ORIGINAL RESEARCH

Effects of Probiotic Supplementation on Nutrient Intake, Ghrelin, and Adiponectin Concentrations in Diabetic Hemodialysis Patients

Yan Zhang, PhD; Xiangdi Meng, MS; Zhaosen Ma, MS; Zhou Sun, MS; Zhixin Wang, PhD

ABSTRACT

Objective • The paper aimed to explore the effect of probiotic supplementation on nutrient intake, Ghrelin, and adiponectin concentrations in diabetic hemodialysis patients.

Methods • A total of 86 patients with diabetic nephropathy who received hemodialysis treatment in the Department of Nephrology of the First People's Hospital of Shanghai from May 2019 to March 2021 were selected as the research subjects, including 52 male patients and 34 female patients, with an average age of 56.57 ± 4.28. According to the research protocol, the patients were divided into the control group (n = 30) and the observation group (n = 56). In the control group, dietary soybean milk was used as a placebo. In the observation group, capsules containing probiotics *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium* were taken with soybean milk. All patients signed an informed consent form before being included in the study. The results of the experimental biochemical analysis and the archived data counted the general data of the patients. Plasma adiponectin concentrations were measured with a commercially available human enzyme immunoassay kit. Ghrelin concentrations were estimated by specific commercial methods. Correlation software was used to calculate patient nutritional intake data. Serum creatinine, insulin resistance, fasting blood glucose, and levels of oxidative stress and inflammatory factors were measured using appropriate biochemical assays.

Results • There was no difference in baseline characteristics between the two groups ($P > .05$). Before treatment, there was no difference in serum adiponectin concentration between the two groups ($P > .05$). After treatment, the serum adiponectin concentration in the observation group was lower than in the control group ($P < .05$). Before treatment, there was no difference in serum ghrelin levels between the two groups ($P > .05$). After treatment, serum ghrelin levels in the observation group were higher than in the control group ($P < .05$). Before treatment, there was no difference in nutrient intake between the two groups ($P > .05$). After treatment, the nutrient intake in the observation group was higher than in the control group ($P < .05$). Serum creatinine, fasting blood glucose, urine protein/creatinine ratio, and HOMA-IR in the observation group were lower than in the control group ($P < .05$). The serum levels of malondialdehyde, C-reactive protein, and TNF-α in the observation group were lower than those in the control group ($P < .05$), and the levels of glutathione in the observation group were higher than those in the control group ($P < .05$).

Conclusion • Supplementation of probiotics in DN dialysis patients can increase serum ghrelin concentration, increase nutrient intake through appetite regulation, and reduce adiponectin level, which is beneficial to blood sugar control, insulin resistance, and renal function. (*Altern Ther Health Med.* 2023;29(4):36-42).

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INTRODUCTION

Diabetic nephropathy (DN) is one of the serious diabetic microvascular complications of diabetes, which is considered the leading cause of end-stage renal disease. It is reported that DN patients worldwide may lead to end-stage renal failure and disability, affecting about 40% of diabetic patients. Intestinal microbiome is associated with obesity, metabolic syndrome, and type 2 diabetes by reducing glucose tolerance and insulin resistance.[1] Probiotics are living microorganisms, and when administered in sufficient amounts, they will bring

health benefits to the host. Supplementing probiotics can improve blood sugar control and anthropometric indicators of adults with type 2 diabetes.[2,3] In addition, probiotic supplements significantly reduced HDL but did not significantly affect cholesterol and triglyceride. Studies have shown that probiotic supplements have beneficial effects on systemic inflammation, oxidative stress, and renal biomarkers in DN subjects.[4,5] The factors related to two metabolic disorders, including diabetes and chronic kidney disease, are adiponectin and Ghrelin.[6,7]

Adiponectin is a cytokine produced by adipose tissue. It is rapidly cleared from the circulation, mainly through the liver, partly through the biliary tract, and secondly through the kidney.[8] Adiponectin activated by AMP-activated protein kinase has been shown to have anti-inflammatory, anti-fibrosis, and anti-oxidation effects on the kidney.[9] Renal tubular cells can express and secrete adiponectin, which may contribute to some extent to the increase of urinary adiponectin. Serum adiponectin levels in patients with type 2 diabetes, obesity, metabolic syndrome, and atherosclerosis are significantly reduced.[10] Although the prevalence of metabolic disorders (insulin resistance) is high, the serum adiponectin level of patients with nephropathy is higher than that of healthy people.[11,12]

Ghrelin is a peptide hormone mainly released by X/A-like cells in gastric mucosa, which plays an important role in regulating hunger. Elevated ghrelin levels can stimulate appetite and lead to obesity and kidney damage. Ghrelin is important in the pathogenesis of protein balance, inflammation, and cardiovascular complications of chronic kidney disease. It has been found that the fasting ghrelin level in patients with stage 2 CKD is increased. On the other hand, recent studies show that the level of total Ghrelin is increased in CKD patients. The purpose of this study was to evaluate the effect of probiotics on nutrient intake, Ghrelin, and adiponectin concentration in diabetic hemodialysis patients.[13,14]

METHOD

Study Design

From May 2019 to March 2021, 86 patients with diabetic nephropathy who received hemodialysis in the Department of Nephrology, First People's Hospital of Beijing were selected as the research objects, including 52 male and 34 female patients, with an average age of 56.57 ± 4.28. According to the research plan, patients were divided into a control group (n = 30) and an observation group (n = 56). The control group took soybean milk as a placebo, while the observation group took capsules containing probiotics *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Bifidobacterium* with soybean milk. All patients signed the informed consent form before being included in the study.

Inclusion Criteria. Inclusion criteria include age 18-80, male or female; diagnosed as diabetic nephropathy according to World Health Organization Type 2 Diabetes Standard 21; Glycosylated hemoglobin (HbA_{1c}) level is between 7% and 10%; Microalbuminuria/creatinine (mAlb/Cr) level ≥30 mg/g/24 h.[15]

Exclusion Criteria. Exclusion criteria include patients with type 1 diabetes; Patients with hypoglycemia coma, diabetic ketoacidosis, hyperosmolar non-ketotic coma, or acute complications of diabetes; Patients with hypertension, drug abuse, alcohol dependence or drug allergy; Patients: Patients who took probiotics or synbiotic supplements and antioxidants and/or anti-inflammatory supplements (vitamin E, vitamin C, and omega-3 fatty acids) within the first three months of this study, and patients who took antibiotics and immunosuppressive drugs within the first three months of the study.

Ethical Considerations

This study was conducted according to the Declaration of Helsinki and approved by the Ethics Committee of the First People's Hospital of the city. All participants provided informed consent forms.

Analysis and Data Collection

Observation Result Test. The main outcome variable in this study was HOMA-IR, which is a marker of insulin resistance in patients with diabetic nephropathy. Secondary variables included biomarkers of metabolic characteristics, inflammation, and oxidative stress. To analyze these variables, 10 mL of venous blood was collected from participants at baseline and the end of the 12th week after the intervention. The blood samples were centrifuged, and the serum was stored at -80°C until analysis. Serum creatinine concentration was measured using a Biosystem Analyzer (Spain), while urinary albumin and creatinine were measured using a Hitachi 902 automatic analyzer (Bollinger Mannheim, Germany). Fasting plasma glucose was measured using commercial kits. The urine protein/creatinine ratio was measured using an American Ariel Alere Afinion® AS100 analyzer, while serum TNF-α concentration was measured using a Boster (USA) assay. High-sensitivity C-reactive protein was measured using turbidimetry (Iatron, Tokyo, Japan), and plasma adiponectin concentration was measured using a human enzyme immunoassay kit (R&D Systems, Minneapolis, Minnesota, USA). Ghrelin was estimated using a specific commercial radioimmunoassay kit (Phoenix Pharmaceutical Company, Belmont, California, USA). The tests were conducted in a blind and paired manner (before and after intervention), in the same analysis run, and random order. This approach was used to reduce errors and variability between measurements. Additionally, blood glucose concentration was measured using an automatic glucose analyzer.

Total Antioxidant Capacity of Plasma. The concentration of total antioxidant capacity (TAC) was measured using the method developed by Benzie and Strain to reduce antioxidant capacity (Benzie and Strain). Total glutathione (GSH) and malondialdehyde (MDA) concentrations were also detected using the Beutler method and the thiobarbituric acid reaction substance spectrophotometry, respectively.

Analysis of Nutritional Intake. Energy and nutrition intake was assessed by a three-day food recording method.[16] Participants were asked to write down the food and drinks they consumed for three days (including a weekend). During the dietitian's visit, each meal's size was accurately assessed.[14] To evaluate the nutrition of patients' diets, Dieta 5.0 software (introduced by the National Institute of Food and Nutrition in Warsaw, Poland) was used.[17] The calculated nutritional data included: energy (kcal), total protein (g) including animal and plant protein (g), carbohydrate (g) including sucrose, glucose, and fructose (g), fiber (g), cholesterol (g), iron (mg) and zinc (mg) intake.

Statistical Analysis

All data were expressed as average SD. The differences in categorical and continuous variables were determined using Chi-squared (χ^2) test and a student *t* test, respectively. The changes in peritoneal transport characteristics, nutritional status, adequacy, and inflammatory status over time were evaluated using repeated analysis of variance (ANOVA). Clinical parameters were calculated by time and group, and a group-by-group effect was analyzed. A pairing test was used to evaluate the difference from baseline in each group, and a *t* test was used to evaluate the differences between groups at the same time point. The trend test evaluated the frequency change of icodextrin use with time in DM patients. The significance of all tests was determined as $P < .05$. SPSS version 11.0 for Windows software was used for statistical analysis.

RESULTS

Analysis Of Baseline Characteristics of Patients

According to experimental biochemical analysis and archival data results, the general data of patients in the control group were counted. The average age of the patients was 55.78 ± 4.26 years, the ratio of male to female was 19:11, the average BMI was 24.15 ± 1.49, the average course of DM was 8.45 ± 2.49 years, the fasting blood glucose was 10.24 ± 2.56 mmol/L, and the glycated hemoglobin content was 8.25 ± 1.34. The microalbuminuria/creatinine ratio was 92.57 ± 17.43 mg/g, the insulin resistance index was 2.63±0.38, and the glomerular filtration rate was ml·min-1. The average age of the observation group was 56.15 ± 5.33 years, the male-female ratio was 33:23, the average BMI was 23.58 ±v2.06, and the average course of DM was 9.36 ± 1.85 years. Glycated hemoglobin content was 8.46 ± 1.74; microalbuminuria/creatinine was 103.54 ± 20.41 mg/g, insulin resistance index was 2.51 ± 0.42, and glomerular filtration rate was 83.55 ± 5.37 ml·min-1. There was no difference in baseline characteristics between the two groups ($P > .05$) (Table 1).

Detection of Serum Adiponectin Level

There was no difference in serum adiponectin concentration between the two groups before treatment ($P > .05$). After treatment, the serum adiponectin concentration in the observation group was lower than in the control group $P < 0.05$) (Table 2).

Detection of Serum Ghrelin Levels

There was no difference in serum ghrelin levels between the two groups before treatment ($P > .05$). After treatment, the serum ghrelin level in the observation group was higher than in the control group ($P < .05$) (Table 3).

Analysis of Nutrient (Vitamin) Intake

Before treatment, there was no difference in the intake of vitamins B1, B2, and C between the two groups ($P > .05$). After treatment, the intake of vitamins B1, B2, and C in the observation group was higher than in the control group ($P < .05$) (Figure 1, Table 4).

Analysis of Nutrient (Element) Intake

Before treatment, the two groups had no calcium, iron, or zinc intake differences ($P > .05$). After treatment, the calcium, iron, and zinc intake in the observation group was higher than in the control group ($P < .05$) (Figure 2, Table 5).

Analysis Of Nutrient (Glycolipid) Intake

Before treatment, the two groups had no difference in carbohydrate, cholesterol, and dietary fiber intake ($P > .05$). After treatment, the intake of carbohydrates and cholesterol in the observation group decreased ($P < .05$), and dietary fiber intake in the observation group increased (Figure 3, Table 6).

Table 1. Analysis of Baseline Characteristics of Patients

Project	Control group (n=30)	Observation group (n = 56)	t/χ^2 value	*P* value
Age (years)	55.78 ± 4.26	56.15 ± 5.33	-0.328	.744
BMI (kg/m^2)	24.15 ± 5.49	23.58 ± 6.06	1.182	.241
Gender (male: female)	17:13	33:23	0.041	.839
Course of DM (years)	8.45 ± 2.49	9.36 ± 1.85	-1.921	.058
Fasting blood glucose (mg/ dL)	146.37 ± 16.56	151.28 ± 18.74	-1.204	.232
Glycosylated hemoglobin (%)	8.25 ± 1.34	8.46 ± 1.74	-0.575	.567
Urine protein/creatinine ratio (mg/g)	92.57 ± 17.43	100.54 ± 20.41	-1.813	.073
HOMA-IR	5.63 ± 0.38	5.51 ± 0.42	1.304	.196
eGFR(ml·min^{-1} × (1.73m^2) $^{-1}$)	72.47 ± 6.46	73.55 ± 5.37	-0.827	.411

Table 2. Detection of serum adiponectin level(s)

Groups	Before treatment (μg/ml)	After treatment (μg/ml)
Control group (n = 30)	6.36 ± 1.94	5.37 ± 2.45
Observation group (n = 56)	6.42 ± 2.02	3.52 ± 1.74
t value	-0.133	4.061
P value	.895	<.001

Table 3. Detection of Serum Ghrelin Level(s)

Groups	Before treatment (pg/ml)	After treatment (pg/ml)
Control group (n = 30)	368.51 ± 46.81	375.38 ± 488.24
Observation group (n = 56)	385.26 ± 55.39	538.41 ± 68.30
t value	-1.408	-2.466
P value	.163	.016

Figure 1. Analysis of Vitamin Intake of Patients

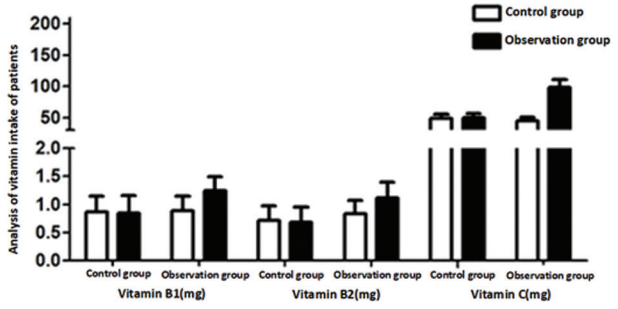


Figure 2. Analysis of Patients' Element Intake

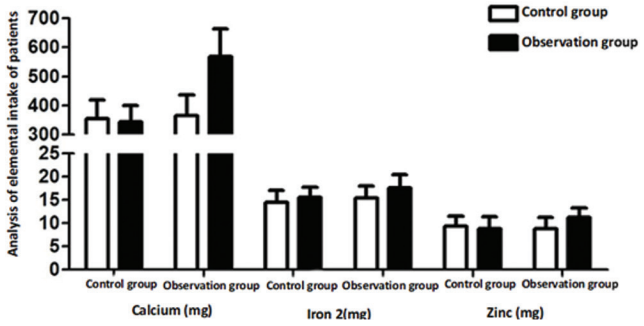


Table 4. Analysis of Vitamin Intake of The Patient (s)

Groups	Vitamin B1(mg)		Vitamin B2(mg)		Vitamin C(mg)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control group (n = 30)	0.87 ± 0.28	0.89 ± 0.26	0.71 ± 0.26	0.83 ± 0.24	48.57 ± 6.42	45.24 ± 5.18
Observation group (n = 56)	0.85 ± 0.31	1.24 ± 0.25	0.68 ± 0.27	1.12 ± 0.28	50.02 ± 6.33	98.57 ± 12.44
t value	0.295	-6.102	0.497	-4.803	-1.007	-22.414
P value	.769	<.001	.620	<.001	.317	<.001

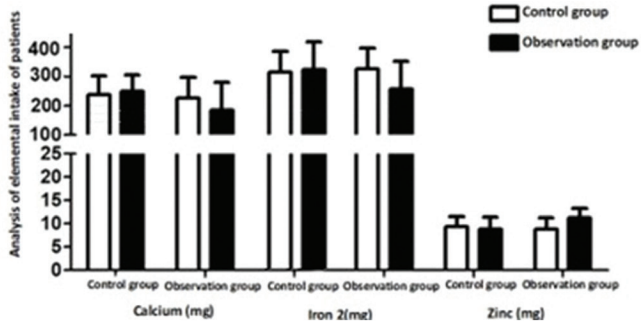
Table 5. Analysis of Elemental Intake of Patient (s)

Groups	Calcium (mg)		Iron 2(mg)		Zinc (mg)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control group (n = 30)	354.27 ± 64.49	365.59 ± 70.43	14.52 ± 2.48	15.37 ± 2.66	9.35 ± 2.14	8.75 ± 2.52
Observation group (n = 56)	342.58 ± 58.27	568.64 ± 94.26	15.63 ± 4.14	17.54 ± 2.87	8.86 ± 2.56	11.24 ± 1.97
t value	0.854	-10.342	-1.343	-3.426	0.894	-5.100
P value	.396	<.001	.183	.001	.374	<.001

Table 6. Analysis of Glucose and Lipid Intake Of Patient(s)

Groups	Carbohydrates (g)		Cholesterol (mg)		Dietary fiber (g)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Control group (n=30)	251.73 ± 37.24	246.29 ± 41.33	327.45 ± 37.46	335.29 ± 31.36	10.36 ± 1.58	9.47 ± 1.94
Observation group (n=56)	262.44 ± 40.51	203.56 ± 21.46	336.75 ± 31.20	266.43 ± 27.24	10.58 ± 1.76	15.36 ± 2.03
t value	-1.201	-11.414	-3.866	10.594	-0.572	-13.020
P value	.233	<.001	<.001	<.001	.569	<.001

Figure 3. Analysis of Patients' Glucose and Lipid Intake



Detection of Biomarkers of Diabetes and Renal Function in Patients

Serum creatinine, fasting blood glucose, urinary protein/creatinine ratio, and HOMA-IR in the observation group were lower than those in the control group ($P < .05$), while the glomerular filtration rate in the observation group was higher than that in the control group ($P < .05$) (Table 7).

Analysis of Inflammation and Oxidative Stress

The levels of serum MDA, C- reactive protein, and TNF-α in the observation group were lower than those in the control group ($P < .05$), while the level of glutathione in the observation group was higher than that in the control group ($P < .05$) (Table 8).

Table 7. Detection of Biomarkers of Diabetes and Renal Function(s)

Group	Serum Creatinine (Mg/Dl)	Egfr (Ml·Min^{-1} × (1.73m^2) $^{-1}$)	Fasting Blood Glucose (Mg/ Dl)	Homa-Ir	Urine Protein/ Creatinine Ratio (Mg/G)
Control group (n = 30)	1.05 ± 0.16	76.35 ± 9.33	139.38 ± 14.58	5.24 ± 1.35	147.36 ± 27.52
Observation group (n = 56)	0.79 ± 0.14	92.52 ± 8.46	114.56 ± 12.52	3.89 ± 1.03	117.53 ± 24.68
t value	8.707	-8.149	8.268	5.186	4.959
P value	<.001	<.001	<.001	<.001	<.001

Table 8. Analysis of Inflammation and Oxidative Stress(s)

Groups	Malondialdehyde (mol/l)	Glutathione (mol/l)	C- reactive protein (mg/L)	TNF-α (pg/ml)
Control group (n = 30)	3.02 ± 0.28	621.47 ± 85.33	5.42 ± 1.36	82.53 ± 23.66
Observation group (n = 56)	2.38 ± 0.30	685.33 ± 105.66	3.75 ± 1.22	52.36 ± 18.44
t value	9.646	-2.848	5.812	6.539
P value	<.001	.006	<.001	<.001

DISCUSSION

Some recent studies have shown that the ecological imbalance of intestinal microbiota may play a role in the development of chronic kidney diseases.[15,18] In particular, bacterial metabolites have been shown to affect the occurrence and progress of chronic kidney disease, while the progression to renal failure will lead to the deterioration of intestinal flora imbalance. For example, intestinal microbiome composition differs in animals and people with chronic kidney disease. In the research of animals and humans with chronic kidney disease, the proportion of *Bifidobacterium*, Bacteroides, and *Lactobacillus* has decreased. In addition, in patients with chronic kidney disease, the proportion of *Platycladus*, *Ruminococcus*, *Rostrum*, and *Festuca* is reduced, while the proportion of *Parabacterium*, *Enterococcus*, and *Enterobacteriaceae* is increased.[19,20] Generally, the proportion of anaerobic bacteria in patients with chronic kidney disease is reduced. In addition, 20% of patients with chronic end-stage renal disease who do not receive dialysis have bacterial DNA in their blood.[19]

Mahabaday studied that in these patients, the same bacterial genus is detected in their intestines, while biomarkers of low-grade inflammation are increased. As the imbalance of intestinal flora will affect many chronic diseases, including type 2 diabetes and its complications, balancing the composition of intestinal flora may be a strategy to control or even prevent diseases.[20] Some studies have analyzed the role of probiotics or synbiotics in regulating intestinal flora in patients with chronic kidney disease.[19,20] In a clinical trial for patients with stage 3 and stage 4 chronic kidney disease, after taking a mixture of *Lactobacillus acidophilus*, *Streptococcus thermophilus*, and *Bifidobacterium longum* for six months, the blood urea nitrogen and uric acid concentrations are decreased. In a similar study, after taking dairy products containing *lactobacillus* for two months, the uric acid nitrogen level in the blood also decreases.[13]

There are differences in intestinal microflora between healthy individuals and patients with type 2 diabetes. In addition, DN is characterized by chronic hyperglycemia, which leads to a metabolic imbalance of carbohydrate, fat, and protein digestion due to insulin sensitivity deficiency. Therefore, blood sugar control is important in treating patients with DN metabolic syndrome. In this study, we evaluated the changes in blood sugar control and showed that probiotics significantly reduced blood sugar levels. Our research results showed that probiotic administration could improve the blood sugar control of DN, including beneficial effects on blood sugar control. It was shown that after 12 weeks, probiotics intake could significantly reduce fasting blood glucose and HOMA-IR. Supplementation of probiotics can reduce serum insulin and insulin resistance and have moderately positive effects on some biomarkers of oxidative stress.[21,22] Another meta-analysis has shown that probiotic supplementation reduces inflammation and oxidative stress and improves biomarkers of renal function in patients with diabetic nephropathy.[23] These results are consistent with our research. It was found that compared with the control group, the levels of C- reactive protein, TNF-α, and malondialdehyde in DN patients taking probiotics decreased, and the plasma glutathione concentration increased.

Studies have shown that supplementation of probiotics can reduce the C- reactive protein level in colorectal cancer patients.[24] Previous studies have reported that six-week probiotic supplementation in women with gestational diabetes mellitus improves C- reactive protein, tricarboxylic acid cycle, malondialdehyde level, and oxidative stress index. In addition, the beneficial effects of probiotics on oxidative damage may be mediated by the production of butyrate in the colon and the reduction of lipid peroxidation.[25] Our research showed that supplementation of probiotics for 12 weeks in DN patients had beneficial effects on blood sugar control and cardiac metabolic risk markers. It indicated that probiotic supplementation might have beneficial therapeutic potential for DN patients. In addition, we estimated the urinary protein/creatinine level before and after taking probiotics and found that the decrease of urinary protein/creatinine level by probiotics represented glomerular filtration function, which was the leading indicator of glomerular filtration capacity. Under normal circumstances, urine microalbumin will not pass through the glomerular

filter membrane due to the selective barrier and electrostatic repulsion of the charge in the filter membrane.

In patients with diabetic nephropathy, the levels of heparan sulfate and sialic acid decrease. This results in a decrease in the charge selectivity of the glomerular filtration membrane, which hinders the affinity of the extracellular basement membrane and proteoglycan. This change also leads to alterations in the diameter enlargement hole of the glomerular filtration membrane filter and the negative charge structure composition in the glomerular filtration membrane. As a result, urinary microalbumin is discharged. Meanwhile, serum creatinine variability strongly influenced eGFR levels in patients with kidney diseases. In previous studies, researchers have proved that compared with healthy individuals, the urine protein/creatinine level of DN patients is increased significantly.[25]

Malnutrition results from a continuous deficiency of energy balance because energy intake and consumption do not match. There is no difference in resting energy consumption between dialysis patients and the healthy matched control group, and insufficient energy intake due to decreased appetite is considered the leading cause of malnutrition. However, glucose absorption in peritoneal dialysis may somewhat improve this situation. Although the mechanism of this loss of appetite is still unclear, recent studies in animal models show that the abnormality of the hypothalamic appetite circuit may be significant. The progress in understanding appetite regulation may make the direct therapeutic operation of food intake possible. In particular, Ghrelin, an intestinal hormone, has been identified as a unique circulating hunger signal, which acts through the hypothalamus and is physiologically related to the beginning of meals and energy intake. Ghrelin can increase the short-term food intake of rodents. It has been previously shown that spontaneous energy intake increases after a single subcutaneous injection of Ghrelin in a group of malnourished patients undergoing maintenance peritoneal dialysis.[26]

Cyclic Ghrelin mainly stimulates NPY and AgRP neurons in the hypothalamus to increase appetite and acts through GHS receptors introduced by the vagus nerve. Ghrelin, therefore, has a direct effect on appetite regulation. Ghrelin can moderately lower blood pressure, consistent with many studies showing that blood pressure decreases by as much as 9-12 mmHg when administered intravenously. In vitro experiments clearly show that Ghrelin inhibits stimulated monocytes and lymphocytes from releasing inflammatory cytokines, and in vivo studies have confirmed this effect in many rodent inflammatory models, including chemically induced colitis, pancreatitis, or arthritis. Ghrelin, especially its bioactive form called acyl ghrelin, has been determined to play the most crucial role in stimulating appetite and promoting gastrointestinal motility, emptying, and gastric acid secretion. In this study, compared with the control group, it was found that the Ghrelin level of patients in the observation group increased. In a previous study of chronic kidney disease patients with peritoneal dialysis, it was also reported that probiotics could promote the increase of Ghrelin levels in vivo. Elevated circulating Ghrelin improves gastrointestinal symptoms, especially anorexia and gastric slow wave.[26]

Diet plays a vital role in maintaining the balance of intestinal flora. It is the source of bacterial fermentation, but it is reported that the dietary fiber intake of patients with chronic kidney disease is low. This fact may lead to an increase in intestinal transit time, thus promoting the fermentation of carbohydrates in the small intestine, thus reducing the utilization of carbohydrates by colon bacteria. Protein absorption in the proximal intestine of patients with chronic kidney disease is impaired, increasing the availability of bacteria protein in the colon. Changes in GM were observed in the early stage of chronic kidney disease. In the small intestine, aerobic and anaerobic bacteria overgrow. The number of actinomycetes, Proteus, and Scleromycetes increases at the colon level.[27]

In the study, relevant software was used to compare and analyze the nutritional intakes of two groups of patients to evaluate the nutritional value of patients' diets. The results showed that probiotic supplements significantly increased the intake of vitamins (vitamin B1, vitamin B2, and vitamin C), elements (iron, zinc, calcium), carbohydrates and dietary fiber in DN patients, which may be related to the increase of Ghrelin level in the observation group. Our research results showed that taking probiotics had a good effect on the kidney and nutrient intake of DN patients. It is considered that Ghrelin is an appetite-enhancing hormone. However, in this study, the form of Ghrelin was not distinguished. It should also be emphasized that there are differences in the level of acyl ghrelin in other studies. Some studies show that the level of chronic kidney disease patients is higher, while others have no difference. In patients with chronic kidney disease, the appetite inhibitor deacyl ghrelin is the main form because it is found that the total deacyl ghrelin level in the plasma of patients with chronic kidney disease is increased, instead of acyl ghrelin. Total Ghrelin mainly represents the deacylated form of Ghrelin, which has anorexia and inhibits food intake.[23]

Several studies have shown that adiponectin has anti-inflammatory effects. C- reactive protein and adiponectin are reported to be interrelated in blood and adipose tissue. Proinflammatory cytokines play an important role in the pathogenesis of metabolic syndrome.[18] The present study shows that the increase in serum adiponectin level is significantly related to the rise in serum creatinine level, the decrease of BMI, the reduction of eGFR, and the decrease in serum albumin level. In other studies, adiponectin level is found to be negatively correlated with visceral adipose tissue storage. Therefore, decreased visceral fat may increase adiponectin levels in overweight subjects. It should be emphasized that although hypoadiponectin is a significant risk factor for metabolic disorders, the adiponectin level in patients with chronic kidney disease is higher and is related to the progression of chronic kidney disease. High plasma adiponectin level in type 2 diabetic nephropathy positively correlates with fasting insulin and HOMA-IR. Probiotics can reduce plasma adiponectin levels in patients with type 2 diabetic nephropathy while fasting insulin HOMA-IR decreases.[28]

A human study found that adiponectin increased in patients with insulin-resistant type 1 diabetes, while lipid content in muscle cells increased.[29] Secondly, proteinuria may be an important factor that links adiponectin with insulin resistance. Increased albuminuria is associated with obesity and diabetes, and is a risk factor for cardiovascular and kidney diseases. Adiponectin has been proven to be the critical regulator of proteinuria and podocyte function. Studies on mice have shown that adiponectin deficiency can cause oxidative stress, podocyte fusion, and urinary albumin excretion. Adiponectin treatment can reverse these abnormalities. However, this phenomenon is somewhat different in the state of kidney disease. For example, adiponectin in patients with nephritis syndrome is significantly increased and related to metabolic risk factors, while severe proteinuria is closely related to the increase of adiponectin level.[29]

Studies on non-diabetic chronic kidney disease show plasma adiponectin positively correlates with the urinary albumin/creatinine ratio. Adiponectin has little effect on renal function, but it is affected by proteinuria.[23,28] In recent years, the crosstalk between adipose tissue and endothelial cells has attracted worldwide attention. Generally speaking, adiponectin is considered an endothelial protective factor because it can prevent TNF-α-driven endothelial injury. However, this is not the case in chronic kidney disease. High adiponectin is self-contradictory to severely damaged endothelial function. After adjusting for other risk factors, adiponectin is directly but not negatively related to endothelial dysfunction. This study showed that probiotics could significantly reduce adiponectin levels in DN patients, and compared with the control group, probiotics can improve eGFR, HOMAIR, serum creatinine, and other indicators, which was consistent with previous reports. Vitamin D deficiency is associated with PC[23] and diabetes.[28] This study did not examine the interaction of diet (e.g., Vitamin D intake) and genetics on PC physical activity that might play a protective role against PC development.[30,31] The role of interaction between physical activity and genes on PC should be explored.

The number of subjects included in this study is small, and the test results lack geographical representation. Our next step will be to increase the sample and conduct this study in multiple centers.

To sum up, our research showed that supplementation of probiotics in DN dialysis patients could increase serum ghrelin concentration, increase nutrient intake through appetite regulation, and reduce adiponectin levels, which was beneficial to blood sugar control, insulin resistance, and renal function.

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