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Effect of fructooligosaccharide on endothelial function in CKD patients: a randomized controlled trial

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ABSTRACT

Background. Microbiota-derived uremic toxins have been associated with inflammation that could corroborate with endothelial dysfunction (ED) and increase cardiovascular risk in patients with chronic kidney disease (CKD). This trial aimed to evaluate the effect of the prebiotic fructooligosaccharide (FOS) on endothelial function and arterial stiffness in nondialysis CKD patients.

Methods. In a double-blind controlled trial, 46 nondiabetic CKD patients were randomized to receive 12 g/day of FOS or placebo (maltodextrin) for 3 months. Total *p*-cresyl sulfate (PCS) and indoxyl sulfate by high-performance liquid chromatography, urinary trimethylamine N-oxide by mass spectrometry, C-reactive protein, interleukin-6 (IL-6), serum nitric oxide and stroma-derived factor-1 alfa were measured at baseline and at the end of follow-up; endothelial function was assessed through flow-mediated dilatation (FMD) and arterial stiffness by pulse wave velocity (PWV).

Results. The mean (± standard deviation) age of the study participants was 57.6 ± 14.4 years, with an estimated glomerular filtration rate of 21.3 ± 7.3 mL/min/1.73 m^2. During the follow-up, regarding the inflammatory markers and uremic toxins, there was a significant decrease in IL-6 levels (3.4 ± 2.1 pg/mL versus 2.6 ± 1.4 pg/mL; P = 0.04) and a trend toward PCS reduction (55.4 ± 38.1 mg/L versus 43.1 ± 32.4 mg/L, P = 0.07) only in the prebiotic group. Comparing both groups, there was no difference in FMD and PWV. In an exploratory analysis, including a less severe ED group of patients (FMD ≥2.2% at baseline), FMD remained stable in the prebiotic group, while it decreased in the placebo group (group effect P = 0.135; time effect P = 0.012; interaction P = 0.002).

Conclusions. The prebiotic FOS lowered circulating levels of IL-6 in CKD patients and preserved endothelial function only in those with less damaged endothelium. No effect of FOS in arterial stiffness was observed.

Keywords: chronic kidney disease, endothelial dysfunction, flow-mediated dilatation, fructooligosaccharide, gut microbiota

INTRODUCTION

Excess inflammation and oxidative stress have been linked to increased cardiovascular risk in chronic kidney disease (CKD). In CKD, endothelial cells assume the pro-inflammatory phenotype and the generation of endothelial nitric oxide (NO) is reduced [1]. Chronic inflammation is known to promote oxidative stress and vice versa by activating transcription factors such as nuclear factor-κB [2]. Pilot studies in non-CKD patients indicate that the gut microbiome could influence the balance of vascular homeostasis [3]. Gut microbiomederived uremic toxins such as indoxyl sulfate (IS) augment the expression of intercellular adhesion molecule-1 and monocyte chemotactic protein-1 [4]. IS and *p*-cresol sulfate (PCS) are associated with reduction in antioxidant capacity and PCS is reported to induce reactive oxygen species in a concentration-dependent manner [5, 6]. In CKD patients gut-derived toxins, such as IS, PCS and trimethylamine N-oxide (TMAO), play a role in endothelial dysfunction (ED), prediction of cardiovascular events and mortality in patients with CKD [7–11].

The scientific community has sought to capitalize on the metabolic potential of the microbiome through probiotics, prebiotics, xenobiotics, nutritional modifications and genetically engineered bacteria, with varying levels of success [12–14]. Preliminary evidence suggests that the modulation of the intestinal microbiota of CKD patients by oral supplementation with pre/probiotics or their combination (synbiotics), may decrease uremic toxins and inflammation, consequently improving endothelial function [15, 16]. Fructooligosaccharide (FOS), a prebiotic, has been widely recognized for its effects on modulating

KEY LEARNING POINTS

What is already known about this subject?

- Inflammation and oxidative stress have been linked to increased cardiovascular risk in chronic kidney disease (CKD);
- gut microbiome-derived uremic toxins play a role in endothelial dysfunction (ED), prediction of cardiovascular events and mortality in patients with CKD; and
- studies suggest that the modulation of the intestinal microbiota of CKD patients by oral supplementation with pre/probiotics or symbiotic, may decrease uremic toxins and inflammation, and consequently, improving endothelial function.

What this study adds?

- Fructooligosaccharide (FOS) therapy lowers plasma interleukin 6 significantly in non-diabetic CKD patients;
- the reduction in treatment-induced *p*-cresyl sulfate did not reach statistical significance, but FOS therapy seems to preserve endothelial function as measured by flow-mediated dilatation in CKD patients with less severe ED.

What impact this may have on practice or policy?

- Supplementation with prebiotic is inexpensive and has potential therapeutic impact on the preservation of endothelial function and consequent decreasing in cardiovascular risk in nondialysis CKD patients.

the microbiota, and promotion of growth, stabilization and proliferation of bacteria beneficial to the maintenance of mucosal integrity of the gastrointestinal tract [17, 18]. Although prebiotic treatments are promising, safe and inexpensive, there is scarce information on their effects in the management of CKD patients. This trial aimed to evaluate the effect of the prebiotic FOS on endothelial function and arterial stiffness in nondialysis CKD patients.

MATERIALS AND METHODS

This is a predefined secondary analysis of a double-blind randomized controlled trial. Part of the study design and results were previously reported [19]. This trial was conducted for 3 months including patients with CKD Stages 3b–5 non-dialysis followed at the outpatient clinic of the Federal University of São Paulo. The inclusion criteria were as follows: age between 18 and 80 years , and estimated glomerular filtration rate (eGFR) between 45 and 15 mL/min/1.73 m^2. We excluded those with diabetes mellitus, malignancy, liver disease, autoimmune diseases, congestive heart failure Classes III/IV, HIV and history of gastrointestinal disease. Other exclusion criteria include use of phosphorus binders, laxatives or prebiotics, probiotics, synbiotics, antibiotics, immunosuppressive and/or anti-inflammatory drugs during 3 months before the enrollment. Previous cardiovascular disease (CVD) was defined as the presence of myocardial infarction, heart failure, angina pectoris, surgical procedures for angina, coronary/peripheral artery disease or stroke.

The study was approved by the Ethical Advisory Committee of the Federal University of São Paulo and registered in ClinicalTrials.gov (NCT02364869). Written informed consent was obtained from all patients.

Study protocol

Briefly, patients were randomized 1:1 to either the prebiotic (FOS, NutraFloraVR, Ingredion, USA) or the placebo (maltodextrin, Mor-Rex, Ingredion, USA) group over a 3-month period. The randomization was computer-generated in blocks of six participants stratified by gender and eGFR. Both supplements had the same characteristics regarding taste, odor and texture, and the participants were advised to take them diluted in water. The daily dose of 12 g was divided into two meals taken after lunch and dinner. During the follow-up patients were evaluated by a doctor and dietitian at baseline, 45 days and 3 months to assess clinical status, dietary monitoring assessed by a 3-day food record, adherence (sachet count) and presence of side effects. All patients received a routine dietary counseling based on 0.6–0.8 g/kg/day of protein intake, low sodium intake and to decrease potassium and phosphorus intake when necessary. The patients were encouraged to maintain stable protein and fiber intake, and not to use laxatives, other prebiotics and/or probiotics during the follow-up.

The subjects underwent an assessment of flow-mediated dilatation (FMD) and pulse wave velocity (PWV) at baseline and at the end of the study. Brachial artery reactivity was evaluated according to the International Brachial Artery Reactivity Task Force guidelines [20]. Briefly, the brachial artery was identified just above the antecubital fossa and was studied and performed using a high-resolution ultrasound system (Sequoia Echocardiography System, version 6.0, Acuson, Siemens, Vernon, CA, USA) equipped with a multifrequency linear transducer (7–12 MHz). We determined the changes of FMD and nitrate-mediated dilatation following physical and pharmacological stimulation, respectively, as previously described [21]. PWV was performed using the Complior SP equipment (Artech Medical, Pantin, France) [22]. Both brachial reactivity and PWV studies were carried out by the same examiner, who was blinded to the group allocation.

In addition, routine chemistry and lipid profile were measured in blood samples collected in a fasting state. C-reactive protein, interleukin-6 (IL-6) and serum stromal cell-derived factor-1 alpha were measured by enzyme immunoassay (Elabscience, Wuhan, Hubei, China). Serum NO was estimated by chemiluminescence, using NO Analyzer (NOATM 280, Sievers Instruments, Inc., Boulder, CO, USA). Serum total concentrations of PCS and IS were quantified by high-performance liquid chromatography with fluorescent detection [23]. Urinary concentration of TMAO was measured by capillary electrophoresis (model 7100, Agilent Technologies, Inc., Santa Clara, CA,

USA) coupled to a model 6430 triple-quadrupole mass spectrometer (Agilent Technologies, Santa Clara, CA, USA) with an electrospray ionization source [24]. Proteinuria was determined in 24-h urine and GFR was estimated by the CKD Epidemiology Collaboration equation.

Statistical analysis

Continuous variables were expressed as mean and standard deviation, median and interquartile range, or frequencies (proportion) as appropriated. The Kolmogorov–Smirnov statistical test was used to investigate the normal distribution of data. Comparisons of continuous variables between groups were performed using Student's *t*-test and the Mann–Whitney U-test, and within groups using Student's *t*-test or Wilcoxon, for normal and skewed data, respectively. Comparisons of proportions were performed using chi-squared analysis, Fischer's exact or McNemer tests, as appropriate. The effect of the intervention on the endothelial function and arterial stiffness was evaluated by the generalized estimation equation (GEE), considering the normal distribution of the dependent variables, and using group and time (before and after intervention) as factors. In the exploratory analysis, GEE was performed in patients with less severe ED defined as FMD $\geq$2.2% (value corresponding to the FMD median of the population at baseline). The GEE models were adjusted for age, use of β-blocker drugs, eGFR, sodium, bicarbonate, ionized calcium, IL-6 and PCS. The value of $P < 0.05$ was established for statistical significance and the analyses were conducted in Statistical Package for Social Sciences software version 20.0 (SPSS software, Chicago, IL, USA) for Windows. The sample size was calculated according to the main objective of the study, as previously described [19].

RESULTS

From the 50 patients recruited, four were discontinued from the protocol due to the need of dialysis therapy ($n = 2$) or personal reasons ($n = 2$). Therefore, the present study included 46 patients who completed the follow-up. Demographic and clinical data are shown in Table 1. The two groups were comparable except that the prebiotic group was older (P = 0.04). The laboratory and vascular parameters at baseline and third month are depicted in Table 2. In the prebiotic group, there was a significant increase in serum sodium (P = 0.005), and a decrease in high-density lipoprotein (HDL)-cholesterol (P = 0.002), ionized calcium (P = 0.004), albumin (P = 0.001), alkaline phosphatase (P = 0.006) and IL-6 (P = 0.04) during the study. Regarding uremic toxins, there was a trend toward a decrease in PCS levels (P = 0.07). In the placebo group, there was a decrease in ionized calcium (P = 0.01) and an increase in HDL-cholesterol (P = 0.04) during the study. Higher serum sodium (P = 0.003) and bicarbonate (P = 0.02) values were observed in the prebiotic group compared with the placebo group at the end of the study (Figure 1). Changes in the laboratory and vascular parameters are depicted in Supplementary data, Table S1. There was no difference in the dietary pattern of the two groups.

In multivariate analysis, by adjusted GEE model, there was no effect of FOS treatment on FMD (group effect P = 0.356; time effect P = 0.460; interaction P = 0.253; Figure 2A) and on PWV (group effect P = 0.089; time effect P = 0.272; interaction P = 0.714; Figure 2B). In an exploratory analysis that included only patients with less severe ED at baseline ($n = 21$, FMD $\geq$2.2%; Figure 3A), there was significant higher FMD at the third month in the prebiotic group (group effect P = 0.135; time effect P = 0.012; interaction P = 0.002; Figure 3B). The comparison between groups of this subpopulation is described in Supplementary data, Tables S2 and S3.

In addition, we compared FMD values at the baseline and at the end of the study in patients with CKD Stages 3b–4 and 5, regardless of treatment group. There was no difference in the FMD between the two groups at baseline (2.8 ± 3.3 versus 3.0 ± 4.2%, Stages 3b–4 and 5, respectively, P = 0.52) or at the end of the study (3.7 ± 3.9 versus 5.8 ± 3.6%, P = 0.10)

The overall medication compliance in both groups was similar [median intake 96.4% (86.7–98.7) versus 94.4% (91.5–99.9) for placebo and prebiotic, respectively P = 0.82]. Only three patients from the placebo and one patient from the prebiotic group consumed <80% of the sachets offered. Only one patient in the prebiotic group complained about abdominal discomfort during the second week of the treatment. For this patient, the supplement dose was decreased to 6 g/day and then progressively increased to 12 g/day by the fourth week.

Table 1. Comparison of demographic and clinical characteristics at baseline of prebiotic and placebo groups

	Prebiotic	Placebo	P-value
	23	23	
Male, %	12 (52)	12 (52)	1.00
Age, years	61.9 ± 11.4	53.4 ± 16.0	0.04
Non-White, %	17 (74)	15 (65)	0.40
Alcohol consumption, %	7 (30)	6 (26)	0.74
Previous smoking, %	8 (35)	5 (22)	0.33
Physical activity, %	9 (39)	7 (30)	0.54
Hypertension, %	22 (97.8)	23 (100)	0.50
Dyslipidemia, %	19 (83)	18 (78)	0.50
Prior CVD, %	2 (9)	3 (13)	0.50
Hypothyroidism, %	3 (13)	6 (26)	0.23
Current medications			
Angiotensin-converting enzyme inhibitor, %	10(44)	6 (26)	0.22
Angiotensin II receptor blockers, %	8 (35)	11 (48)	0.37
Diuretics, %	18 (78)	17 (74)	0.73
Calcium channel antagonist, %	12 (52)	11 (48)	0.77
Beta blocker, %	6 (26)	12 (52)	0.07
Acetylsalicylic acid, %	4 (17)	3 (13)	0.50
Statins, %	19 (83)	16 (70)	0.30
Systolic blood pressure, mmHg	130 (120–140)	120 (120–150)	0.85
Diastolic blood pressure, mmHg	80 (80–90)	80 (80–90)	0.57
Body mass index, kg/m^2	27.1 ± 5.3	28.1 ± 4.9	0.51

Data are presented as mean ± SD, median (interquartile range) or *n* (%).

DISCUSSION

In a double-blind controlled trial involving 46 nondiabetic CKD patients, we examined the effect of the prebiotic treatment on endothelial function and arterial stiffness in CKD patients not on dialysis, and noted a significant reduction in plasma IL-6 levels and a trend toward reduction in PCS levels. We did not

Table 2. Biochemical parameters of both groups at baseline and at the end of the study

	Prebiotic ($n = 23$)			Placebo ($n = 23$)			Comparison between groups	
	Baseline	Third month	P-value	Baseline	Third month	P-value	P^{T0}	P^{T3}
Creatinine, mg/dL	2.83 (2.27–3.38)	2.96 (2.00–3.53)	0.25	2.90 (2.35–3.63)	2.83 (2.34–3.75)	0.73	0.46	0.93
Urea, mg/dL	89 (71–115)	86 (64–117)	0.52	89 (66–120)	101 (70–113)	0.46	0.90	0.77
Sodium, mEq/L	139 ± 2.4	140 ± 1.6	0.005	138 ± 2.2	138 ± 2.2	0.60	0.57	0.003
Potassium, mEq/L	4.7 ± 0.5	4.7 ± 0.6	0.76	4.7 ± 0.6	4.7 ± 0.6	0.98	0.96	0.81
eGFR, mL/min	21.6 ± 8.4	21.8 ± 8.8	0.81	21.0 ± 6.1	21.2 ± 7.0	0.77	0.78	0.80
Proteinuria, g/24 h	0.66 (0.29–1.44)	0.64 (0.30–1.77)	0.15	0.63 (0.19–1.60)	0.71(0.15–2.31)	0.22	0.48	0.88
Hemoglobin, g/dL	12.7 ± 1.5	12.5 ± 1.4	0.15	13.0 ± 2.0	13.1 ± 1.8	0.85	0.57	0.19
Glucose, mg/dL	86 (79–94)	85 (80–97)	0.71	85 (81–92)	84 (78–98)	0.99	0.82	0.66
Total cholesterol, mg/dL	161(127–191)	168(143–197)	0.11	178(141–212)	172(152–213)	0.41	0.41	0.41
HDL-cholesterol, mg/dL	47 (41–59)	45 (36–53)	0.002	45 (41–53)	51 (38–56)	0.04	0.52	0.32
LDL-cholesterol, mg/dL	87 ± 35	95 ± 31	0.17	102 ± 25	102 ± 29	0.74	0.12	0.48
Triglycerides, mg/dL	135 (93–177)	136 (100–187)	0.37	151 (115–235)	139 (102–184)	0.15	0.09	0.83
Bicarbonate, mmol/L	24.9 ± 3.9	24.5 ± 2.6	0.72	23.3 ± 3.9	22.5 ± 3.1	0.41	0.18	0.02
Albumin, g/dL	4.40 ± 0.39	4.20 ± 0.27	0.001	4.35 ± 0.31	4.30 ± 0.31	0.43	0.65	0.12
Ionized calcium, mmol/L	1.28 ± 0.08	1.19 ± 0.15	0.004	1.31 ± 0.82	1.21 ± 0.17	0.01	0.24	0.54
Phosphorus, mg/dL	3.73 ± 0.67	3.8 ± 0.73	0.64	3.55 ± 0.83	3.8 ± 0.61	0.15	0.41	0.98
Intact-parathormone, pg/mL	201 (115–353)	269 (129–347)	0.71	226 (147–389)	205 (127–400)	0.19	0.40	0.67
Alkaline phosphatase, U/L	69 (55–104)	67 (53–87)	0.006	73 (61–93)	79 (60–96)	0.74	0.98	0.37
25(OH) vitamin D, ng/mL	27 (20–30)	26 (22–31)	0.27	26 (21–29)	27 (23–32)	0.03	0.97	0.45
CRP, mg/dL	1.11 ± 2.76	0.40 ± 0.42	0.59	0.42 ± 0.49	0.32 ± 0.42	0.15	0.84	0.37
IL-6, pg/mL	3.4 ± 2.1	2.6 ± 1.4	0.04	3.5 ± 2.3	3.1 ± 2.2	0.71	0.80	0.65
Serum IS, mg/L	6.3 ± 4.4	6.4 ± 4.2	0.91	5.5 ± 2.8	5.7 ± 2.9	0.81	0.91	0.80
Serum PCS, mg/L	55.4 ± 38.1	43.1 ± 32.4	0.07	51.5 ± 29.4	52.9 ± 30.7	0.63	0.70	0.23
Urinary TMAO, µmol/L	176.0 ± 78.7	200.6 ± 179.9	0.80	169.6 ± 87.1	188.9 ± 108.4	0.48	0.44	0.75
SDF-1α, pg/mL	308 (195.5–420)	272 (171–509.5)	0.68	213 (18–520.5)	260 (65–426.5)	1.00	0.38	0.27
Serum NO, µM/L	361.9 ± 217.3	312.1 ± 127.3	0.57	301.9 ± 133.6	330.2 ± 192.9	0.18	0.27	0.71
PWV, m/s	8.6 ± 1.9	8.7 ± 1.6	0.63	7.7 ± 1.4	7.9 ± 1.6	0.58	0.14	0.08
FMD, %	2.95 ± 3.04	4.30 ± 4.70	0.20	2.93 ± 3.84	2.64 ± 3.50	0.82	0.97	0.15

Data are presented as mean ± SD or median (interquartile range). LDL, low-density lipoprotein; CRP, C-reactive protein; SDF-1α, stroma-derived factor-1 alfa.

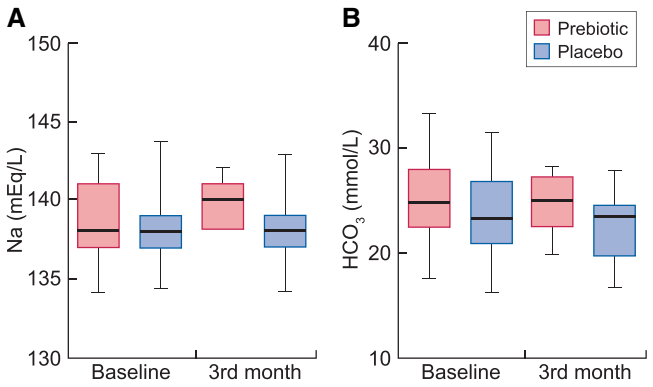

FIGURE 1: Graph showing the comparison of serum sodium (**A**) and bicarbonate (**B**) levels in the prebiotic and placebo groups at baseline and the third month.

observe a significant difference in FMD and PWV between the two groups, but in a subset of patients with less severe ED FMD remained stable in the prebiotic group, but it decreased in the placebo group.

Experimental and clinical studies have demonstrated controversial effects of microbiota modulating agents on the endothelial function, cardiovascular markers, inflammation and CKD progression [25–30]. No superiority has been described among these agents, while the prebiotics and fibers found in some food have the advantage of stimulating the individual's own microbiota [31]. Of note, few studies evaluating the effect of prebiotics on endothelial function of CKD patients have been performed. In an experimental study, Catry *et al.* demonstrated an enhancement of endothelial function after prebiotic administration in mice [27]. In a clinical trial, Cosola *et al.* observed an increase of FMD after a prebiotic supplementation in healthy individuals [25]. Our data suggest that a protective role for FOS in the endothelium may be implicated. In fact, this prebiotic seemed to attenuate the deterioration of endothelial function in a less damaged endothelium subgroup. It is important to mention that the patients in the less damaged endothelium subgroup who received FOS were older than those of the placebo group. This fact could diminish the expected magnitude of the endothelial response to FOS. On the other hand, the worsening of FMD in the placebo group contrasting with the maintenance of this parameter in the prebiotic group may corroborate with this assumption. One could speculate that the lack of effect of FOS in the whole population was due to the presence of patients with severely damaged endothelium, who were unable to respond to the intervention.

Studies in the literature have suggested that microbiota-modulating agents could bring about the reduction of inflammation and uremic toxins, leading to an improvement of the endothelial function [32, 33]. In fact, Vaziri *et al.* observed that the consumption of prebiotics decreased inflammation and oxidative stress in nephropathic rats [34]. Similarly, we observed in the prebiotic group a significant decrease in serum IL-6, a marker of inflammation. This could suggest a potential role of

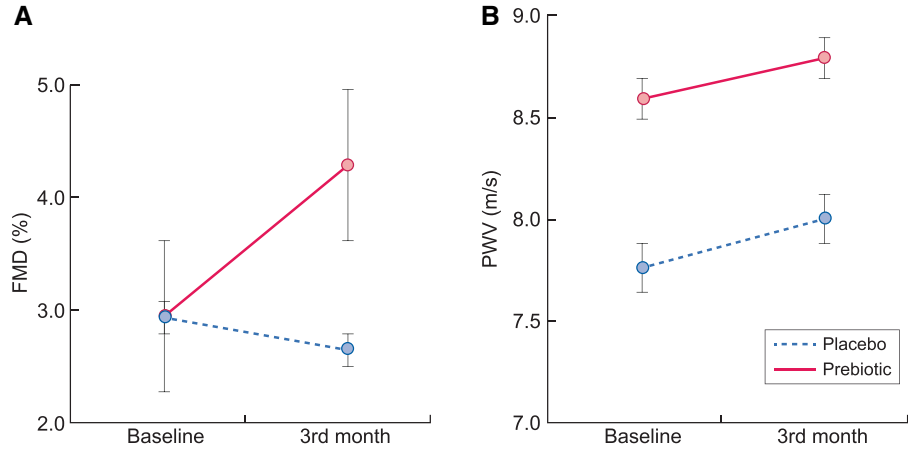

FIGURE 2: (**A**) FMD (group effect P = 0.356; time effect P = 0.460; interaction P = 0.253); (**B**) PWV (group effect P = 0.089; time effect P = 0.272; interaction P = 0.714) in the prebiotic and placebo groups.

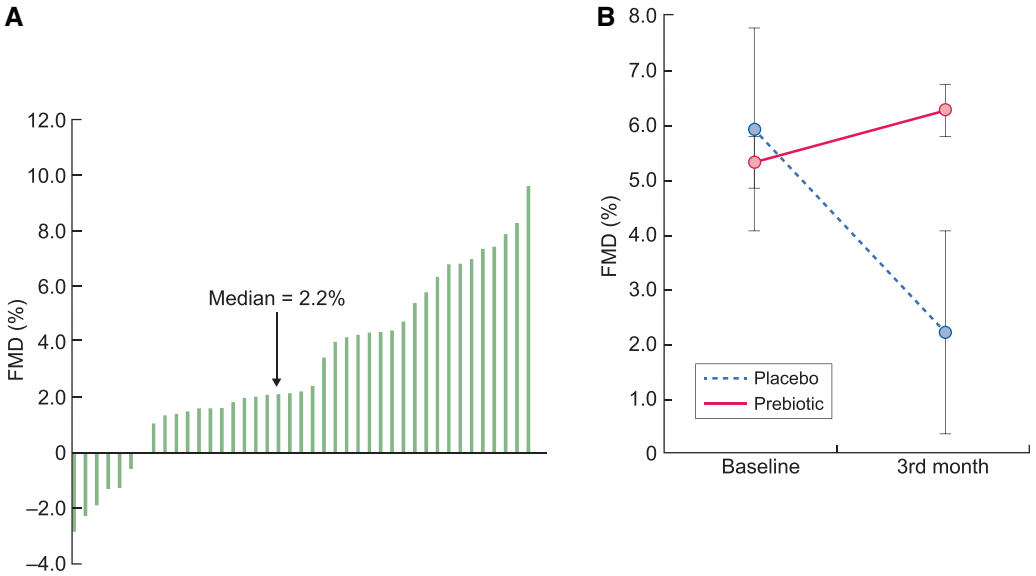

FIGURE 3: (**A**) Behavior of FMD variable at baseline (median 2.2%). (**B**) FMD in the prebiotic and placebo groups (group effect P = 0.135; time effect P = 0.012; interaction P = 0.002).

FOS in decreasing inflammation, consequently leading to a protective effect on endothelial function in these patients. Moreover, two experimental studies have demonstrated that dietary prebiotics were able to decrease IS serum levels [35, 36]. In addition, clinical studies have demonstrated that prebiotic therapy decreased PCS levels in predialysis and dialysis CKD patients [26, 37]. In our study, a downward trend of PCS after the prebiotic intervention was observed, but no direct association between this change and the endothelial function amelioration could be demonstrated.

Some unexpected results without plausible explanations were observed in the prebiotic group. First, an increase in serum sodium levels was observed, probably reflecting a greater fecal excretion of water due to changes in the intestinal habits related to the use of FOS [38]. One could speculate that the increase in sodium levels might have impaired the effect of FOS in this study. While some recent clinical studies have shown a direct role of sodium in the ED regardless of blood pressure [39, 40], we could not observe any effect of sodium on FMD. Moreover, serum sodium remained within the normal levels and nonworsening of blood pressure was observed. Second, although a decrease of inflammation has been observed, there was a slight decrease in the serum albumin levels. Since albumin is an acute negative phase protein [41, 42] we would not expect that paradoxical result. Third, there was a decrease in serum levels of alkaline phosphatase, which could point to a potential beneficial effect of FOS. Accordingly, a recent study including essential hypertensive patients demonstrated that those with lower levels of alkaline phosphatase had less severe ED [43]. The reason for this potential relationship remains to be clarified. Finally, the last unexpected result was the observation of a decrease in serum HDL levels in CKD patients whose kidney function remained stable. Moreover, an increase of HDL under prebiotic supplementation was demonstrated in non-CKD patients [44].

Some limitations of this study may be listed, such as the relatively small sample size, short intervention time and low dose of FOS administration. We also did not collect detailed dietary information during the study period, which could have a

significant impact on the microbiome and their metabolites. On the other hand, this study has some strengths, including the use of an accessible, with few or no side effects, therapy in CKD nondialysis patients. In addition, this study suggests that early FOS therapy could be helpful in preserving endothelial function in this population.

CONCLUSIONS

In this double-blind randomized controlled trial, we found that FOS therapy lowers plasma IL-6 significantly in nondiabetic CKD patients. The reduction in treatment-induced PCS did not reach statistical significance. In the entire cohort, FMD and PWV were unaffected by prebiotic treatment, but FOS therapy seems to preserve endothelial function as measured by FMD in CKD patients with less severe ED. These encouraging signals from this pilot study should be rigorously examined in large-scale well-designed studies.

SUPPLEMENTARY DATA

Supplementary data are available at ndt online.

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CONFLICT OF INTEREST STATEMENT

None declared.

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