel movement during the past week? (This urgent need to open your bowel makes you rush to the toilet.)
(15) When going to the toilet during the past week, have you had the feeling of not completely emptying your bowels? (The feeling that after finishing a bowel movement, there is still more stool that needs to be passed.)

Response Scale:
(1) No discomfort at all.
(2) Slight discomfort.
(3) Mild discomfort.
(4) Moderate discomfort.
(5) Moderately severe discomfort.
(6) Severe discomfort.
(7) Very severe discomfort.
APPENDIX C
SIMPLIFIED NUTRITIONAL APPETITE QUESTIONNAIRE

Subject ID: ___________________________ Date: __________

Simplified Nutritional Assessment Tool
SNAQ

Instructions: Please complete the questionnaire by circling the correct answers.

My appetite is

a. very poor
b. poor
c. average
d. good
e. very good

When I eat

a. I feel full after eating only a few mouthfuls
b. I feel full after eating about a third of a meal
c. I feel full after eating over half a meal
d. I feel full after eating most of the meal
e. I hardly ever feel full

Food tastes

a. very bad
b. bad
c. average
d. good
e. very good

Normally I eat

a. less than one meal a day
b. one meal a day
c. two meals a day
d. three meals a day
e. more than three meals a day

Tally the results based on the following numerical scale: a = 1, b = 2, c = 3, d = 4, e = 5. The sum of the scores for the individual items constitutes the SNAQ score. SNAQ score ≤14 indicates significant risk of at least 5% weight loss within six months.

---

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## Epworth Sleepiness Scale

**Name:**

**Date:**

**Your age:** (Yr) __________________________  **Your sex:** □ Male □ Female

How likely are you to doze off or fall asleep in the situations described below, in contrast to feeling just tired?

This refers to your usual way of life in recent times.

Even if you haven't done some of these things recently try to work out how they would have affected you.

Use the following scale to choose the **most appropriate number** for each situation:

0 = would never doze
1 = Slight chance of dozing
2 = Moderate chance of dozing
3 = High chance of dozing

<table>
<thead>
<tr>
<th>Situation</th>
<th>Chance of dozing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sitting and reading</td>
<td></td>
</tr>
<tr>
<td>Watching TV</td>
<td></td>
</tr>
<tr>
<td>Sitting, inactive in a public place (e.g. a theatre or a meeting)</td>
<td></td>
</tr>
<tr>
<td>As a passenger in a car for an hour without a break</td>
<td></td>
</tr>
<tr>
<td>Lying down to rest in the afternoon when circumstances permit</td>
<td></td>
</tr>
<tr>
<td>Sitting and talking to someone</td>
<td></td>
</tr>
<tr>
<td>Sitting quietly after a lunch without alcohol</td>
<td></td>
</tr>
<tr>
<td>In a car, while stopped for a few minutes in the traffic</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
</tr>
</tbody>
</table>

**Score:**

0-10  Normal range
10-12  Borderline
12-24  Abnormal
APPENDIX E
TELEPHONE SCRIPT

Hello, my name is ___________, with the fiber study.
(Potential participant indicates they are calling about the study)
Great. I would be happy to give you more information about the study. The purpose of this study is to determine whether the providing adequate fiber to patients with CKD will result in improved gastrointestinal function and quality of life. If you qualify and decide to participate, you will be randomly assigned to a intervention or control group, but you will not be told which group you are in until completion of the study. Both groups will be given cookies, cereal bars and breakfast cereal and to consume daily for a period of 6 weeks (42 days), with the intervention group receiving high fiber food. During the course of the study you would be asked to come in our clinical lab on four separate occasions to have your blood drawn and fill out questionnaires. These appointments should take no more than an hour. The questionnaires will ask questions regarding your quality of life, appetite, and gastrointestinal symptoms. Participants will also be asked to provide three stool samples during the study.
Foods provided to participants provide nutrients and energy to all participants. Individuals selected for the fiber-fortification group may experience improved gastrointestinal function and quality of life due to the fiber. Does this sound like something you would be interested in doing?
(Responds Yes)
Great, now I will read the inclusion/exclusion criteria for the study to make sure you qualify. Please wait until I finish reading through this list, then you can let me know if you are still interested.
(Read inclusion/exclusion criteria without pausing)
Does this still sound like something you would like to take part in?
(Responds Yes)
Great, then we can schedule an initial appointment for you to receive more detailed information on the study and review a consent form. Is there a date and time that works best for you?
(Schedule appointment and obtain best way to contact patient)
Inclusion Criteria
Participants Must:
- Be 18 years of age or older
- Have GFR of ≤ 50 mL/min/1.73 m² (stage 3, 4 and 5 but who are not on dialysis)

Exclusion Criteria
- Have you been diagnosed with acute kidney injury (AKI)
- Have you been diagnosed with glomerulonephritis (GN)?
- Are you on immunosuppressant/steroid medications?
- Are you taking a probiotic supplement and refuse to discontinue it?
- Are you scheduled for dialysis within 3 months of study initiation?
- Do you have a history of liver disease?
- Have you been on dialysis?
- Have you undergone renal transplantation?
- Are you breastfeeding?
- Do you have active gastrointestinal bleeding?
- Have a change in medications over the past 4 weeks?

If you have answered any of the above questions with “yes”, you are not eligible to take part in the study.
Are you still interested in taking part in the study?
APPENDIX F
DAILY DIARY

Daily Diary
Subject #_____

- This is a daily journal that will include questions about your bowels, medication use, study foods and fiber use.
- Please fill out each day **before bed time**.
- It is important to collect accurate and complete information in order to successfully analyze it.
- Make sure to fill out each day.
- If you have any question, please feel free to call the study number **352-682-4883** or **352-870-9086**.
Daily Diary

1. How many bowel movements did you have today?
   - 0
   - 1
   - 2
   - 3
   - 4
   - 5
   - >6

2. Did you experience diarrhea today?
   - yes
   - no

3. Did you take laxative today?
   - yes
   - no

4. Are you currently taking antibiotics?
   - yes
   - no

5. Did your medication change?
   - yes
   - no

6. How many servings of study foods did you consume today?
   - 0
   - 1
   - 2
   - 3
   - 4
   - 5
   - >6

7. Did you consume a fiber supplement today? If so, what did you take?
APPENDIX G
FOOD RECORD

Please be as specific as possible when recording foods and beverages. Include types of breads (wheat, rye, etc), preparations of foods (grilled, raw, canned, etc), any added dressings or condiments, or brand names when applicable. A meal will require more than one entry if multiple foods were eaten.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Type of Meal or Snack</th>
<th>Food Eaten (include preparation, brand names, or specific types of bread where applicable)</th>
<th>Amount of Food Eaten (cups, ounces, number of items, etc)</th>
<th>Additional Notes or Comments</th>
</tr>
</thead>
</table>

120
## Measurement Guide

<table>
<thead>
<tr>
<th>Hand Symbol</th>
<th>Equivalent</th>
<th>Foods</th>
<th>Calories</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fist</td>
<td>1 cup</td>
<td>Rice, pasta, Fruit, Veggies</td>
<td>200 75 40</td>
</tr>
<tr>
<td>Palm</td>
<td>3 ounces</td>
<td>Meat, Fish, Poultry</td>
<td>160 160 160</td>
</tr>
<tr>
<td>Handful</td>
<td>1 ounce</td>
<td>Nuts, Raisins</td>
<td>170 65</td>
</tr>
<tr>
<td>2 Handfuls</td>
<td>1 ounce</td>
<td>Chips, Popcorn, Pretzels</td>
<td>150 130 130</td>
</tr>
<tr>
<td>Thumb</td>
<td>1 ounce</td>
<td>Peanut butter, Hard cheese</td>
<td>170 100 100</td>
</tr>
<tr>
<td>Thumb Tip</td>
<td>1 teaspoon</td>
<td>Cooking oil, Mayonnaise, butter, Sugar</td>
<td>40 15 15</td>
</tr>
</tbody>
</table>

- 1 cup (200 ml) = cup of liquid | 1 cup (240 ml) = cup of ice cream, macaroni, pasta
- 1 cup (300 ml) = cup of soda, milk, juice
- 1 cup (400 ml) = cup of water
- 1 cup (500 ml) = cup of soup
- 1 slice (100g) = slice of bread, cake
- 1 egg (50g) = egg
- 1 tablespoon (15ml) = tablespoon of butter, oil
- 1 teaspoon (5ml) = teaspoon of salt, sugar

Symbols:
- Fist: 1 cup
- Palm: 3 ounces
- Handful: 1 ounce
- 2 Handfuls: 1 ounce
- Thumb: 1 ounce
- Thumb Tip: 1 teaspoon

Examples:
- 1 cup of water
- 1 cup of pasta
- 1 cup of rice
- 1 cup of milk
- 1 cup of ice cream
- 1 cup of soda
- 1 cup of soup
- 1 slice of bread
- 1 egg
- 1 tablespoon of butter
- 1 teaspoon of salt

Calories for different food items are also provided in the table.
Food Record Procedure Manual
Example Food Record

- Sample Diet: Provide as much detail as possible
- Type of Meal: Breakfast 8:00am
- Food Eaten: 3 Whole Eggs scrambled
  - 1 Tablespoon of butter
  - Pinch of salt
  - 2 Slices of Arnold whole wheat Bread
  - 2 Tablespoons of Welch’s brand grape jelly
  - 1.5 cups of Tropicana Orange Juice
MEASURING CUPS (C): Use the measuring cups to estimate the capacity of mugs, bowls or glasses, and to estimate sizes of portions or servings. For example, these cups would be used to estimate quantities of measure of liquids (such as juice or milk) and solids (such as potato salad or corn chips).

MEASURING SPOONS (tsp, tbsp): Use the measuring spoons to estimate the capacities of cooking spoons, serving spoons, or household spoons, and to estimate small amounts. Always have the respondent estimate level spoons.

RULER (""): Use the ruler to estimate dimensions in inches. For example, the ruler would be used to estimate the length, width, and height of a piece of cornbread; and the length and width of a piece of meat (height would be estimated using the thickness sticks).
3-Day Food Records

- Instructions:
  Please provide a descriptive record of everything you have consumed for 3 consecutive days for each week.

Step 1: Record Date food or drink was consumed
Step 2: Record Time
Step 3: Type of Meal or snack (Breakfast, lunch, etc.)
Step 4: Describe what was consumed (brand names, read off packaging, condiments, spices, homemade) Use one space for each type of food or drink. Remember additions to foods such as milk on cereal, cream in coffee.
Step 5: Describe how much food or drink was consumed (Use measurement tools)
Step 6: List any additional comments to better describe the food or drink consumed.
APPENDIX I
TELEPHONE SCRIPT

(study coordinator calling after potential participant indicated preference to be called at specific number and during certain time)

Hello, my name is__________, and I am calling from the Food science and Human Nutrition department at University of Florida. May I speak with (the name of participant). We were informed by your doctor office that you were interested to receive more information about a fiber study, is that correct?

Participant, yes.

So do you have few minutes to discuss the study?

Participant, yes.

Great. I would be happy to give you more information about the study. The purpose of this study is to determine whether providing adequate fiber to patients with CKD will result in improved gastrointestinal function and quality of life, and clinical markers. If you qualify and decide to participate, you will be randomly assigned to a intervention or control group, but you will not be told which group you are in until completion of the study. Both groups will be given study foods (eg. crackers) and fiber powder supplement that can be added to foods and beverages to be consumed daily for a period of 12 weeks (84 days), with the intervention group receiving high fiber food. During the course of the study you would be asked to go to a Quest Diagnostic facility near you to have your blood drawn on six separate occasions. Additionally, you will be asked to come to our clinical lab at University of Florida campus in Gainesville on two separate occasions approximately twelve weeks apart to have blood drawn. These appointments should take no more than 30 minutes. Throughout the study you will be receiving questionnaires in the mail in a pre-paid return envelopes. These questionnaires will be asking questions regarding your quality of life, bowel frequency, and food intake.

Foods provided to all participants will provide nutrients and energy. Individuals selected for the fiber-fortification group may experience improved gastrointestinal function and quality of life due to the fiber. Does this sound like something you would be interested in doing?

(Responds Yes)

Great, now I will read the inclusion/exclusion criteria for the study to make sure you qualify. Please wait until I finish reading through this list, then you can let me know if you are still interested.

(Read inclusion/exclusion criteria without pausing)

Does this still sound like something you would like to take part in?

(Responds Yes)

Great, then we can schedule an initial appointment for you to receive more detailed information on the study and review a consent form. Is there a date and time that works best for you?

(Schedule appointment and obtain best way to contact patient)
**Inclusion Criteria**
Participants Must:
- Be 18 years of age or older
- Have GFR of ≤ 50 mL/min/1.73 m² (stages 3, 4 and 5 but who are not on dialysis)

**Exclusion Criteria**
- Have you been diagnosed with acute kidney injury (AKI)
- Have you been diagnosed with glumerulonephritis (GN)?
- Are you on immunosuppressant/steroid medications?
- Are you taking a probiotic supplement and refuse to discontinue it?
- Are you scheduled for dialysis within 3 months of study initiation?
- Do you have a history of liver disease?
- Have you been on dialysis?
- Have you undergone renal transplantation?
- Are you breastfeeding?
- Do you have active gastrointestinal bleeding?

If you have answered any of the above questions with “yes”, you are not eligible to take part in the study.
Are you still interested in taking part in the study?
University of Florida Study
Pea Hull Fiber

Orange Blueberry Muffin No Pea Fiber
(Control)

**Nutrition Facts**

| Amount Per Serving | Calories 180 | Calories from Fat 40
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% Daily Value</td>
<td></td>
</tr>
<tr>
<td>Total Fat 4.5g</td>
<td>7%</td>
<td></td>
</tr>
<tr>
<td>Saturated Fat 2.5g</td>
<td>13%</td>
<td></td>
</tr>
<tr>
<td>Trans Fat 0g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cholesterol 25mg</td>
<td>8%</td>
<td></td>
</tr>
<tr>
<td>Sodium 135mg</td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>Total Carbohydrate 30g</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>Dietary Fiber 1g</td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td>Sugars 14g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Protein 3g</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin A 4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitamin C 2%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Calcium 4%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Iron 6%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.

<table>
<thead>
<tr>
<th>Amount Per Gram</th>
<th>Fat 5 g</th>
<th>Carbohydrate 4 g</th>
<th>Protein 4 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calories</td>
<td>2,000</td>
<td>2,500</td>
<td></td>
</tr>
<tr>
<td>Total Fat</td>
<td>Less than 5g</td>
<td>Less than 5g</td>
<td>Less than 5g</td>
</tr>
<tr>
<td>Saturated Fat</td>
<td>Less than 5g</td>
<td>Less than 5g</td>
<td>Less than 5g</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Less than 30mg</td>
<td>Less than 30mg</td>
<td>Less than 30mg</td>
</tr>
<tr>
<td>Sodium</td>
<td>Less than 2,400mg</td>
<td>Less than 2,400mg</td>
<td>Less than 2,400mg</td>
</tr>
<tr>
<td>Total Carbohydrate</td>
<td>30g</td>
<td>Less than 17g</td>
<td>Less than 17g</td>
</tr>
<tr>
<td>Dietary Fiber</td>
<td>2g</td>
<td>10g</td>
<td></td>
</tr>
<tr>
<td>Protein</td>
<td>2g</td>
<td>10g</td>
<td></td>
</tr>
</tbody>
</table>

**Ingredients:** Enriched Flour (Bleached Wheat Flour, Malted Barley Flour, Niacin, Reduced Iron, Thiamine Mononitrate, Riboflavin, Folic Acid), Reduced Fat Milk, Blueberries, Sugar, Unsalted Butter, Eggs, Orange Juice Concentrate. Baking Powder (Cornstarch, Sodium Bicarbonate, Sodium Aluminum Sulfate, Monocalcium Phosphate), Natural Flavor, Salt.

**Allergens:** Contains Egg, Milk, Wheat
University of Florida Study
Pea Hull Fiber

Orange Blueberry Muffin with Pea Fiber
AC125-72-2d

NUTRITION FACTS
Serving Size (90g)
Serving Per Container

Amount Per Serving
Calories 170  
Calories from Fat 50%

% Daily Value
Total Fat 8g  9%
  Saturated Fat 3.5g  18%
  Trans Fat 0g

Cholesterol 45mg  16%

Sodium 320mg  13%

Total Carbohydrate 26g  9%
  Dietary Fiber 10g  40%
  Sugars 10g

Protein 3g

Vitamin A 4%  •  Vitamin C 4%

Calcium 6%  •  Iron 4%

Percent Daily Values are based on a 2,000 calorie diet. Your daily values may be higher or lower depending on your calorie needs.

Calories: 2,000  •  2,500

Total Fat: Less than 65g  •  57g
  Saturated Fat: Less than 10g  •  16g
  Cholesterol: Less than 300mg  •  300mg
  Sodium: Less than 2,400mg  •  2,400mg
  Total Carbohydrate: 300g  •  250g
  Dietary Fiber: 210g  •  100g

Calories per gram:
  Fat 9  •  Carbohydrate 4  •  Protein 1

Ingredients: Reduced Fat Milk, Blueberries, Sugar, Enriched Flour (Bleached Wheat Flour, Malted Barley Flour, Niacin, Reduced Iron, Thiamine Mononitrate, Riboflavin, Folic Acid), Pea Hull Fiber, Eggs, Unsalted Butter, Orange Juice Concentrate, Baking Powder (Cornstarch, Sodium Bicarbonate, Sodium Aluminum Sulfate, Monocalcium Phosphate), Natural Flavor, Sodium Bicarbonate, Salt, Xanthan Gum.

Allergens: Contains Egg, Milk, Wheat
Frutafit® HD

Description

Frutafit® HD is a native inulin/fructo-oligosaccharide (FOS). It is a natural powdered food ingredient extracted from chicory roots. Frutafit® HD can be applied in a wide range of food products.

Inulin from chicory is a polydisperse mixture of linear fructose polymers with mostly a terminal glucose unit, coupled by means of β(1-2) bonds. The number of units (degree of polymerisation) can vary between 2 and 60.

Specification

(Method of analysis: available on request)

Physical aspects
Dry matter content 95-99%

Composition on dry matter
Carbohydrates ≥ 99.5%
Inulin (DP2-DP60) ≥ 90%
Fructose, glucose, sucrose ≤ 10%
Average chain length 8-13 monomers
Ash ≤ 0.2%
Heavy metals Pb, As each ≤ 0.1 mg/kg
Cd, Hg each ≤ 0.01 mg/kg

Microbiology
Aerobic plate count (30°C) ≤ 1000 CFU/gram
Aerobic plate count (55°C) ≤ 1000 CFU/gram
Moulds ≤ 20 CFU/gram
Yeasts ≤ 20 CFU/gram
Bacillus cereus ≤100 CFU/gram
Listeria monocytogenes ≤100 CFU/gram
Enterobacteriaceae absent in 1 gram
Staphylococcus aureus absent in 1 gram
Salmonella absent in 25 grams

Nutritional information

All values are averages expressed per 100 grams

Frutafit® HD:

Carbohydrates:
- digestible (fructose, glucose, sucrose) 97 grams
- non-digestible (dietary fibre, inulin) 7 grams
Proteins 0 gram
Fats 0 gram
Dietary fibres (AOAC 997.08) 90 grams
Moisture 3 grams

Minerals:
- Sodium 40 mg
- Calcium 11.5 mg
- Potassium 7.5 mg
- Iron 0.4 mg
- Other minerals negligible

Vitamins negligible
Cholesterol absent
Gluten absent
Lactose absent
Folate absent
Insecticides, pesticides absent
Enzymatic activity absent
Colour, flavour, preservatives absent

Calorific value 1.2 kcal/gram
Glycaemic response 14

1 Calculated value based on 1 kcal/gram pure inulin. Please check local legislation and adapt if necessary.
2 The effect on the blood glucose level of 25 gram carbohydrate coming from Frutafit® HD is compared with the effect on blood glucose level of 25 gram glucose (control=100).
APPENDIX L
BASELINE QUESTIONNAIRE

Address:
Phone number:
D.O.B: ____________
Sex (circle one):    M     or     F
Please describe your race/ethnicity. Check all that apply.

Race and Ethnicity
○ American Indian or Alaska Native
○ Black or African American
○ Asian
○ White
○ Native Hawaiian or other Pacific Islanders
○ Hispanic
○ Other___________
○ Hispanic
○ Other___________

Please verify health history and medication use

Medical history
Are you currently or have been diagnosed with any of the following conditions?
○ Diabetes I
○ Diabetes II
○ Hypertension
○ Wasting disease
○ Inflammatory bowel disease
○ Other; please indicate:

Medication History
Are you currently taking any of the following medications (please indicate by circling)
○ Insulin injections
○ Diabetes medication
○ Antibiotics or Trimethoprim or similar class medications (e.g. cimetidine, cefoxitin)
○ Hypertension medications (Antihypertensive agents, diuretics, beta blockers, or calcium channel blockers)
○ ACE inhibitors (e.g. Trandolapril, Benazepril (Lotensin), Captopril (Capoten) etc.
○ Other, please indicate;

If you are currently taking an antibiotic or have taken antibiotics in the past 2 weeks, please indicate:
When did you start?
If finished, when did you finish?
How long is/was your prescription for (when is/was your last day) ?
Name of prescription: _______________
Dosage: _______________

Have you had a change of medication recently?
No Yes, if yes:
What medication did you stop using?

What medication did you start using?

Diet and Supplement Use
Are you currently taking any supplements or nutritional foods? Please indicate foods/beverages that are supplemental to the diet (i.e. ensure, special foods, fiber formula, vitamins, minerals etc.) Specifically, indicate any fiber or prebiotics/probiotics supplements or foods you are taking:
Yes No , if yes:
What are you taking? (please specify type, name and brand if possible)

How long have you been taking it for?
How long do you intend to take it for?
Name of supplement/nutritional food: ______________________
Dosage/amount: ___________________

Are you on a special diet? (I.e. vegetarian, gluten free etc.) Yes No , if yes
Please list:
APPENDIX M
FOOD RECORD

3-day Food Record

Study No: __________________________ Study Phone No: 352-870-9086

**Tips for Completing an Accurate Food Record**

**Complete it for three days.** Your food record should be for three days of intake. Include the day and date at the top of each form. The three days can be consecutive or non-consecutive and should include at least one weekend day.

**Use a separate form.** Use a separate sheet for each day of the food record. Multiple sheets are included.

**Carry it with you.** Carry the food record with you during the day and document your meals and snacks soon after you eat. It is very difficult to recall what you ate days or hours later.

**Describe combination foods.** If you are eating combination foods, such as pizza with various toppings, make sure to record these ingredients.

**Estimate serving size.** Estimate the serving size to the best of your ability. Use the serving size on the food label if available. If you are uncertain, estimate using familiar objects. For example, you can use “palm of your hand” to estimate the size of a chicken breast or “baseball” to estimate an ice cream serving.

**Write down beverages.** Make sure to record all beverages that you consume in the food and beverage description. This also includes no-calorie drinks such as diet sodas and unsweetened ice tea.

**Eating out.** Indicate the name of the restaurant or franchise if possible and indicate the food you ate from the menu by writing the exact menu name and amount.

**Approximate water intake.** Record your total daily estimated water intake at the bottom of each daily food record. Include other beverages (e.g. juice, soda) in the Food & Beverage Description section.

**Write clearly and use extra room or sheet.** Use the extra sheet or the back of the sheet page if you need more room.

**Mail back.** When done please mail it back as soon as possible using the prepaid mail provided.

**Call Us.** Don’t hesitate to call the phone number provided on the top of this page if you have any question at anytime.
Food Record – Day #
Subject No ___________  Day/Date______________

Serving Size/Food & Beverage Description

Breakfast /Snack

Lunch /Snack

Dinner /Snack
APPENDIX N
BOWEL FREQUENCY QUESTIONNAIRE

Date: ____________ Study #: ______________

1. How many bowel movements did you have today?
   □ 0
   □ 1
   □ 2
   □ 3
   □ 4
   □ 5
   □ >6

2. Did you experience diarrhea today?
   □ Yes
   □ No

3. Did you take laxative today?
   □ Yes
   □ No

4. Are you currently taking antibiotics?
   □ Yes
   □ No

5. Did your medication change?
   □ Yes
   □ No

6. Did you consume a fiber supplement today? If so, what did you take?
   □ Yes  __________________________
   □ No


15. Physical Activity and Health. Atlanta, GA: Division of Nutrition, Physical Activity and Obesity, National Center for Chronic Disease Prevention and Health Promotion, 2011.


BIOGRAPHICAL SKETCH

Younis Salmean earned his Bachelor of Science degree in nutrition from California State University Fresno in 2005. He was the recipient of a highly sought after scholarship awarded by Kuwait University. He went on to join the Department of Food Science and Human Nutrition at the University of Florida, where he earned his Master of Science degree in 2008. Younis subsequently joined the doctoral program at the University of Florida and earned his Doctor of Philosophy degree in August, 2013. Younis Salmean has presented his dissertation research at conferences such as the Food & Nutrition Conference & Expo in San Diego, 2011. As well as Experimental Biology in 2010, and 2013. Additionally, Younis Salmean has published part of his research in the Journal of Renal Nutrition.