Probiotic Supplementation in Chronic Kidney Disease: A Double-blind, Randomized, Placebo-controlled Trial

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Objective: The objective of the study was to evaluate the effects of probiotic supplementation on the gut microbiota profile and inflammatory markers in chronic kidney disease patients undergoing maintenance hemodialysis (HD).

Design and Methods: This was a randomized, double-blind, placebo-controlled study. Forty-six HD patients were assigned to receive 1 of 2 treatments: probiotic (n = 23; Streptococcus thermophilus, Lactobacillus acidophilus e Bifidobacteriumlongum, 90 billion colony-forming units per day) or placebo (n = 23) daily for 3 months. Blood and feces were collected at baseline and after intervention. The inflammatory markers (C-reactive protein and interleukin-6) were analyzed by immunoenzymatic assay (enzyme-linked immunosorbent assay). Uremic toxins plasma levels (indoxyl sulfate, p-cresyl sulfate, and indole-3-acetic acid) were obtained by Reversed-Phase High-Performance Liquid Chromatography. Routine laboratory parameters were measured by standard techniques. Fecal pH was measured by the colorimetric method, and the gut microbiota profile was assessed by Denaturing Gradient Gel Electrophoresis analysis.

Results: Sixteen patients remained in the probiotic group (11 men, 53.6 ± 11.0 year old, 25.3 ± 4.6 kg/m²) and 17 in the placebo group (10 men, 50.3 ± 8.5 year old, 25.2 ± 5.7 kg/m²). After probiotic supplementation there was a significant increase in serum urea (from 149.6 ± 34.2 mg/dL to 172.6 ± 45.0 mg/dL, P = .02), potassium (from 4.4 ± 0.4 mmol/L to 4.8 ± 0.4 mmol/L, P = .02), and indoxyl sulfate (from 31.2 ± 15.9 to 36.5 ± 15.0 mg/dL, P = .02). The fecal pH was reduced from 7.2 ± 0.8 to 6.5 ± 0.5 (P = .01). These parameters did not change significantly in placebo group. Changes in the percentage delta ($\Delta$) between groups were exhibited with no statistical differences observed. The inflammatory markers and gut profile were not altered by supplementation.

Conclusions: A probiotic supplementation failed to reduce uremic toxins and inflammatory markers. Therefore, probiotic therapy should be chosen with caution in HD patients. Further studies addressing probiotic therapy in chronic kidney disease patients are needed.

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Introduction

Research has shown that the imbalance of the microorganisms living in the gut community and the impairment of the colonic epithelium are related to inflammation and oxidative stress in chronic kidney disease (CKD). In fact, CKD patients are constantly exposed to various factors such as malnutrition, edema, stress (physical, psychological, or pharmacological), constipation, dietary restriction, and uremia among others, which compromise the intestinal homeostasis.1-4

Alterations in the microbiota composition of CKD patients have been associated with the growth of bacterial species involved in the generation of harmful compounds called uremic toxins such as indole-3 acetic acid (IAA), indoxyl sulfate (IS), and p-cresyl sulfate (p-CS).5 CKD patients present a progressive retention of these uremic toxins,
with a negative impact on many body functions and increase in cardiovascular mortality. The bacterial fermentation of the amino acid tryptophan in the colon leads to production of indoles that are metabolized to IAA in the intestine and IS in the liver. On the other hand, the fermentation of tyrosine and phenylalanine leads to synthesis of p-cresol and finally p-CS.

Probiotic supplementation has been suggested as an adjuvant therapy to improve the balance of the gut microbiota contributing to intestinal barrier integrity and metabolic control of these patients. Probiotics are "natural or genetically modified microorganisms, expressing specific exogenous enzymes, which are able to survive stomach acid and bile, to increase the colon concentration of symbionts, and confer a health benefit." The mechanisms by which probiotics exert their effects involve changes in intestinal pH, antagonism of pathogens by production of antibacterial components, competition for available nutrients, conjunction with mutagens and carcinogens preventing their actions, and improved intestinal barrier functions.

Studies evaluating the effectiveness of probiotics on CKD have reported discrepant results. Thus, the present study evaluated the effects of a probiotic formulation on the biochemical and inflammatory parameters, uremic toxins levels, and gut microbiota profile in CKD patients on hemodialysis (HD).

Methods

Recruitment of Participants

Forty-six HD patients were included in this randomized, double blind, placebo-controlled study (23 received probiotic supplement and 23 received placebo). Patients aged 18 years, undergoing HD for at least 6 months, were included. Patients with inflammatory diseases, cancer, AIDS, autoimmune disease, smokers, use of a central catheter for hemodialysis access, amputated limbs, pregnancy, AIDS, autoimmune disease, smokers, use of a central catheter for hemodialysis access, amputated limbs, pregnancy, and patients who had used catabolic drugs, antioxidant vitamin supplements pre, pro, and symbiotic and antibiotics in the last 3 months before starting this study were excluded.

Dialysis duration was 3 to 4.5 hours per session, 3 times per week, with a blood flow greater than 250 mL/minute and a dialysate flow of 500 mL/minute. The study protocol was reviewed and approved by the Ethics Committee of the School of Medicine–Universidade Federal Fluminense (083/11), and all the patients were asked to sign the informed consent.

The random sequence of treatment (probiotic and placebo) was manually generated for a simple randomization. None of the subjects involved in the study had access to the allocation sequence until the end of the statistical analyses. The participants and the researchers who interviewed and visited the subjects were blinded to the contents of the bottles, which contained probiotic or placebo capsules. All laboratory measurements were centralized and performed in a blinded manner.

Intervention

Twenty-three patients were randomly allocated in the probiotic group and 23 patients were allocated in the placebo group. The dosage was 3 capsules of probiotic or placebo per day for 3 months. Each capsule of probiotic contained 30 billion live bacteria, totaling 90 billion colony-forming units (CFU) per day and included the following strains: *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacteria longum*. Patients were contacted weekly, by phone calls, during the trial by study staff to encourage adherence to supplementation and also to monitor side effects. Adherence was measured by the number of capsules returned to researchers subtracted from the number of capsules dispensed at the previous visit. This number was divided by the number of capsules the patient should have taken during 3 months and then multiplied by 100 to obtain the adherence percentage.

Analytical Procedures and Sample Processing

Blood samples were taken from each subject in the morning, at the start of a dialysis session (after overnight fasting), into a syringe containing ethylenediaminetetraacetic acid (1.0 mg/mL) as anticoagulant. Plasma was separated (15 minutes, 3,000 g, 4°C), and stored at −80°C until analysis.

Total concentrations of IS, IAA, and p-CS were quantified by Reversed-Phase High-Performance Liquid Chromatography with fluorescent detection as previously described. Briefly, plasma samples were processed as described and injected into a high-performance liquid chromatography system (Shimadzu Prominance) consisting of a Rheodyne injector (model 7125), a quaternary pump (Shimadzu LC-20AD), and a fluorescence detector (Shimadzu RF-20A) all controlled by LC Solution software.

High-sensitivity C-reactive protein and interleukin-6 were analyzed by immunoenzymatic assay (enzyme-linked immunosorbent assay). Routine laboratory parameters were measured by standard techniques.

The fecal samples were collected in sterile containers and provided to the laboratory on the day of collection before and after the follow-up. The fecal pH was measured by the colorimetric method with homogenized stool samples in distilled water and DNAs were extracted to Denaturing Gradient Gel Electrophoresis (DGGE) analysis.

DNA Extraction and Polymerase Chain Reaction of the 16S Ribosomal RNA

DNA extraction and polymerase chain reaction were analyzed as previously described. Briefly, the DNAs were extracted using the Xpedition Soil/Fecal Miniprep DNA (Zymo, Irvine, CA). The integrity and quality of extracted DNA were analyzed by gel electrophoresis in 0.8%
Probiotics) consuming more than 80% of the capsules.

Denaturing Gradient Gel Electrophoresis

DGGE of the amplified gene sequences was performed using a DCode system (Universal Mutation Detection system), Bio-Rad (Richmond). The polymerase chain reaction products were applied directly to the gel electrophoresis in 6% polyacrylamide. After electrophoresis, the gel was stained with SYBR Gold I (Molecular Probes Invitrogen) and scanned in the image capture system STORM 860 Imaging System (GE Healthcare). The results were presented as dendrograms constructed after image capture and analysis by Dice correlation coefficients (r). The cluster analysis was performed by the unweighted pair group method with average linkages (UPGMA) using BioNumerics Software (Applied Maths, Belgium).

Nutritional Assessment

The following anthropometric parameters were measured: body weight (kilogram), height (meter), waist circumference (centimeter), mid-arm circumference (centimeter), and skinfold (millimeter; biceps and triceps). Arm muscle area was calculated according to the following formula: [MAC [cm] = 3.14 × triceps skinfold [in mm]²/4r] − n, where n = 10 for male and 6.5 for female. Body mass index was calculated as body weight divided by squared stature and arm muscle area was calculated from triceps skinfold thickness and mid-arm circumference. A trained staff member performed all the measurements after the HD session.

Statistical Analysis

The distribution of variables was analyzed by the Kolmogorov–Smirnov test. The normally distributed variables were expressed as mean ± standard deviation or mean (95% confidence intervals). The differences of the variables were analyzed using nonparametric tests (paired tests, Wilcoxon) or parametric tests (Independent Samples t-test or Paired Samples t-test). The percentage delta (Δ) was also used to check the differences in the variables before and after supplementation. Regression analysis was performed to determine variables that had independent associations with a number of bands (DGGE). A statistical significance level of 5% was accepted. The statistical analyses were performed using SPSS 19.0 software (Chicago, IL).

Results

Seven patients in the probiotic group and 6 in the placebo group left the study (Fig. 1). Patient’s characteristics at baseline are shown in Table 1. There were no differences between groups at baseline for all assessed variables (all P > .05).

Adherence was high with 82% of patients (placebo and probiotics) consuming more than 80% of the capsules.

Table 2 shows the effects of probiotic supplementation or placebo on biochemical and inflammatory parameters. Pre-dialysis serum urea and potassium plasma levels increased after 3 months of probiotic. In addition, a reduction in fecal pH was observed in the probiotic group, and there was no significant change in inflammatory parameters. Table 3 shows the effects of probiotic supplementation or placebo on plasma levels of toxins. IS plasma levels increased after probiotic intervention (Fig. 2), but it is important to note that the levels in the placebo at baseline were higher than in probiotic group, even without statistical difference. The percentage delta (Δ) between groups are reported also in Table 3. Changes were exhibited with no statistical differences observed.

The gut microbiota profile of 20 patients (10 in each group) were assessed before (T1) and after (T2) probiotic supplementation or placebo (Fig. 3). The average number of bands (indicative of bacterial profile) was not different between the probiotic (21.2 ± 4.9) and placebo groups (23.3 ± 7.3; P = .48) at baseline. There was no significant difference in the average number of bands, comparing T1 versus T2, neither in the probiotic group (22.4 ± 6.7, P = .62) nor in the placebo group (24.5 ± 5.0, P = .58).

A negative correlation between the average number of bands at baseline and fecal pH (r = −0.5; P = .038) and serum urea (r = −0.49, P = .038) was observed. A linear regression showed that urea was an independent predictor for the number of bands (β = −0.66, P = .027) after adjustment for age, gender, body mass index, % body fat, and pH fecal. Figure 4 summarizes the hypothetical effects of probiotic supplementation in CKD patients according to the results observed in this study.

Discussion

In the present study, after 3 months of probiotic supplementation, no benefits in the biochemical parameters and inflammatory markers in CKD patients on HD were observed. In addition, there was a significant increase in pre-HD urea, potassium, and IS after probiotic supplementation; however, a reduction in fecal pH occurred in this group. No change was observed in the gut microbiota profile after probiotic or placebo supplementation.

Probiotics have a long history in clinical practice as a safe strategy for several diseases. Global health organizations, such as World Health Organization, attribute health benefits to this approach. Over recent years, researchers have supported the hypothesis that probiotics may be a promising adjuvant therapy for CKD, given the role of the gut microbiota alterations in uremic toxicity and systemic inflammation.

Some studies have shown benefits of probiotic supplementation in CKD patients. In a randomized controlled clinical trial, there was a decrease in the serum urea concentrations in Stages 3 and 4 CKD patients (n = 30) with a 16 billion CFU/day dose of Lactobacillus casei shirota over a
2 month period.13 In a 6-month prospective, randomized, double-blind, crossover, placebo-controlled study, 46 Stages 3 and 4 CKD patients received 90 billion CFU/day of a probiotic formulation and the blood urea nitrogen levels were decreased significantly in 29 patients (63% of the sample), whereas creatinine and uric acid levels reduction was not significant.12 On the other hand, Natarajan et al. (2014) observed no effect on uremic toxins and C-reactive protein levels in 22 HD patients after 2 months of supplementation with a 180 billion CFU/day dose of probiotic formulation.14

In a recent review, Vaziri et al. (2015)26 argued that factors associated with CKD (such as uremia, dietary, and medicinal regimens) create an unfavorable biochemical milieu for the introduction of probiotic bacteria. Especially, HD patients who have significant uremia which leads to higher urea influx into the intestinal lumen.

In the intestinal lumen, urea creates a hospitable milieu for the expansion of urease-possessing bacteria providing a greater rate of urea hydrolysis and large amounts of ammonia \([\text{CO(NH}_2\text{)}_2 + \text{H}_2\text{O} \rightarrow \text{CO}_2 + 2\text{NH}_3]\) and ammonium hydroxide \([\text{NH}_3 + \text{H}_2\text{O} \rightarrow \text{NH}_4\text{OH}]\) which are harmful to the intestinal barrier. Consequently, the disruption of the epithelial barrier facilitates the translocation of uremic toxins and other microbial substances from the gut lumen into the systemic circulation, resulting in worsening of systemic inflammation.26,27

At baseline, a negative correlation between the average number of bands and predialysis urea plasma levels was found, suggesting indirect evidence of the role of biochemical milieu in modulating the gut microbiome.2,28 In a previous study published by our group, a negative association between the average number of bands and vascular cell adhesion molecule 1 plasma levels was observed in nondialysis CKD patients, suggesting a possible relationship between gut microbiota and cardiovascular risk in CKD patients.21

Ureolytic bacteria belong to both symbiotic and pathogenic microbiota. In gut microbiome, the level of urease activity is diverse.29 Even some strains of species considered constitutively nonureolytic may present urease activity under specific conditions, suggesting that the environment may take a role in modulating the enzymatic activity of the bacteria.20-32

Figure 1. Flow chart of the study subjects.

Table 1. Baseline Characteristics of Probiotic and Placebo Groups of Maintenance Hemodialysis Patients

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Probiotics (n = 16)</th>
<th>Placebo (n = 17)</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>53.6 ± 11.0</td>
<td>50.3 ± 8.5</td>
<td>.30</td>
</tr>
<tr>
<td>Gender (M/F) (H/M) (Homem/Mulher)</td>
<td>11/5</td>
<td>10/7</td>
<td>.81</td>
</tr>
<tr>
<td>Time on dialysis (mo)</td>
<td>60 (38.2-105)</td>
<td>36.5 (24.2-72)</td>
<td>.09</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>25.3 ± 4.6</td>
<td>25.2 ± 5.7</td>
<td>.90</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>90.0 ± 9.5</td>
<td>93.7 ± 17.9</td>
<td>.48</td>
</tr>
<tr>
<td>Arm muscle area (cm²)</td>
<td>38.2 ± 7.5</td>
<td>45.6 ± 15.9</td>
<td>.17</td>
</tr>
</tbody>
</table>
Thus, probiotic supplementation may have failed to promote benefits in this study because of the unfavorable environment in which such strains were introduced. In parallel, CKD patients present factors that impair the maintenance of the gut pH within the proper range, making it less acid. In addition to uremia per se, patients usually have a lower intake of fiber, resulting from the restrictions of fruits and other vegetables to prevent hyperkalemia, leading to a lower production of short chain fatty acids, which contribute to the maintenance of normal gut pH.1-4

The colonic pH may be related to the rate of ammonia that will be absorbed or converted into ammonium hydroxide. At a lower pH, there is higher generation of nonionized ammonia, which easily crosses the gut epithelium reaching the intrahepatic portal circulation. In a more acidic environment, the conversion rate of ammonia into ammonium hydroxide is higher and, this metabolite can then be excreted in the feces.33 Under physiological conditions, colonic pH is in the range of 5.7 to 6.7, and in this case, the ammonium hydroxide can be eliminated without causing damage because it is produced in small amounts.33 Some studies have documented the probiotics effects on pH in several situations. Tejero-Sariñana et al. (2012)35 observed, in an in vitro study, that probiotics led to an increased production of organic acids reducing pH and this beneficial alteration was associated with the inhibition of pathogens. In another study, Bull-Otterson et al. (2013)36 observed that a probiotic supplementation decreased significantly the fecal pH in rats with alcoholic liver disease. Natarajan et al. (2014)14 showed that probiotic supplementation decreased pH fecal of dialysis patients compared with placebo controls. In the present study, a significant decrease in fecal pH in the group supplemented with probiotics was also observed. Despite this significant reduction, the average value only managed to reach the upper limit of the range considered normal.

Our results also show a significant increase in potassium and urea plasma levels after probiotic supplementation. Although we have recognized that other factors unrelated to probiotic supplementation may have been responsible for the change in potassium and urea, these results have made us speculate about how the gut microbiota in CKD patients may be involved in their homeostasis. It is important to emphasize that food intake record was not performed, and there is lack of information about protein, fibers, and potassium intake by these patients, even knowing that they usually do not change eating habits.

In CKD, fecal excretion of potassium has been considered to contribute significantly to potassium homeostasis.37 This adaptive mechanism becomes essential to maintain the potassium balance in these patients. In a study in peritoneal dialysis patients, 25% of potassium intake was eliminated in the feces.38 Mathialahan et al. (2005)39 showed that rectal potassium secretion was almost 3-fold greater in CKD patients in the end-stage renal disease than normal renal

### Table 2. Effects of Probiotic Supplementation or Placebo on Biochemical and Inflammatory Parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline (n = 16)</th>
<th>After</th>
<th>Δ</th>
<th>P Value</th>
<th>Baseline (n = 17)</th>
<th>After</th>
<th>Δ</th>
<th>P Value</th>
<th>P Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea predialysis (mg/dL)</td>
<td>149.6 ± 34.2</td>
<td>172.6 ± 45.0</td>
<td>22.9 (−0.8; 46.7)</td>
<td>.05</td>
<td>152.2 ± 45.3</td>
<td>155.9 ± 38.6</td>
<td>3.7 (−21.7; 29)</td>
<td>.75</td>
<td></td>
</tr>
<tr>
<td>Urea post-dialysis (mg/dL)</td>
<td>52.8 ± 18.8</td>
<td>51.3 ± 19.7</td>
<td>−1.5 (−15.4; 12.4)</td>
<td>.82</td>
<td>47.3 ± 18.9</td>
<td>49.5 ± 12.7</td>
<td>2.1 (−8.9; 13.2)</td>
<td>.68</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>14.3 ± 0.8</td>
<td>9.6 ± 7.7</td>
<td>−4.6 (−81.5; 72.2)</td>
<td>.58</td>
<td>9.3 ± 1.5</td>
<td>10.3 ± 0.6</td>
<td>1.0 (−7.2; 9.3)</td>
<td>.35</td>
<td></td>
</tr>
<tr>
<td>Potassium (mg/dL)</td>
<td>4.4 ± 0.4</td>
<td>4.8 ± 0.47</td>
<td>0.4 (0.07; 0.8)</td>
<td>.02</td>
<td>4.6 ± 0.48</td>
<td>5.0 ± 0.52</td>
<td>0.4 (−0.08; 1.0)</td>
<td>.09</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>11.6 ± 1.3</td>
<td>11.1 ± 1.8</td>
<td>−0.4 (−1.3; 0.4)</td>
<td>.27</td>
<td>11.0 ± 1.4</td>
<td>10.9 ± 1.4</td>
<td>−0.6 (−1.3; 0.1)</td>
<td>.01</td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dL)</td>
<td>4.2 ± 0.23</td>
<td>4.2 ± 0.29</td>
<td>0.06 (−0.1; 0.2)</td>
<td>.53</td>
<td>4.2 ± 0.22</td>
<td>4.2 ± 0.27</td>
<td>0.0 (−0.2; 0.2)</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Globulin (g/dL)</td>
<td>2.8 ± 0.4</td>
<td>2.9 ± 0.4</td>
<td>0.12 (−0.1; 0.3)</td>
<td>.19</td>
<td>3.0 ± 0.5</td>
<td>2.9 ± 0.4</td>
<td>−0.1 (−0.3; 0.0)</td>
<td>.25</td>
<td></td>
</tr>
<tr>
<td>C-reactive protein (mg/dL)</td>
<td>4.1 (1.4; 9.2)</td>
<td>5.5 (2.6; 11.7)</td>
<td>2.7 (−3.1; 8.5)</td>
<td>.71</td>
<td>3.1 ± 0.9</td>
<td>6.7 ± 0.8</td>
<td>3.4 (−6.4; −0.5)</td>
<td>.35</td>
<td></td>
</tr>
<tr>
<td>Interleukin-6 (pg/mL)</td>
<td>41.2 ± 18.9</td>
<td>38.4 ± 20.1</td>
<td>32.8 (−50.6; 116.2)</td>
<td>.71</td>
<td>32.9 ± 13.8</td>
<td>30.3 ± 18.5</td>
<td>−2.6 (−21.1; 15.7)</td>
<td>.73</td>
<td></td>
</tr>
<tr>
<td>pH fecal</td>
<td>7.2 ± 0.8</td>
<td>6.5 ± 0.5</td>
<td>−0.6 (−1.1; −0.1)</td>
<td>.01</td>
<td>6.8 ± 0.8</td>
<td>6.8 ± 0.7</td>
<td>0.05 (−0.6; 0.7)</td>
<td>.88</td>
<td></td>
</tr>
</tbody>
</table>

Data were presented as mean ± standard deviation or mean (95% confidence intervals). The P value is reflecting the differences of the variables between baseline and after intervention moment in each group.

### Table 3. Effect of Probiotic Supplementation or Placebo on Uremic Toxins Plasma Levels in CKD Patients on HD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Baseline (n = 16)</th>
<th>After</th>
<th>Δ</th>
<th>P Value</th>
<th>Baseline (n = 17)</th>
<th>After</th>
<th>Δ</th>
<th>P Value</th>
<th>P Δ</th>
</tr>
</thead>
<tbody>
<tr>
<td>IS (μg/L)</td>
<td>31.2 ± 15.9</td>
<td>36.5 ± 15.0</td>
<td>5.2 (0.8; 9.6)</td>
<td>.02</td>
<td>39.1 ± 8.2</td>
<td>42.5 ± 11.0</td>
<td>3.3 (1.1; 7.9)</td>
<td>.12</td>
<td></td>
</tr>
<tr>
<td>p-CS (μg/L)</td>
<td>50.4 ± 29.0</td>
<td>46.3 ± 32.7</td>
<td>4.1 (−18.8; 10.5)</td>
<td>.55</td>
<td>57.0 ± 28.7</td>
<td>57.5 ± 29.8</td>
<td>0.4 (8.4; 9.2)</td>
<td>.91</td>
<td></td>
</tr>
<tr>
<td>IAA (μg/L)</td>
<td>451 ± 280</td>
<td>456.8 ± 199</td>
<td>5.6 (−115.5; 126)</td>
<td>.92</td>
<td>678.4 ± 376</td>
<td>744.9 ± 309</td>
<td>66.4 (48.2; 181)</td>
<td>.22</td>
<td></td>
</tr>
</tbody>
</table>

CKD, chronic kidney disease; IAA, indole-3-acetic acid; IS, indoxyl sulfate; HD, hemodialysis; p-CS, p-cresyl sulfate.

Data were presented as mean ± standard deviation or mean (95% confidence intervals). The P value is reflecting the differences of the toxins plasma levels between baseline and after intervention moment in each group.
function controls. When an inhibitor of the potassium channel was placed in the gut lumen, potassium secretion was reduced by 45% in CKD patients, and no effect was observed in the control group. Greater potassium channel protein expression was observed in colonocytes from CKD patients. These data suggest that the increased expression of potassium channels is an adaptive mechanism to increase the potassium secretion in the colon of CKD patients.

Considering that the introduction of probiotic bacteria in a uremic environment may exacerbate the damage to gut barrier, hypothetically potassium channels could be 1 of the structural components affected, compromising the gastrointestinal excretion of potassium. In an in vitro study, Vaziri et al. (2012)\textsuperscript{40} showed that human enterocytes incubated with uremic plasma showed depletion of claudin-1 and occludin, key components of the junctional complex of the gut barrier, which confirms the role of uremia in the dysfunction of gut barrier components.

Our results also show a significant increase in IS plasma levels after probiotic supplementation, which could be a consequence of increased permeability of the gut barrier allowing greater diffusion of uremic toxins from the gut lumen into the bloodstream. On the other hand, the other toxins analyzed (p-CS and IAA) showed no significant change.

Hida et al. (1996)\textsuperscript{9} in a study involving 25 HD patients showed that a supplementation of a probiotic formulation (Bifidobacterium infantis, Lactobacillus acidophilus, and Enterococcus faecalis) for 4 weeks led to a reduction in fecal p-cresol, whereas the reduction in p-cresol plasma levels was insignificant.

A nonrandomized, placebo-controlled trial evaluated Bifidobacterium longum supplementation for 5 weeks in 11 HD patients and found a significant reduction in IS plasma levels.\textsuperscript{10} In a latter study, the same group evaluated the effects of the same strain for 12 weeks in 27 HD patients and confirmed the previous results.\textsuperscript{41}

Hyun et al. (2013)\textsuperscript{42} in a study involving 16 HD pediatric patients observed no significant effects on the IS and p-CS plasma levels after 12 weeks by probiotic preparation containing 8 species of bacteria (Lactobacillus casei, Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus Delbrueckii subsp. bulgaricus, Bifidobacterium longum, Bifidobacterium breve, Bifidobacterium infantis, and Streptococcus salivarius subsp. thermophilus).

According to Urben et al. (2014),\textsuperscript{43} it is difficult to reach conclusions regarding studies with probiotics. Factors such as the diversity of strains, heterogeneity of the study populations, limited sample size, different experimental designs, different treatment periods, among others make studies difficult to be compared.

In addition, although probiotics are widely regarded as safe, there are significant concerns about safety in particular populations.\textsuperscript{24} According to the World Health Organization and the Food and Agriculture Organization (2002),\textsuperscript{16} “Probiotics may theoretically be responsible for 4 types of side effects such as systemic infections, deleterious metabolic activities, excessive immune stimulation in susceptible individuals and gene transfer.” A review study by the Agency for Healthcare Research and Quality, based on 622 studies concluded that, “the current literature is not well equipped to answer questions on the safety of probiotics in intervention studies with confidence.”\textsuperscript{44}

The current scenario brings several evidences that the intestine plays a critical role in CKD. Thus, it is expected that this organ will gain increasing prominence as a promising therapeutic target in the CKD context. Over the next years, the authors hope that studies have results about strategies capable of reestablishing the gut’s biochemical environment to provide more favorable conditions for the maintenance of a balanced intestinal microbiota and healthy colonic epithelium.

In view of our results and considering the peculiarities of CKD patients, it is plausible to consider that a probiotic or symbiotic supplementation can promote a synergistic action and to be more effective to modulate the gut dysbiosis in CKD patients.\textsuperscript{26,45} Prebiotics may contribute to modulation of the gut microbiota and to improve the integrity of the intestinal epithelial barrier, decreasing uremic toxins production and attenuating local and systemic inflammation, thus promoting a more favorable scenario for the introduction of probiotic microorganisms.\textsuperscript{46} Besides the prebiotics, other therapeutic strategies for have already emerged to improve the gut imbalance in CKD such as physical exercise\textsuperscript{47} and the use of oral adsorbents.\textsuperscript{26}

The present study presents a number of limitations. First, the small sample size may have limited other statistically significant effects of the probiotic supplementation. Second, the inflammatory markers used in this study could be influenced by a variety of factors (however, randomization should control this). Third, the food intake
of the patients of this study was not recorded. These au-
thors believe that a larger, long-term, and crossover with a
washout study is crucial to better characterize the probi-
otics effects.

Figure 3 summarizes the hypothetical effects of probiotic
supplementation in CKD patients according to the results
observed in this study, emphasizing that many other factors
should be considered.

Conclusions
In conclusion, the importance that gut microbiota has in
CKD is clear as are the deadlocks associated with the use of
probiotics in this population. The potential risks of probi-
otic supplementation in CKD patients should be consid-
ered because of the peculiarities of this disease. Further
studies, with a larger sample, concerning probiotic interac-
tions in CKD patients are needed.


**Figure 4.** Hypothetical effects of probiotic supplementation in chronic kidney disease patients. High urea plasma levels in hemodialysis patients lead to increased urea influx into the colon lumen promoting biochemical alterations in the colon environment. These conditions may alter the enzymatic activity of bacteria (including probiotics) providing a greater urea hydrolysis rate and large amounts of ammonia. Part of the ammonia may be absorbed reaching the intrahepatic portal circulation and enter the urea cycle which produces urea. Another part of the ammonia may be converted to ammonium hydroxide, a caustic product for intestinal barrier components. The damage to the intestinal barrier could affect the gastrointestinal excretion of potassium and turn the membrane permeable to the diffusion of substances from the intestinal lumen into the bloodstream, such as IS.

**Practical Application**

Increased uremic toxins production from gut microbiota and their accumulation are associated to high cardiovascular disease risk in CKD patients on hemodialysis. Some strategies to restore the gut microbiota balance like probiotics treatment are advised to these patients. However, complexities in the composition of the gut microbiota in CKD patients should be taken into account when strains are given to these patients as a strategy to reestablish the gut microbiota equilibrium. We need a higher understanding about how the patient’s gut microbiota interacts with the given probiotic.

**References**


4. Rossi M, Johnson DW, Campbell KL. The kidney-gut axis: implications for therapeutics to restore the gut microbiota balance like probiotics treatment are advised to these patients. However, complexities in the composition of the gut microbiota in CKD patients should be taken into account when strains are given to these patients as a strategy to reestablish the gut microbiota equilibrium. We need a higher understanding about how the patient’s gut microbiota interacts with the given probiotic.


