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## The Effect of Dietary Fiber on Epigenetic Regulation of Gene Expression in Hemodialysis Patients

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# THE EFFECT OF DIETARY FIBER ON EPIGENETIC REGULATION OF GENE EXPRESSION IN HEMODIALYSIS PATIENTS

ISABELLE STRACK

40 Pages

**Objective:** More than 2/3 of those with kidney failure use hemodialysis as a treatment option. The purpose of this pilot study is to increase the understanding of the epigenetic changes that occur in hemodialysis patients through dietary fiber intake.

**Design and Methods:** Gene expression through epigenetic changes of dietary fiber intake and soluble fiber intake were analyzed in this cross-sectional study. Data was collected from questionnaires and blood samples ( $n=20$ ). Block Dialysis Food Frequency Questionnaire was used to calculate dietary fiber intake. Blood analysis counted the top 100 most prevalent microRNAs (miRNA). Pearson  $r$  correlation was used to determine level of significance ( $p$ -value  $< 0.05$ ).

**Results:** There was no significant relationship between BMI and fiber intake or length on dialysis (in months). There was a significant, positive relationship between dietary fiber intake and mir-886 (Pearson  $r = 0.68$ ), mir-941-2 (Pearson  $r = 0.52$ ), and mir-148b (Pearson  $r = 0.54$ ). Soluble fiber intake and mir-886 had a significant, positive relationship (Pearson  $r = 0.66$ ). There are 4 genes associated with mir-886, no genes associated with mir-941-2, and mir-148b has 19 genes strongly associated with it.

**Conclusion:** Fiber intake affects the regulation of gene expression through altered miRNA production, thus fiber intake (epigenetic modifications) may serve as a potential treatment beneficial for hemodialysis patients despite dietary restrictions. Further research is needed to validate this research.

**KEYWORDS:** Kidney, Fiber, Hemodialysis, Epigenetic, miRNA

THE EFFECT OF DIETARY FIBER ON EPIGENETIC REGULATION OF GENE  
EXPRESSION IN HEMODIALYSIS PATIENTS

ISABELLE STRACK

A Thesis Submitted in Partial  
Fulfillment of the Requirements  
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Department of Family and Consumer Sciences

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THE EFFECT OF DIETARY FIBER ON EPIGENETIC REGULATION OF GENE  
EXPRESSION IN HEMODIALYSIS PATIENTS

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## CHAPTER I: INTRODUCTION

Chronic kidney disease (CKD) has become more prominent, with an overall prevalence of 14% of the United States population.<sup>1</sup> This is a disease that can be debilitating and worsen over time. At this moment, there are no known cures for CKD, but there are methods of treatment to prolong the lives of those diagnosed including hemodialysis, kidney transplantation, and lifestyle changes. There are five stages to CKD and there are different preventative measures taken in each stage. The fifth stage is called kidney failure and it occurs when both kidneys no longer function well enough to keep the body healthy. Patients with kidney failure need dialysis, a treatment given to remove toxins that are not excreted through urination, to help maintain their function and keep them living. There are two types of dialysis, hemodialysis and peritoneal dialysis. Hemodialysis is a medical procedure using a dialyzer that acts as an “artificial kidney” and draws blood from the body to remove toxins and waste before returning the filtered blood to the body. Peritoneal dialysis infuses dialysate into the patients’ peritoneal membrane to filter the blood, correct electrolyte imbalances, and then empties the dialysate with waste at the end of treatment.<sup>1,2</sup>

Nutrition is crucial to lifestyle changes because it aids in symptom management and disease retardation of CKD. The kidneys work to remove waste in the body and by managing what goes into the body through nutrition, kidney function can be better maintained. Sodium and potassium are nutrients that the kidney has difficulty filtering when in a diseased state, and these minerals are limited in the kidney diet to help the kidney work optimally in its diseased state.<sup>3,4</sup> Fiber is the dietary material that is resistant to the action of digestive enzymes and feeds the gut bacteria.<sup>5</sup> Dietary fiber has been linked with positive effects on cell turnover, metabolism, and eating behavior as it feeds the commensal bacteria in the gut, promoting growth and longevity of the gut

microbiome.<sup>6</sup> The aims of this study were to explore the amount of dietary fiber intake and how it affects the genes associated with gut microbiome in those with CKD.

Dietary fiber has been proven to have an effect on shaping epigenetic biomarkers of the gut microbiome.<sup>7,8,9</sup> Epigenetic refers to the changes in gene expression caused by nongenetic influences which can include nutrition, physical activity, or medications. Epigenetic biomarkers refer to the measurement of epigenetic modifications in tissues or peripheral fluids that are used as markers of disease detection, progression, and therapy response.<sup>10</sup> A commonly used epigenetic biomarker is microRNA, which is a small part of RNA that prevents protein development by binding to and destroying mRNA. Many studies have identified a correlation with the gut microbiome and the establishment of metabolic memory, the lingering effects of poorly controlled blood glucose levels that effect metabolism long-term, in CKD due to changes in appetite and meal routine.<sup>4,7,11</sup> This can eventually lead to diabetes, a comorbidity, and is an example of how diet influences epigenetic changes in the body. Most of the studies that have researched the correlation between dietary fiber and the gut microbiome have only been performed with the general population and do not distinguish populations with a low fiber intake due to dietary restrictions in a disease such as CKD. There are a few studies that mention dietary fiber intake in hemodialysis patients, but these studies have focused on the cardiovascular system rather than the gut microbiome.<sup>12,13</sup>

Dietary fiber intake in relation to the epigenetic markers in patients receiving hemodialysis has not been fully explored. Dietary fiber can influence the healthiness of the gut microbiome.<sup>7,12,14</sup> It is unclear if there is a direct effect of dietary fiber on slowing the progression of CKD, however it is possible that there is a correlation between the two through a mediating effect of the gut microbiome that is nourished with fiber. Currently there is no ideal fiber intake identified for

hemodialysis patients, there is only a restriction of types of foods that may be consumed. It is estimated that hemodialysis patients consume 11-12 g/day of dietary fiber.<sup>4</sup> This is very low compared to the suggested intake for the general population, 25 g/day for women and 38 g/day for men, or 14 grams per every 1000 kcal consumed in a day.<sup>5</sup>

The purpose of this research is to determine if there is a correlation between dietary fiber intake and epigenetic changes of gene expression in patients receiving hemodialysis. The hypothesis is that as fiber intake is decreased, there will be a corresponding downregulation of genes associated with fermentation. The independent variable is the consumption of dietary fiber. The dependent variable is the epigenetic biomarkers (microRNAs) that may be affected by the consumption of dietary fiber.

## **Methods**

### **Sampling Procedure**

The study was cross-sectional using survey data, along with collection of blood from 20 patients in a local dialysis clinic. A local dialysis clinic was contacted and asked to participate in this study. Flyers were placed in the clinic with inclusion and exclusion criteria and contact information for study personnel. All interested subjects contacted the researcher directly or left their information at the clinic for the researcher to contact them. Power analysis was used to find the final analytic aim.

To be included in this study patients must be English speaking, between 18 and 90 years old, receiving maintenance hemodialysis for at least one year, and experiencing two or more kidney disease related symptoms for at least one year. Participants were excluded if they were receiving peritoneal dialysis, had a previous kidney transplantation, had a diagnosed

gastrointestinal illness such as inflammatory bowel disease, or had received antibiotics or probiotics in the past three months.

### **Data Collection**

Two appointments were set with each participant, based on their convenience. These appointments occurred at the dialysis center. At the first appointment, written informed consent was reviewed with participants and they were informed that they may choose to leave the study at any time. For the participants who continued, the consent form was signed and dated. The participants were given the option of releasing electronic health records that included health characteristics such as comorbidities and medications as well as the most recent lab data (chemistry profile, complete blood count, inflammatory markers). A blood specimen was collected before the dialysis session by a dialysis nurse. After collection, these specimens were transported to Illinois State University School of Biological Sciences (ISUSBC) by a research member where they were stored at -80° Celsius until analysis. The blood specimens were used to study epigenetic biomarkers via microRNAs. During the dialysis session, the participant filled out the study questionnaires which can be answered in a total of two hours or less. These surveys include anthropometric data, socio-demographic data, and dietary behavior. The primary goal of the second appointment was to secure an opportunity to meet with the participants to discuss any significant changes in health or health behaviors since the first appointment.

### **Instrumentation**

The Block Dialysis Food Frequency Questionnaire (BDFFQ) from NutritionQuest takes dietary restriction and day-to-day variability in dialysis patients into consideration. Approximately 100 questions are in the BDFFQ and inquire on food types, frequency, and amount of food

consumed in the past three months. These questions were used to establish the diet of the participant to use in comparison with collected data from blood samples. MiRNA was collected using the blood samples.

## **Analysis**

Blood analysis required three steps. First, total RNA was extracted and purified using PAXgene Blood miRNA Kit. Second, the miRNAs within the total RNA were amplified and sequenced using NEBNext Multiplex Small RNA Sample Prep Set and Illumina HiSeq 4000 in Roy J. Carver Biotechnology Center at University of Illinois Urbana Champaign. Third, the results were counted in each of the 20 samples.

The BDFEQ was returned to NutritionQuest for processing via an updated nutrient database that estimates nutrient composition and averages before returning in an electronic file for further analysis. The total dietary fiber consumption and soluble fiber intake was measured in grams. The 100 most prevalent miRNA found in the blood samples was cross analyzed with the total dietary fiber intake and the soluble fiber intake. Pearson  $r$  correlation was used via SPSS Statistics for Windows, Version 26.0 using a p-value  $\leq 0.05$  for statistical significance. A curated database of miRNA interactions, miRTarBase, was used to find all correlated genes that expressed statistically significant miRNA.<sup>15</sup> For statistical measures SPSS was used to conduct a Pearson  $r$  correlation.<sup>16</sup> A Pearson  $r$  correlation with a p-value  $\leq 0.01$  was used for BMI vs. total dietary fiber intake and length of dialysis (in months) vs. total dietary fiber intake.

## **Results**

### **Demographics**

A total of 20 participants completed this study. Table 2 shows the majority of the participants were Caucasian (74%), followed by 16% of the participants being African American,

5% Hispanic/Latino, and 5% American Indian/Alaskan native (Table 2). The majority of the participants identified as male (79%) and 21% of the participants were female as shown in Table 3. Table 4 includes descriptive statistics including age, weight, BMI, and length on dialysis. The average age of the participants was 62. There was a 62-year age gap between the oldest and youngest participants. Out of these participants the average BMI was 28, classifying the average participants as overweight. The average length of dialysis in months was 32 months out of the 19 participants that filled out the BDFEQ.

### **Fiber Intake**

The total dietary fiber intake and soluble fiber intake was averaged out of 19 participants that filled out the BDFEQ and can be found in Table 4. Average total dietary fiber intake calculated from the BDFEQ was 12.55g daily. Maximum total dietary fiber intake was 27.01g daily while minimum dietary fiber intake was 1.44g daily. Average soluble fiber intake was 4.36g soluble fiber daily. Maximum soluble fiber intake was 8.57g soluble fiber daily while minimum soluble fiber intake was 0.44g soluble fiber daily. There was no statistically significant relationship between BMI and total dietary fiber or soluble fiber intake (Figure 1 and 2).

### **miRNA Statistics**

When investigating the potential relationship between miRNA prevalence and total dietary fiber intake, three out of the top one hundred most prevalent miRNA were found to have a statistically significant relationship with total dietary fiber intake as shown in Table 5. There was a significant, positive, and strong relationship between total dietary fiber intake and mir-886,  $r(14) = 0.68$ ,  $p < 0.01$  (Figure 3). There were four genes associated with mir-886 in the miRTarBase database: MCL1, CXCL12, CDC6, and PLK1. There was a significant, positive, and moderate



relationship between total dietary fiber intake and mir-941-2,  $r(14) = 0.52, p < 0.05$  (Figure 4). There were no genes found to be associated with mir-941-2. There was a significant, positive, and moderate relationship between total dietary fiber intake and mir-148b,  $r(14) = 0.54, p < 0.05$  as demonstrated in Figure 5. There are 19 genes strongly associated with mir-148b in the miRTarBase database: HLA-G, CCKBR, ITGA5, ROCK1, PIK3CA, NRAS, CSF1, ALCAM, PRKAB1, ACVR1, MAP3K9, HOTAIR, MLH1, PIK3R3, WNT1, SLC2A1, CYBB, and MCL1. One miRNA out of the top one hundred most prevalent miRNA was found to have a statistically significant relationship with soluble fiber intake. There was a significant, positive, and moderate relationship between soluble fiber intake and mir-886,  $r(14) = 0.66, p < 0.01$  (Figure 6).

## Discussion

The purpose of this study was to increase the understanding of dietary fiber intake and the epigenetic changes that occur in hemodialysis patients. Results of this study could be utilized by the entire healthcare team that works with hemodialysis patients. This could potentially change how dialysis clinics educate patients about food avoidance and consumption. Currently, many fruits and vegetables are restricted for hemodialysis patients because of their high potassium levels. The kidney diet is very restrictive, as it generally restricts dietary intake to include low salt, low potassium, and a high protein content. As a pilot study, this is a novel experiment that could detect a relationship between fiber and epigenetic changes in individuals with CKD.

## Microbiome

By the age of 60, the gut microbiome becomes less diverse as beneficial microbes such as *Lactobacillus* and *Bifidobacterium* are lost.<sup>17,18</sup> Instead enterobacteria populations increase, increasing risk for infections and need for antibiotics that decrease bacterial activity in the gut.<sup>18</sup>

It was a requirement that participants did not have any antibiotics within six months of participation to eliminate the potential confounding factor. Researches have proposed that changes to the microbiota occur gradually over time and increase diversity with chronological age.<sup>19,20</sup> The elderly are more likely to have a habitual diet which decreases overall richness in the gut microbiome and allows unhealthful bacteria to thrive in this environment preventing the production of various proteins.<sup>21</sup> This habitual diet may lead to a deteriorated relationship between the host and gut microbiome, resulting in gut dysbiosis. This proposes that young individuals are more likely to have a more diverse diet and a greater representation of miRNA from the diet. The majority of the participants in this pilot study (58%) were >60 years old, which may have resulted in less diverse microbial populations.

### **Fiber and Diet Restrictions**

A diet rich in fruits, vegetables, and whole grains is associated with better outcomes in CKD patients, through achievement of a lower body weight maintaining lean body mass in obese patients, improving blood pressure, and reducing cardiovascular risk.<sup>22,23</sup> Cardiovascular complications are the number one cause of death in patients on hemodialysis.<sup>4</sup> It is common for people living with kidney failure to not get enough fiber as a kidney diet is a strict diet, restricting foods many fruits, vegetables, and whole grains due to mineral restriction of potassium and phosphorus. In this study, 0% of the population consumed the recommended amount of dietary fiber intake daily (compared to 25 g/d for females and 38 g/d for males respectively).<sup>25</sup> The average consumption of fiber in this study was 36% of the recommended amount of dietary fiber. Since many forms of dietary fiber are restricted for kidney patients, this is likely an accurate representation of this population. This study found that the relationship between dietary fiber

intake and length of dialysis was not significant, indicating that patients do not increase or decrease their fiber intake as their disease progresses.

A kidney diet has many restrictions in it as many food sources of fiber are high in potassium and phosphorus. Potassium is needed for muscle contraction, but too much potassium can cause muscle cramps, weakness, irregular heartbeat, or a heart attack. In kidney failure, the kidneys have difficulty filtering potassium, leaving many hemodialysis patients with hyperkalemia. There are ways to limit this without strictly using the diet, including use of potassium-binding medications- a common practice in this population.<sup>25</sup> Too much phosphorus in the body can cause hyperphosphatemia which leads to a bone disease which causes bones to become weak and break easily.<sup>26</sup> Limiting the amount of phosphorus in the diet aids in the prevention of this problem. The threat of hyperkalemia may prevent increasing consumption of fiber. Electrolytes are carefully monitored in kidney failure, but decreased consumption of fiber has adverse health outcomes in this population such as constipation, increased inflammation in the body, and increased triglyceride levels.<sup>27</sup> Several studies have shown that a diet rich in fiber and potassium is associated with a reduced risk of CKD prevalence, progression, and kidney failure.<sup>28,29</sup>

### **miRNA and Genes**

When a miRNA is increased, it increases protein production by activating genes or decreases protein production through deactivating genes. Among the top 100 most prevalent miRNA, there were five that have been related to kidney disease in literature: mir-142, mir-146a, mir-15b, mir-192, and mir-29c. The consumption of dietary fiber did not affect the prevalence of these miRNAs, indicating that fiber consumption does not play a large role in kidney failure in this sample population.

Out of the three significant miRNAs in this study, mir-941-2 did not have any genes associated with it in literature. This shows that there is a plethora of information to be learned about how the human body works and it is not fully mapped out yet. There are four genes associated with mir-886 in literature: MCL1, CXCL12, CDC6, and PLK1. These genes are associated with auto-immune diseases such as human immunodeficiency virus type 1, Meier-Gorlin syndrome 5, and monoclonal paraproteinemia.<sup>30</sup> They are not associated with any diseases related to the gut or metabolism. The results suggest that increasing fiber could help be protective to the immune system and prevent these diseases. There are 18 genes associated with mir-148-b in literature: HLA-G, CCKBR, ITGA5, ROCK1, PIK3CA, NRAS, CSF1, ALCAM, PRKAB1, ACVR1, MAP3K9, HOTAIR, MLH1, PIK3R3, WNT1, SLC2A1, CYBB, and MCL1. Many of these genes code for proteins that help to maintain function of the GI tract. Fiber aids in GI health and motility, indicating an increase in fiber would stimulate mir-148-b and stimulate these genes to increase protein production for metabolism in the gut. Mir-148-b is known to suppress HLA-G expression, a promising gene for BK polyomavirus replication (BKPyV) which is associated with nephropathy and premature kidney transplant failure.<sup>31,32</sup> Drugs are used to suppress BKPyV to decrease the chances of kidney rejection. Incorporating an adequate amount of fiber in the diet could aid in maintaining kidney function through increasing mir-148-b expression, decreasing HLA-G expression, and decreasing nephropathy.

CCKBR codes for hydrochloric acid secretion and acts as a tumor suppressor inhibiting kidney carcinoma cell growth.<sup>33</sup> Fiber could help to decrease the risk of kidney carcinoma through increasing CCKBR expression and preventing the side effects of CKD that may occur with kidney carcinoma. NRAS helps to produce GTPases that play important roles in cell division, cell differentiation, and apoptosis. This helps to maintain health at a cellular level and prevent

cancerous cells from forming. An increase in fiber could increase NRAS expression to maintain normal cell mitosis and reduce risk of cancer. HOTAIR is another gene that aids in prevention of cancer, specifically of the gastrointestinal system. An increase in dietary fiber can potentially increase HOTAIR expression and prevent gastrointestinal cancer by regulating bowel movements. Mir-148-b inhibits glycolysis through inhibiting SLC2A1, a vital gene for carbohydrate metabolism as it provides instruction for production of GLUT1 (glucose transporter protein type 1) that limits glucose uptake.<sup>34</sup> Decreased production of SLC2A1 can decrease production of GLUT1 and decrease glucose absorption into the brain and cause neurological damage. An adequate amount of fiber in the diet could inhibit GLUT1 production and aid in the maintenance of blood sugar levels over a longer period of time as it limits the amount of glucose uptake from the blood into the brain.

## **Conclusion**

This study suggests that fiber intake affects the regulation of genes by altering the production of miRNA. There have been propositions on how to modify the current kidney diet guidelines, as there is a movement for guidelines to educate more on the role of dietary fiber, potassium, cholesterol, and sodium in this diet instead of using restrictions.<sup>35</sup> There needs to be more research to find a safe and practical way to rewrite nutrition guidelines for CKD and kidney failure. For the majority of miRNA in this study, there was no significant relationship with fiber intake. For the three miRNA with a positive significant relationship with fiber intake, there was data about their effects on genes for two out of three. The relationship of mir-941-2 is unknown, indicating a need for further research. This study found proinflammatory genes targeted by significant miRNA (mir-886) that may help to decrease inflammation. This can also be explained by our knowledge of fiber increasing SCFA production which is anti-inflammatory. Mir-148b

plays a role in gene production to maintain normal cell cycle that is linked with reduced cancer risk. Increased fiber has potential in decreasing nephropathy and kidney transplant rejection through decreasing mir-148b and BK Polyomavirus replication. More miRNA needs to be mapped in comparison to the genes they upregulate and downregulate to be able to make assumptions with more certainty. Further research including a larger population more representative of the American hemodialysis population is required to determine validity of this study.

### **Practical Application**

Ultimately, this pilot study is promising in that there is a relationship between fiber and epigenetic changes. A limitation to this study is the small sample size. Further research is required to be considered statistically significant and applicable. In the future, it would be beneficial to include earlier stages on CKD in a longitudinal study to monitor how fiber intake affects miRNA expression. This could help determine if dietary fiber intake has a role in kidney disease progression over time.

Research would be beneficial to improve the care of those with CKD by optimizing the gut microbiome and its subsequent epigenetic role through the modifiable factor of fiber in the diet. With more research, dietary standards can be set to aid medical professionals in helping their patients. Patients would benefit from more accurate nutrition counseling and advice from dietitians.

Table 1 Study Demographics (N =20)

Participant ID	Age	Weight (kg)	Height (cm)	Length of Dialysis (in months)
1	62	68	173	104
2	78	80	183	21
3	33	132	173	39
4	29	71	165	23
5	58	128	198	19
6	64	65	170	-
7	61	87	150	20
8	67	84	185	21
9	91	75	178	13
10	79	60	175	34
11	64	94	175	25
12	43	63	183	14
13	66	122	178	21
14	43	105	178	81
15	57	110	175	20
16	41	51	158	62
17	81	87	185	12
18	75	105	168	31
19	57	94	170	14
20	87	76	152	42

Table 2 Ethnicity of Study Participants (N = 19)

	Frequency	Percent
White/Caucasian	14	70
Black/African American	3	15
Hispanic/Latino	1	5
American Indian/Alaskan Native	1	5

Table 3 Gender of Study Participants (N = 19)

	Frequency	Percent
Male	15	75
Female	4	20

Table 4 Descriptive Statistics (N = 19)

	N	Minimum	Maximum	Mean	Std. Deviation
Age	20	29.0	91.0	61.8	17.4
Weight (kg)	20	51.0	132.0	88.0	23.1
BMI (kg/m2)	19	17.3	44.1	28.1	7.5
Months on Dialysis	19	3.0	95.0	23.4	24.7
Total Dietary Fiber Intake	19	1.4	27.0	12.6	6.9
Soluble Fiber Intake	19	.4	8.6	4.4	2.2

Table 5 miRNA Prevalence (N = 16)

	Dietary Fiber R Correlation	Dietary Fiber Significance	Soluble Fiber R Correlation	Soluble Fiber Significance	Mean	Std. Deviation
mir_146a	-.183	.497	-.204	.450	3464	1110
mir_15b	.056	.836	-.013	.963	1875	627
mir_192	-.054	.844	-.071	.794	16670	8204
mir_29c	-.191	.479	-.215	.424	1443	734
mir_142	-.247	.357	-.258	.334	6180	3518
mir_100	.333	.207	.315	.235	6532	8924
let_71_2	.168	.535	.137	.612	3045	3045
mir_125b_1	.282	.289	.253	.345	3070	2941
mir_125b_2	.232	.386	.223	.406	669	628
mir_127	-.032	.906	-.055	.841	566	522
mir_409	-.121	.656	-.149	.582	939	759
mir_30b	-.278	.297	-.271	.311	6545	4936
mir_886	.677**	.004	.659**	.005	795	573
U106	-.252	.346	-.206	.444	828	566
let_7b	.230	.391	.236	.379	9756	6537
mir_618	-.042	.876	-.130	.631	1241	816
mir_99a	.028	.917	.000	.999	4000	2558
U48	-.008	.976	.031	.909	2817	1732
mir_4326	.154	.568	.115	.671	1502	922
mir_16_2	.170	.530	.083	.761	7361	4400
mir_148a	.363	.167	.307	.247	9418	5505
let_7f_2	.101	.711	.029	.915	4329	2433
mir_19b_1	-.313	.238	-.344	.191	1630	892
mir_363	.440	.088	.353	.180	10288	5529
U63	-.099	.716	-.072	.790	1058	565
let_7i	.293	.272	.204	.448	41116	22756
mir_181a_1	-.022	.934	-.045	.869	1466	779
mir_1180	-.039	.885	-.024	.930	2318	1202
U27	-.306	.250	.290	.275	1333	691

(Table Continues)



Table 5 Continues

	Dietary Fiber R Correlation	Dietary Fiber Significance	Soluble Fiber R Correlation	Soluble Fiber Significance	Mean	Std. Deviation
mir_182	.203	.450	.207	.441	26895	13797
mir_150	-.275	.302	-.211	.434	17251	8522
U84	-.129	.633	-.073	.789	9052	4391
mir_125a	.027	.920	.054	.843	3426	1617
mir_151	.118	.663	.112	.678	41083	19141
mir_140	-.058	.831	-.080	.768	68949	31644
mir_7_1	.408	.117	.334	.207	2486	1131
mir_199a_1	-.410	.115	-.411	.114	1503	665
mir_92a_1	.121	.657	.070	.796	532748	234666
mir_92b	.071	.794	.060	.826	1754	749
mir_145	-.093	.732	-.105	.698	1587	678
mir_342	-.213	.429	-.163	.547	3196	1363
HBII_210	-.186	.490	-.136	.617	10284	4382
mir_1979	-.225	.403	-.252	.347	21801	9199
mir_181a_2	.210	.436	.186	.491	6056	2549
mir_103_1_as	.414	.111	.331	.210	4538	1901
HBII_202	-.432	.095	-.380	.146	1565	652
U95	-.260	.330	-.199	.461	7060	2936
mir_194_2	.236	.379	.196	.466	4069	1684
mir_223	.080	.769	-.017	.949	12967	5358
mir_106b	.495	.051	.415	.110	2944	1214
mir_576	-.153	.571	-.195	.470	2329	948
mir_339	-.341	.197	-.378	.149	6778	2532
mir_25	.420	.105	.338	.201	100124	39821
mir_128_1	.415	.110	.329	.213	7445	2945
mir_652	.391	.134	.281	.292	4247	1674
mir_320a	-.226	.399	-.210	.434	19736	7740
mir_328	-.081	.764	-.061	.823	9010	3529
let_7g	.210	.434	.142	.599	12085	4710
mir_194_1	.420	.105	.354	.178	2661	1035
mir_942	.027	.920	-.073	.787	3755	1428
mir_30e	.291	.274	.173	.521	2335	884
mir_92a_2	.158	.559	.101	.708	60435	22772
U57	-.246	.359	-.234	.384	3156	1189
U31	-.005	.985	-.008	.978	1994	744
mir_941_2	.513*	.042	.476	.062	1943	718
mir_186	-.063	.817	-.111	.681	31012	11278
let_7a_3	-.074	.785	-.147	.587	6082	2312
mir_574	-.132	.626	-.068	.802	1614	580
mir_148b	.537*	.032	.465	.069	2732	973
mir_103_2_as	.433	.094	.346	.190	7134	2519
mir_361	.042	.877	.033	.905	23362	7861
mir_197	-.315	.234	-.280	.294	6065	2007
mir_210	.362	.168	.288	.280	1605	530
mir_451	-.367	.163	-.386	.140	3134560	1030538
mir_1307	.332	.208	.284	.287	2542	834

(Table Continues)

Table 5 Continues

	Dietary Fiber R	Dietary Fiber Significance	Soluble Fiber R	Soluble Fiber Significance	Mean	Std. Deviation
U104	-.261	.330	-.219	.416	20112	6590
U74	.053	.845	.074	.786	3831	1253
mir_26a_1	.430	.096	.368	.161	27668	9359
mir_130b	.007	.978	-.032	.905	1944	651
mir_30c_2	.000	.999	-.043	.873	80397	25558
mir_22	.363	.167	.311	.241	37079	11787
mir_425	.135	.618	.095	.727	59304	18659
mir_23a	-.119	.661	-.186	.490	2063	646
mir_625	.014	.959	-.033	.903	1755	538
let_7d	.146	.590	.074	.786	3527	1079
mir_93	.471	.066	.351	.183	5475	1662
mir_30d	.277	.299	.179	.508	62747	18804
mir_191	.453	.078	.437	.090	403133	114184
mir_18a	-.078	.775	-.137	.613	5626	1541
let_7f_1	.101	.710	.002	.993	2463	653
mir_532	.448	.082	.357	.175	9217	2361
mir_1975	-.285	.285	-.250	.351	3210	804
mir_484	.214	.425	.132	.627	103676	22514
mir_1826	.051	.850	-.005	.985	2421	517
mir_3158_1	-.218	.416	-.247	.356	5305	1108
mir_1358_2	-.218	.416	-.247	.356	5305	1108
mir_486	.248	.355	.186	.492	4643190	942560
mir_185	.369	.160	.249	.352	90754	17397
mir_3184	.151	.577	.147	.587	151194	28166

\*. Correlation is significant at the 0.05 level (2-tailed).

\*\*. Correlation is significant at the 0.01 level (2-tailed).

Figure 1 Total Dietary Fiber Intake vs. BMI

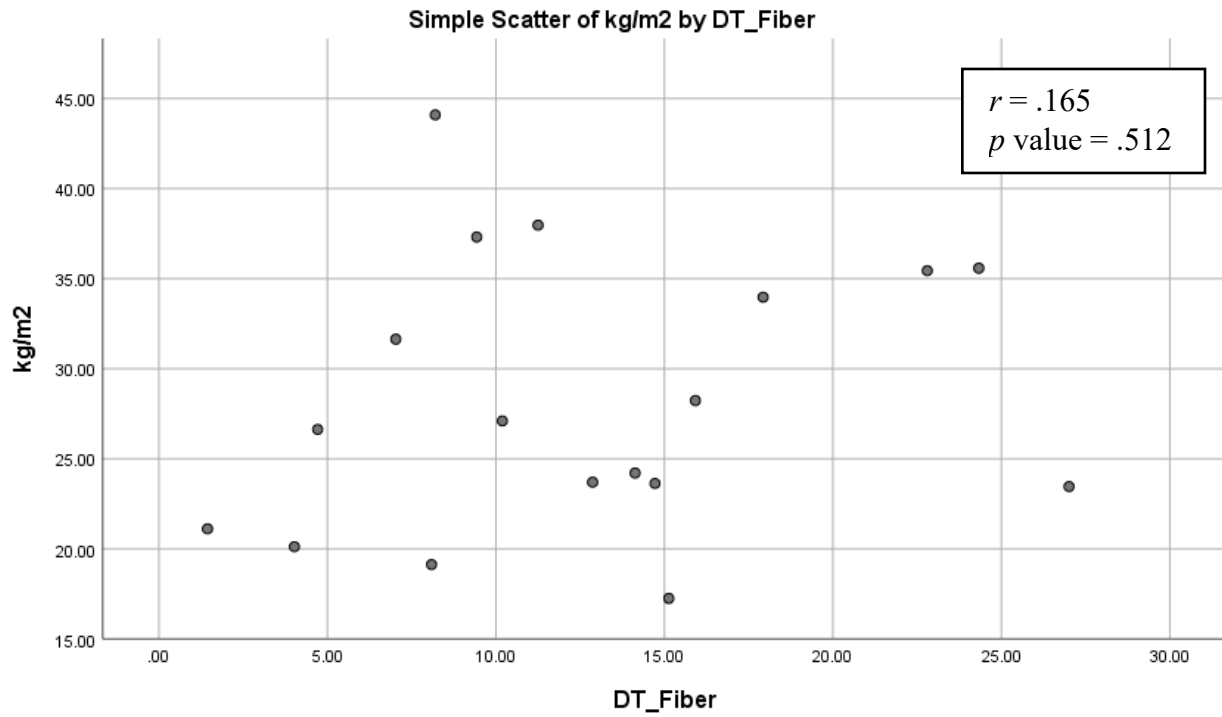


Figure 2 Soluble Fiber Intake vs. BMI

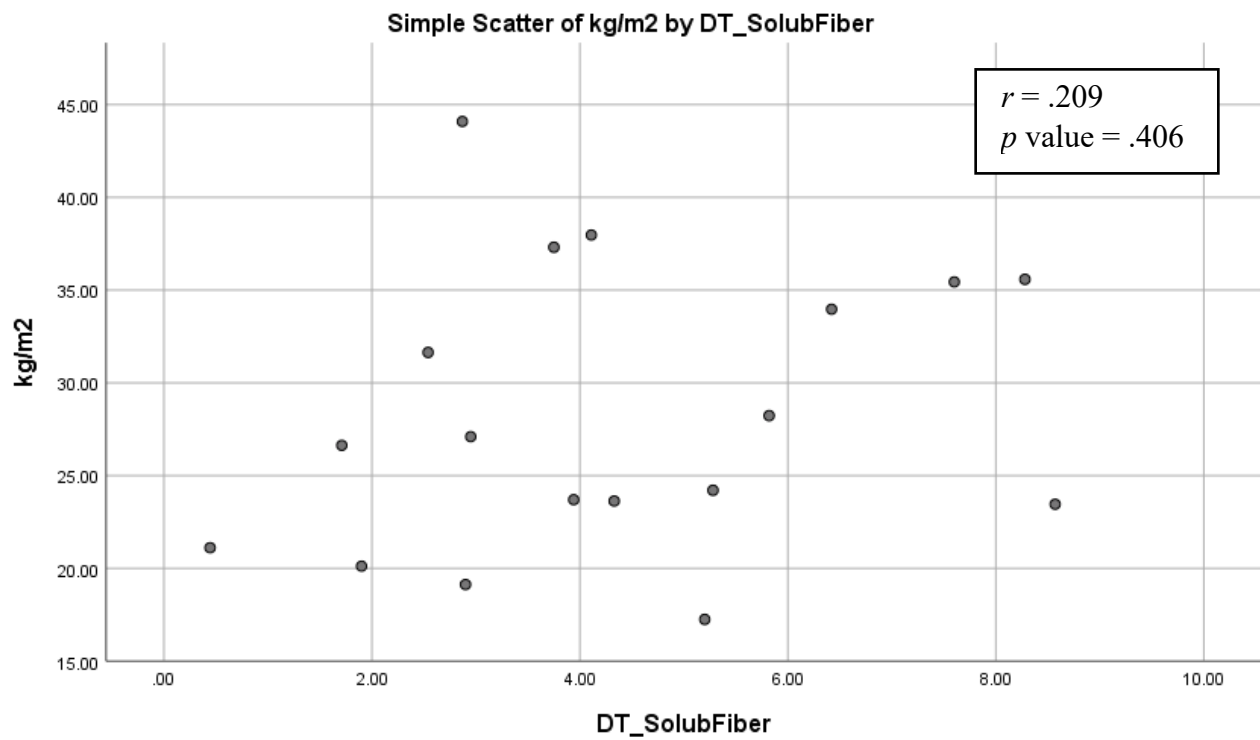


Figure 3 Total Dietary Fiber vs. mir\_886

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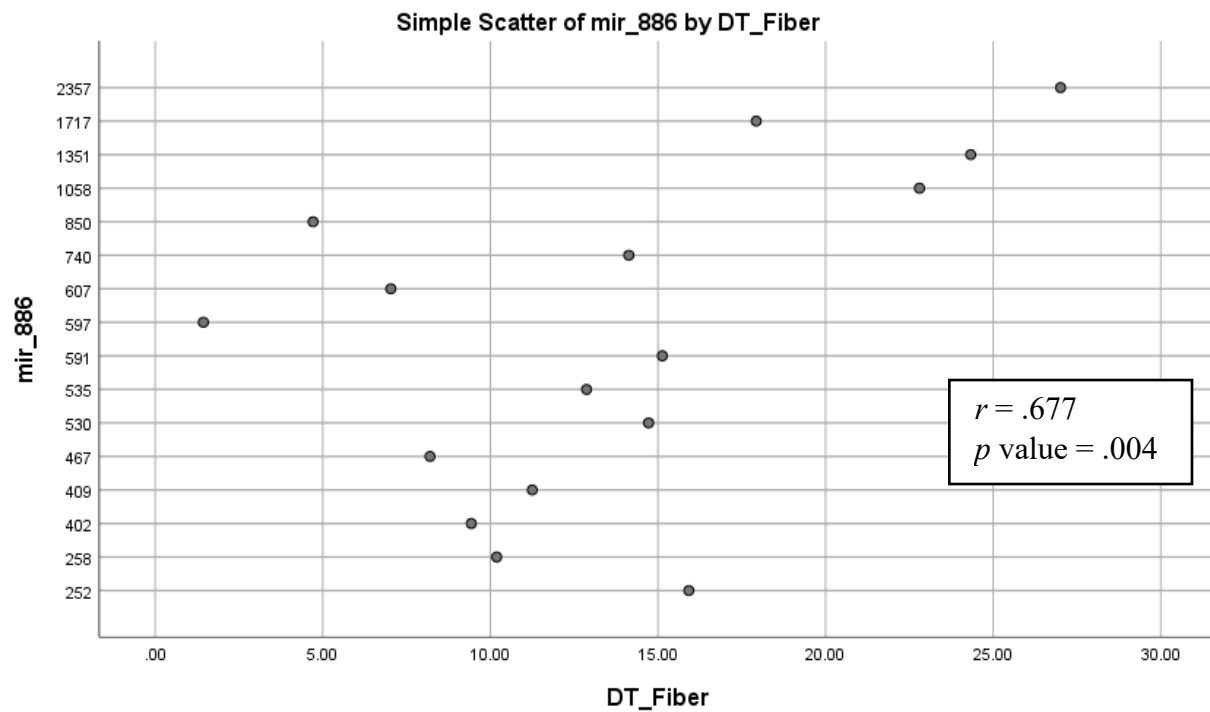


Figure 4 Total Dietary Fiber vs. mir\_941\_2

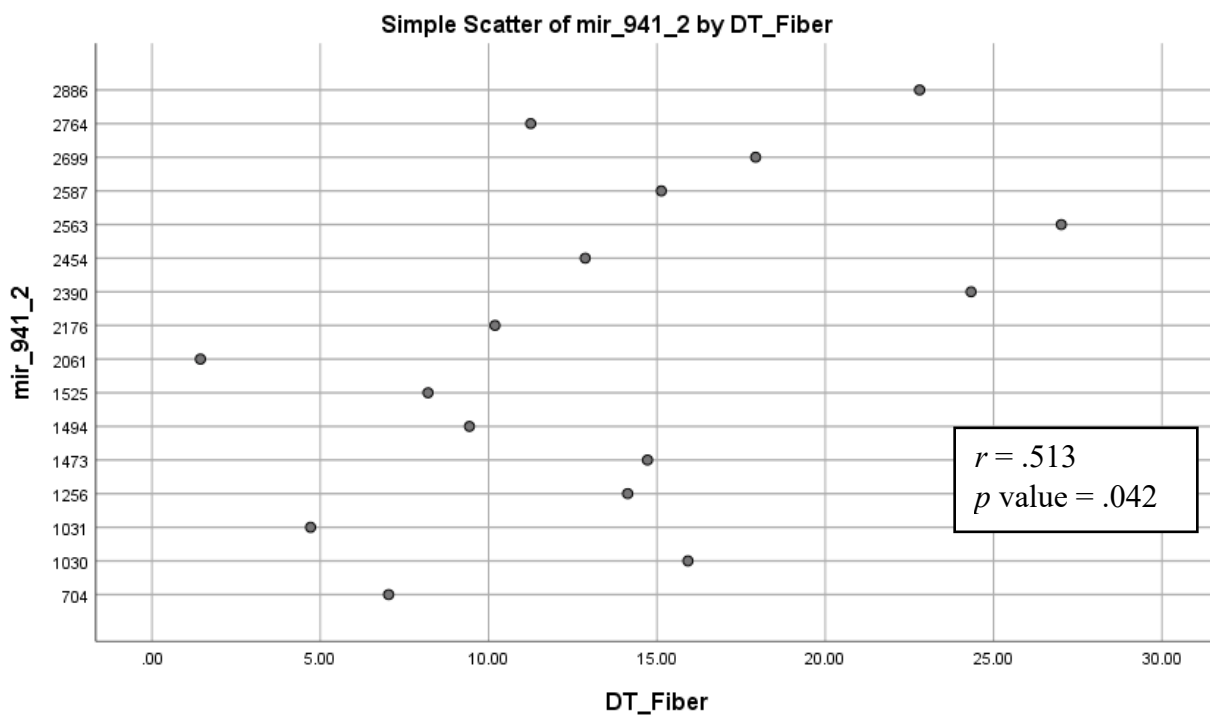


Figure 5 Total Dietary Fiber vs. mir\_148b

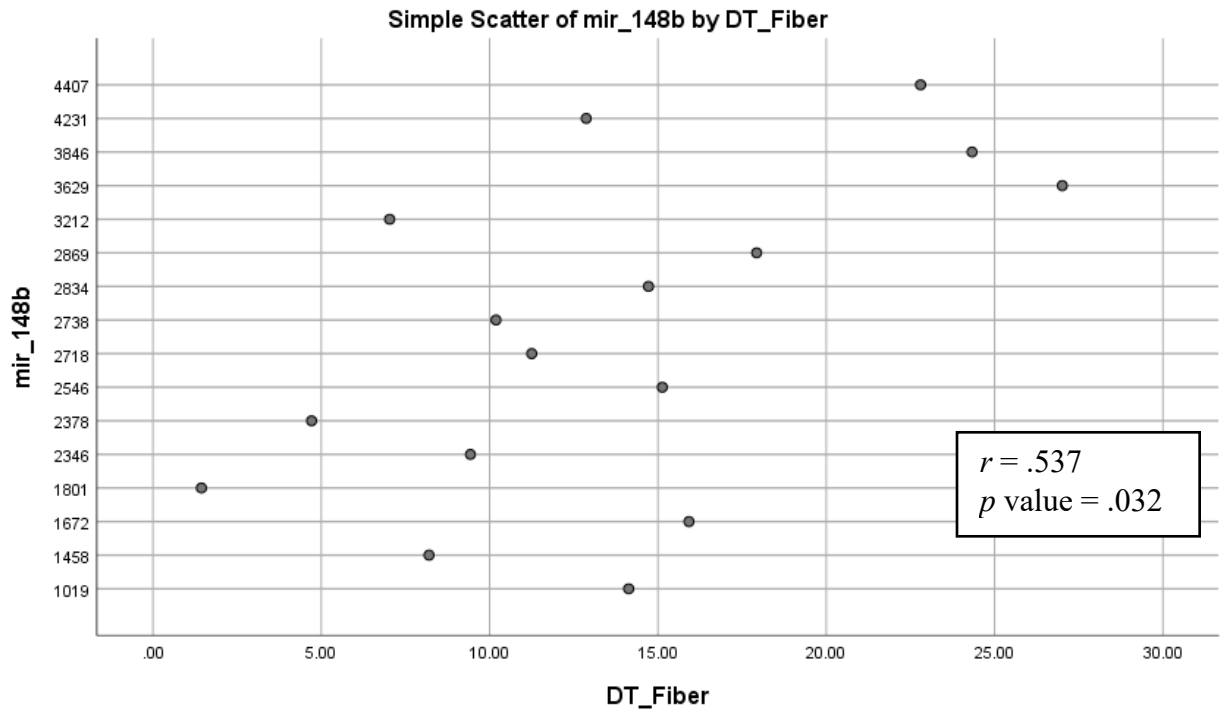
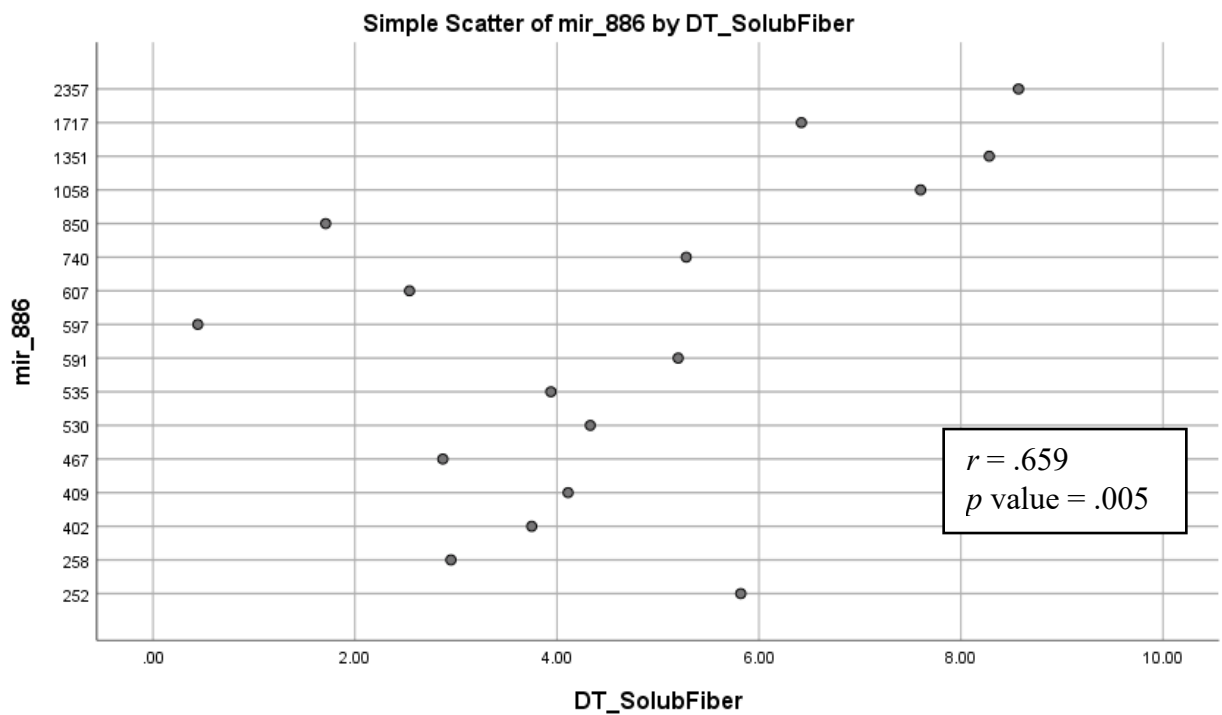


Figure 6 Soluble Fiber vs. mir\_886



## CHAPTER II: LITERATURE REVIEW

### **Kidney Disease**

Over 660,000 Americans are affected annually by kidney failure, and of these over 460,000 individuals are on dialysis.<sup>1</sup> Each year kidney disease kills more people than breast or prostate cancer.<sup>1</sup> General kidney disease refers to any conditions that involve the kidney or any problems related to its function. The main job of the kidney is to filter extra water and wastes from the blood and produce urine, but the kidney also plays a role in controlling blood pressure, making red blood cells, and keeping bones strong by hormone regulation. There are many symptoms related to kidney disease and related comorbidities, including fatigue, pain, anorexia, irritability, priuritis, oliguria, malnutrition, type 2 diabetes and high blood pressure.<sup>36,37</sup> Chronic Kidney Disease (CKD) is classified as a gradual loss of function in the kidney over a long period of time. This damages the kidney's ability to effectively filter blood and causes wastes to build up in the body.<sup>1</sup> A progression of CKD can lead to an advanced state of loss of function in the kidney and cause need for kidney replacement therapy.

The two types of kidney replacement therapy include peritoneal dialysis and hemodialysis, which may extend life expectancy by 5-10 years.<sup>38</sup> These are both blood treatments that help to control blood pressure by removing excess waste, water, and chemicals such as potassium, sodium, and bicarbonate. Peritoneal dialysis uses the lining of the abdomen to filter the blood by slowly filling the peritoneal cavity with dialysate, a solution that attracts waste from the body, and then emptying the dialysate through a catheter daily. Hemodialysis requires a machine that acts as an artificial kidney to remove blood from the body, filter the blood, and then return it to the body. Peritoneal dialysis can be conducted at home or at work, whereas hemodialysis requires going to a dialysis center three times per week for four-five hours at a time.<sup>38,39</sup>

## **Kidney Diet Restrictions**

When the kidneys do not function properly it can lead to an imbalance in minerals such as potassium, phosphorus, and sodium because the body is not filtering and eliminating them. For this reason, there are special diet recommendations for those in kidney failure. As hemodialysis does not occur daily, it requires a stricter diet to help minimize the buildup of waste between treatments. Potassium is needed for muscle contraction, but too much potassium can cause muscle cramps, weakness, irregular heartbeat, or a heart attack.<sup>26</sup> All fruits and vegetables have some potassium, but certain fruits and vegetables have more than others and should be limited or totally avoided. Phosphorus works with calcium and vitamin D to maintain bone density. An excess amount of phosphorus in the body may lead to hyperphosphatemia. The excess amount of phosphorus will bind with free calcium, stimulate parathyroid hormone (PTH) secretion, and decrease production of calcitriol.<sup>25</sup> This decreases intestinal calcium absorption and results in hypocalcemia, a bone disease called kidney osteodystrophy and hyperparathyroidism.<sup>40</sup> Limiting the amount of phosphorus in the diet aids in the prevention of these problems. To limit phosphorus intake, patients are counseled to avoid milk, cheese, yogurt, whole grains, and high fiber foods.<sup>41</sup>

Sodium helps the body maintain the correct amount of fluid in the body. When sodium concentration rises, the body retains fluid causing an increase in blood pressure. Using less salt and avoiding salty foods is recommended to help control blood pressure and reduce fluid weight gains. Finally, individuals in kidney failure cannot create urine and eliminate excess fluid. Having too much fluid in the body can cause high blood pressure, swelling, trouble breathing, and heart failure. Those on dialysis need a high protein diet (1.2 g protein/kg actual body weight per day) to keep blood protein levels consistent, support lean body mass, heal wounds faster, and strengthen the immune system.<sup>1,38,41</sup>

## Effects on Kidney Disease

Kidney failure causes dysbiosis in the body.<sup>42,43</sup> The gut microbiota is a collection of all microorganisms residing in the gut, while the gut microbiome is the entire collection of the genes encoded by these microbes. The gut microbiota has many functions which include stimulating the immune system, maintaining intestinal epithelial homeostasis, synthesizing Vitamins B1, B2, B6, B9, B12 and K, enhancing the gastrointestinal tract motility and function, inhibiting pathogens by creating colonization resistance, promoting angiogenesis and fat storage, modulating human bone mass density, metabolizing fiber, and producing short-chain fatty acids (SCFA).<sup>44</sup> The development of noncommunicable diseases such as obesity, cancer, diabetes, cardiovascular disease, asthma, and kidney disease are increasingly being linked to alterations in the gut microbiota.<sup>45,46</sup>

In previous studies, kidney disease has been associated with decreased dietary fiber intake, frequent use of antibiotics, constipation, slow colonic transit, and intestinal wall edema. All these affiliations can be linked to changes in the gut microbiome by the following processes: impacting intestinal junctions, causing an increased intestinal permeability, and making it so that bacteria may cross the intestinal barrier.<sup>14,43</sup> Nitrogenous waste products raise the body's pH through ammonia and lead to a decreased immune system. This means that the bacteria and metabolic products have been translocated out of the gut and into the body, which can induce inflammation as well as leukocyte stimulation. The immune response is triggered by a foreign substance entering the human body that does not fit and under normal circumstances the body would be able to identify and break down any translocated bacteria, but in kidney failure the body is in a disease state and is not functioning properly. Bacteria translocation occurring over a prolonged period can alter the gut microbiome and epigenetics.



Epigenetics is the study of how gene expression is affected by the environment and human behaviors. Gene expressions are altered by affecting how genes are read and not by altering genetic codes. A common type of epigenetic change is DNA methylation, a process of adding/removing a chemical group to DNA to determine if the gene will be turned “on” or “off”.<sup>47</sup> Epigenetics change throughout the lifespan as part of normal development and aging in response to the environment. Health can be affected by epigenetics through infections and dietary choices. There are many minerals, herbs, and phytochemicals that are known to demonstrate powerful epigenetic modification in cells such as resveratrol, quercetin, and curcumin.<sup>48,49</sup> Because these changes to gene expression are affected by the environment, they are oftentimes reversible.

Emerging evidence shows the increasing regulatory control that kidney disease holds over gut microbiome alterations. The gut microbiome of individuals with CKD were altered when compared to healthy controls, with approximately 190 significantly different microbial operational taxonomic units.<sup>9,50</sup> Loss of kidney function results in retention of substances that would normally be excreted or metabolized by the kidney. As the kidney declines, there is an increase in blood concentrations resulting in retention of molecules that cause uremia. For the majority the uremic retention molecules come endogenously from mammal consumption, but microbial metabolism further contributes to the development of uremic retention molecules. These microbial alterations can cause increased uremic toxin levels that contribute to CKD progression, creating a feedback loop that further exacerbates CKD progression overtime.<sup>45</sup>

Hemodialysis filters blood from the body to decrease uremic toxins and related syndrome, a condition that involves high levels of waste products in the blood due to kidney failure and is a symptom common to individuals with CKD. Hemodialysis has aided over a million people in the world with kidney failure in survival and increases kidney clearance capacity by a supplemental

10-15%.<sup>51,52</sup> It is common for patients with kidney failure to experience a decline in skeletal muscle mass from inflammation, malnutrition, and nutrient loss during each hemodialysis session. In a study by Hendricks et al., it was found that a patient loses on average 12g of amino acids in a single hemodialysis session.<sup>53</sup> This could result in protein malnutrition and increase the patients' risk of comorbidities.

While hemodialysis removes waste products such as creatinine and urea, one study has found a significant increase in inflammatory biomarkers and uremic retention toxins in individuals participating in hemodialysis compared to non-dialysis patients.<sup>36,54</sup> This is relevant to the microbiome because the inflammatory biomarkers indicate that the ecosystem is being disrupted. Uremic retention toxins are associated with an increased mortality and other adverse effects consistent with CKD.

### **Fiber in Relation to Gut Microbiota**

Dietary fiber is found in plant-based foods such as fruits, vegetables, legumes, beans, nuts, and whole grains. Fiber is also known as roughage or bulk as it cannot be digested by human enzymes or absorbed but rather passes through the proximal portion of the gastrointestinal tract and enters the large intestine. There are two types of fiber: soluble and insoluble. Soluble fiber dissolves in water and has been found to decrease blood cholesterol and serum glucose levels. Insoluble fiber does not dissolve, instead acts as a stool thickener and promotes bowel movements. The benefits of a high fiber diet include normal bowel movements, maintaining bowel health, lower cholesterol levels, controlled blood glucose, and achieving healthy weight.<sup>5</sup> Dietary fiber ferments in the hindgut with the help of the bacteria in the gut microbiome to produce SCFAs. SCFAs are fatty acids that have one to six carbon atoms and are produced from anaerobic bacteria in the microbiome through fermentation. Some studies found that the level of SCFAs present can

be an indicator of the health of the microbiome.<sup>12,55</sup> A study by Wang et al., found that SCFAs were reduced in CKD patients and even suggested that butyrate supplementation might delay CKD progression.<sup>12</sup>

Dietary fiber is digested and fermented in the colon by gut microbes into SCFAs such as *n*-butyrate, acetate, and propionate which are then sensed by G protein-coupled receptors GPR41 and GPR43.<sup>55</sup> In rats, *n*-butyrate has been shown to stimulate leptin production in adipocytes, signaling the brain to decrease energy intake. SCFA also induce glucagon-like peptide-1 (GLP-1) secretion by the intestinal enteroendocrine L cells, increasing insulin secretion.<sup>56</sup> *n*-Butyrate has been found to increase expression of tight junction proteins in colon epithelia and exhibits anti-inflammatory effects through the reduction of cytokine and chemokine release from human immune cells. Acetate and propionate are used as substrates for oxidation, lipid synthesis, and energy metabolism through gluconeogenesis.<sup>55</sup>

In a study by Chen et al. piglets were fed different supplements of dietary fiber, insoluble and soluble for 28 days and then the difference in microbial composition, content of SCFAs, and the barrier function of the colon were analyzed. It was found that the group receiving soluble fiber had an increased amount of SCFAs, microbial diversity, and community richness compared to the insoluble fiber group. These characteristics aid in the overall maintenance of the intestinal barrier function.<sup>42</sup>

Prebiotics and probiotics are dietary interventions to promote gut health. Probiotics are beneficial bacteria that when eaten are introduced into the gut microbiome. Prebiotics are the food for the bacteria in the gut and the most important prebiotic is fiber which can be found in fruits, vegetables, and whole grains.<sup>46</sup> Recent studies have proposed that prebiotic supplements such as dietary fiber may inhibit the progression of CKD.<sup>58</sup> Serum creatinine and urea have been used as

markers of health in CKD, and some studies have resulted in reduced serum creatinine and urea levels when dietary fiber is increased.<sup>36,58</sup> Another study by Wang et al. noted that dietary fiber is not digested in the body, but instead is broken down into SCFAs, which then feed and positively alter the composition of the gut microbiota.<sup>12</sup> This positive alteration to the composition of the gut microbiota causes beneficial effects on cell turnover, metabolism, and eating behavior.<sup>6</sup> SCFAs have been shown to stimulate gut motility and intestinal transit.<sup>36</sup>

In another recent prospective cohort study with 219 prevalent hemodialysis patients, dietary fiber intake was associated with less inflammation, less myocardial hypertrophy, injury, and a reduced risk of major adverse cardiovascular events.<sup>4</sup> It is estimated that hemodialysis patients consume 11-12 g/day of dietary fiber.<sup>4</sup> This is very low compared to the suggested intake for the general population which is about 25 g/day for women and 38 g/day for men, or 14 grams per every 1000 kcal consumed in a day.<sup>5</sup> Currently, there is no recommendation for fiber intake recommendation for CKD or dialysis patients.

### **Modifiable Factors Influencing Gut Microbiome**

A microbiome is made of a collection of microorganisms that coexist in a specific environment. The gut microbiome is made up of a mixture of human and microbial cells located in the intestines, making this ecosystem gene-rich. At birth, this environment is sterile with colonization beginning from the mother's vaginal microbiota along with environmental interactions in the first days of life. This ecosystem is made up of a large population of many species and contains a high growth rate, meaning that there could be evolution occurring in the gut during a single lifespan.<sup>58</sup> The composition of the core gut microbiota is considered to be essentially stable throughout adulthood, harboring over 100 trillion microbial cells and over 1000 different bacterial species.<sup>45</sup> There is an ongoing cycle of communication between the gut microbiota and the host immune system from

birth where they each help to shape and develop the other. A wide variety of signaling pathways and chemical interactions are used to transmit communications between the microbes and the immune system to act upon multiple organ such as the gut, liver, muscle, and brain. These host-microbe interactions cause a chain of communications that can modulate metabolic reactions, resulting in various focuses in producing combinatorial substrates such as bile acids, choline, and SCFAs. These metabolites contribute to host metabolic phenotype and resulting disease risk.<sup>55</sup>

The gut microbiota produces small molecules during the metabolism of food that play critical roles in shuttling information between host cells and the host's microbial symbionts. This diverse array of genes means that there is a variety of genes available to be expressed and opportunities of modification in their expression. As of right now, there is no universal diagnostic gene panel because of the wide variability and ethnic specificity of the identified genes. Some studies suggest that the GI microbiota may be used as a therapeutic target in the restoration of the homeostasis of the gut and repopulate the ecosystem to a healthier state.<sup>13,59,60</sup>

There are biologically and metabolically flexible components of the gut microbiome that respond to environmental changes or changes in diet. This means behavior modifications including dietary choices can alter the environment of the microbiome and make epigenetic changes. The right environment can aid in the reduction of complications associated with CKD by altering gene expression. This is beneficial to prevent disease progression and aid in increasing lifespan.<sup>12,36</sup>

In recent years, fecal microbiota transplants have attracted much attention. A fecal microbiota transplant is a transplantation of fecal microbiota from a healthy donor into a diseased individual. The sample taken from the donor is mixed in saline and transplanted via colonoscopy, endoscopy, sigmoidoscopy, enema, or pill form.<sup>61</sup> This form of targeting the gut microbiota is considered as an option to improve human health. Evidence is showing that fecal microbiota transplantations can

help those with reoccurring *C. difficile* and with many gastrointestinal diseases, but it is currently undergoing many studies in the various ways it can be used.<sup>46</sup> These transplantations are thought to be able to aid in chronic conditions such as obesity, hypertension, chronic liver disease, cancer therapy, metabolic syndrome, diabetes, Parkinson's disease, and microbial dysbiosis- many of these are comorbidities for those with CKD.<sup>59</sup>

The microbiota can be altered from various lifestyle and environmental factors including diet, toxins, drugs, and pathogens.<sup>45</sup> The current consensus is that the most effective way to modify the gut microbiome is through lifestyle factors, with the most prominent lifestyle factors being diet and physical activity.<sup>1</sup> Physical activity triggers the autonomic nervous system to signal peristalsis and movement in the gut, which promotes movement of food for the bacteria and the production of SCFAs.

Epigenetic markers are new tools that are aiding in the personalization of treatment.<sup>3,62,63</sup> It is the hope that through the use of epigenetics, the GI microbiota may be altered to produce a healthier gut microbiome. Before this can become a therapeutic option, changes need to be identified and the pathways in CKD understood to create a personalized epigenetic treatment. Current studies are investigating the presence of epigenetic biomarkers on weight loss predictions and sickness level.<sup>62</sup>

### **Epigenetic Role of GI Microbiota**

Micro RNAs (miRNAs) are short non-coding RNA that regulate physiological and pathological processes by post-transcriptional gene expression, meaning they control protein production by binding to and disabling longer messenger RNA (mRNA) templates.<sup>64</sup> This leads to a decrease in expression from the mRNA and ultimately decrease protein expression.

There has been a large amount of evidence supporting the role of GI microbiota on metabolism, but the role of epigenetics and the mechanism behind it have yet to be fully understood. There is evidence suggesting that miRNAs may be helpful in understanding disease pathology by being used as a stable biomarker in mapping out the molecular mechanisms that further the disease.<sup>64</sup> Evidence suggests that miRNAs are involved in many processes including cell proliferation, differentiation, and cancer.<sup>65</sup> miRNAs are seen at stable levels in a healthy individual, but these levels change in states of stress. The most effective way to monitor these levels is through the blood, making this a reasonable method to monitor kidney function and disease.

Some miRNAs that might be beneficial to monitoring kidney function are mir-142, mir-146, mir-15b, mir-192, and mir-29c. As mentioned earlier, inflammation is triggered by microorganism translocation. Mir-142 and mir-146 are expressed in these immune cells, meaning that they will likely be altered by an increase in inflammation.<sup>66,67</sup> A study by Kramer et al. found that any permanent change in the expression of mir-142 may lead to primary immunodeficiency.<sup>66</sup> Mir-15b targets insulin receptors, leading to inactivation of these receptors and causing decreased insulin sensitivity.<sup>68</sup> An upregulation of this miRNA may possibly lead to production of more comorbidities related to kidney disease such as diabetes. Mir-192 has shown a relationship to kidney disease, but the relationship has not been fully elucidated yet. A study by Meng, Chung, & Lan found an upregulation of mir-192 was associated with increased kidney fibrosis.<sup>69</sup> Another miRNA that has been found to correlate with degree of kidney function is mir-29c, a study by Lin-Li et al. saw a downregulation of miRNA with worsened degree of histological fibrosis and kidney function.<sup>70</sup>

There is a plethora of information to be learned about how the human body works and it is not fully mapped out yet. There are some miRNA that do not have any genes associated with them in the available literature such as mir-941-2, and others have been mapped out at least partially such as mir-886 and mir-148-b. Each miRNA plays a different role and can regulate a wide variety of genes. There are four genes associated with mir-886 in literature: MCL1, CXCL12, CDC6, and PLK1. These genes are associated with auto-immune diseases such as human immunodeficiency virus type 1, Meier-Gorlin syndrome 5, and monoclonal paraproteinemia.<sup>30</sup> They are not associated with any diseases related to the gut or metabolism. There are 18 genes associated with mir-148-b in literature: HLA-G, CCKBR, ITGA5, ROCK1, PIK3CA, NRAS, CSF1, ALCAM, PRKAB1, ACVR1, MAP3K9, HOTAIR, MLH1, PIK3R3, WNT1, SLC2A1, CYBB, and MCL1. Many of these genes code for proteins that help to maintain function of the GI tract. Fiber aids in GI health and motility, indicating an increase in fiber would stimulate mir-148-b and stimulate these genes to increase protein production for metabolism in the gut. Mir-148-b is known to suppress HLA-G expression, a promising gene for BK polyomavirus replication (BKPyV) which is associated with nephropathy and premature kidney transplant failure.<sup>31,32</sup> Drugs are used to suppress BKPyV to decrease the chances of kidney rejection. The increase of fiber in the diet increases mir-148-b expression, decreasing HLA-G expression, and decreasing nephropathy.

CCKBR codes for hydrochloric acid secretion and acts as a tumor suppressor inhibiting kidney carcinoma cell growth.<sup>33</sup> NRAS helps to produce GTPases that play important roles in cell division, cell differentiation, and apoptosis. This helps to maintain health at a cellular level and prevent cancerous cells from forming. HOTAIR is another gene that aids in prevention of cancer, specifically of the gastrointestinal system. Mir-148-b inhibits glycolysis through inhibiting SLC2A1, a vital gene to carbohydrate metabolism as it provides instruction for production of



GLUT1 (glucose transporter protein type 1) that limits glucose uptake.<sup>34</sup> Decreased production of SLC2A1 can decrease production of GLUT1 and decrease glucose absorption into the brain and cause neurological damage. There is still more to learn about each of these miRNAs and the genes that they are associated with as there is weak evidence for an additional 381 genes associated with mir-148-b.<sup>15</sup>

In light of the research stated above, there is a lack of knowledge in a recommended amount of fiber intake for hemodialysis patients to fully support their gut microbiome, this can contribute to a dysbiosis in the gut. This dysbiosis leads to a change in the gene expression of miRNAs that is yet to be fully elucidate on and may influence the rate of progression of CKD.

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