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ORIGINAL ARTICLE

Amylose resistant starch (HAM-RS2) supplementation increases the proportion of *Faecalibacterium* bacteria in end-stage renal disease patients: Microbial analysis from a randomized placebo-controlled trial

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Abstract

Introduction: Many of the deleterious effects associated with chronic kidney disease (CKD) are secondary to the resultant systemic inflammation. The gut microbial changes caused by CKD are thought to perpetuate systemic inflammation. Therefore, strategies aimed at modulating the gut microbiota may be helpful in reducing complications associated with CKD. We hypothesized that supplementation with high-amylose maize resistant starch type 2 (HAM-RS2) would beneficially alter the gut microbiome and lead to lower levels of systemic inflammation.

Methods: A double-blind, parallel, randomized, placebo-controlled trial was performed comparing dietary supplementation of HAM-RS2 with placebo in patients with end-stage CKD. Fecal microbial data were obtained from a subset of patients after DNA extraction and 16s sequencing.

Findings: Supplementation of HAM-RS2 led to a decrease in serum urea, IL-6, TNFα, and malondialdehyde ($P < 0.05$). The *Faecalibacterium* genus was significantly increased in relative abundance following HAM-RS2 supplementation (HAM-RS2-Day 0: 0.40 ± 0.50 vs. HAM-RS2-Day 56: 3.21 ± 4.97 $P = 0.03$) and was unchanged by placebo (Control-Day 0: 0.72 ± 0.72 vs. Control-Day 56: 0.83 ± 1.57 $P = 0.5$).

Discussion: Supplementation of amylose resistant starch, HAM-RS2, in patients with CKD led to an elevation in *Faecalibacterium* and decrease in systemic inflammation. Microbial manipulation in CKD patients by using the prebiotic fiber may exert an anti-inflammatory effect through an elevation in the bacterial genera *Faecalibacterium.*

Keywords: Inflammation, microbiology, metabolism

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INTRODUCTION

Chronic kidney disease (CKD) is a worldwide health problem associated with various disease processes, including cardiovascular disease.[1] CKD results in systemic inflammation that mediates many of its adverse consequences.[2] The gastrointestinal tract has emerged as one of the major sources of CKD-associated systemic inflammation.[3] This is in part due to a profound transformation of the gut microbiome, a change that may disrupt the intestinal epithelial barrier and promote local and systemic inflammation. The generation of harmful microbial metabolites and the disruption of the intestinal epithelial barrier by dysbiotic microbial community contribute to inflammation[4] by facilitating entry of microbial and toxic gut luminal contents through the intestinal barrier and into systemic circulation.[5]

Short-chain fatty acids (SCFAs), particularly butyrate, produced from fermentation of complex carbohydrates by symbiotic microbes are essential nutrients for colonic epithelial cells and anti-inflammatory regulatory T lymphocytes.[6] Depletion of the gut's SCFA-producing microbial species can contribute to the disruption of the colonic epithelial barrier and subsequent inflammation. Supplementation with the prebiotic high-amylose maize resistant starch type 2 (HAM-RS2) fiber in CKD patients has been shown to attenuate oxidative stress, repair uremia-induced disruption of colonic epithelial tight junction,[7] and in murine models increase the population of SCFA-producing bacteria.[8] A recent double-blind, parallel, randomized, placebo-controlled trial conducted by our group demonstrated that HAM-RS2 supplementation significantly lowers plasma urea and potassium concentrations and reduces biomarkers of inflammation and oxidative stress in end-stage renal disease (ESRD).[9] Using fecal samples collected from a subgroup of patients enrolled in this trial, we sought to determine the effect of HAM-RS2 supplementation on specific SCFA-producing genera, namely *Bifidobacteria*, *Prevotella*, *Parabacteroides*, *Ruminococcus*, and *Faecalibacterium*, which are known to be abnormal in CKD patients, and have been shown to be altered by HAM-RS2 supplementation in CKD animals.[8]

METHODS

Patients

Twenty ESRD patients undergoing hemodialysis at Bahman Hemodialysis center in Tabriz, Iran, were enrolled in the study. Individuals with gastrointestinal disease, diabetes, active inflammatory disorders, infections, and malignancy and patients who had been treated with antibiotics within 3 months before the enrollment in the study were excluded. The underlying causes of ESRD in the study population included hypertensive nephrosclerosis in eight patients, glomerulonephritis in five, postrenal and urolithiasis in two, polycystic kidney disease in one, chronic pyelonephritis in one, and CKD of unknown etiology in three patients.

Patients were randomized to receive biscuits containing 20 g/d of HAM-RS2 (Ingredion ANZ Pty Ltd, Lane Cove, NSW, Australia), an insoluble, fermentable fiber, or regular wheat flour (placebo) during the first month and 25 g/d during the second month. Twenty subjects were chosen at random to be included in the subgroup of microbial analysis. After simple randomization, the HAM-RS2-treated group included six men and three women aged 53.8 ± 11.8 years and the placebo-treated group consisted of seven men and four women aged 57.6 ± 9 years. The trial was approved by the Human Subjects Institutional Review Board at the Tabriz University of Medical Sciences and was conducted in compliance with the Declaration of Helsinki. The study was registered on the Iranian Registry of Clinical Trials (IRCT2016062628644N1), and all participants provided informed consent prior to enrollment.

Laboratory tests

Blood samples were collected at the onset and the end of the study period and processed by Tabriz University Medical Center's central laboratory for determination of urea nitrogen, uric acid, calcium, phosphorus, and parathyroid hormone. In addition, plasma concentrations of TNFα and IL-6 were measured using ELISA kits purchased from the Bioassay Technology Laboratory, Shanghai Crystal Day Biotech Co Ltd. Malondialdehyde (MDA) level was measured using the thiobarbituric acid reactive substances method.

Microbial analysis

Fecal samples were stored and processed at Tabriz University Medical Center's central laboratory. Microbial DNA from the fecal samples was extracted by bead beating using 0.1 mm zirconia/silica beads (BioSpec) and then followed by DNA purification using the QIAamp DNA Stool Mini Kit (Qiagen Inc. Valencia, CA, USA). Total DNA from the fecal samples was extracted, and 16s DNA sequencing was performed as previously described.[10]

Table 1 Serum concentrations of cytokines and metabolites by treatment groups

	HAM-RS2 group		Control group	
	Before	After	Before	After
BUN (mg/dL)	57.90 ± 15.3	**49.20 ± 12.0**	60.60 ± 13.6	62.20 ± 12.4
Uric acid (mg/dL)	7.00 ± 0.89	6.73 ± 1.25	7.65 ± 0.82	7.1 ± 1.23
Calcium (mg/dL)	8.56 ± 0.50	8.81 ± 0.12	8.44 ± 0.56	8.63 ± 0.48
Phosphate (mg/dL)	4.01 ± 0.75	4.50 ± 0.64	4.98 ± 1.07	5.37 ± 1.02
iPTH (ng/mL)	289.40 ± 145.5	365.20 ± 212.0	232.60 ± 113.8	214 ± 92.2
IL-6 (ng/mL)	157.52 ± 83.1	**118.52 ± 81.6**	152.76 ± 95.0	185.10 ± 103.2
TNFα (ng/mL)	318.69 ± 168.7	**261.26 ± 164.1**	304.26 ± 215.1	372.54 ± 215.1

Significant values are given in bold.

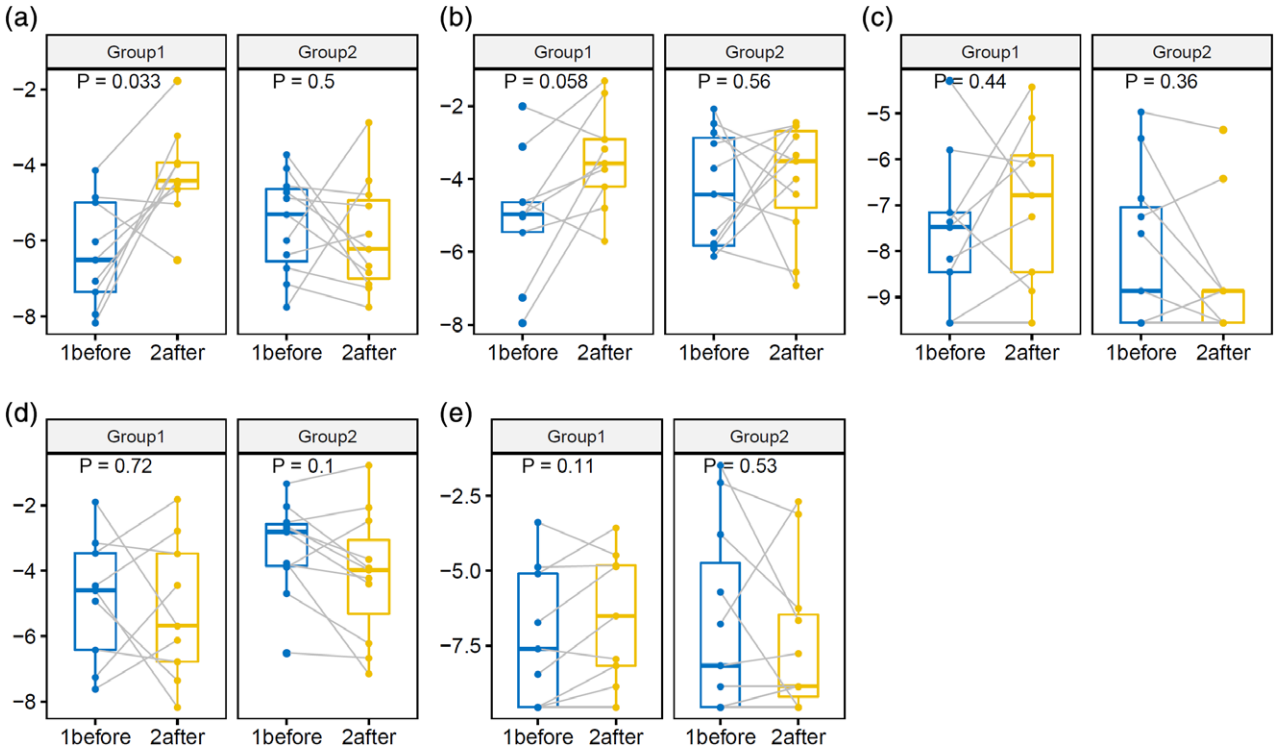

Figure 1 The relative frequencies of different bacterial genera before and after dietary supplementation with the prebiotic fiber HAM-RS2: (a) g—*Faecalibacterium*; (b) g—*Ruminococcus*; (c) g—*Parabacteroides*; (d) g—*Bifidobacterium*; and (e) g—*Prevotella*

Statistical analysis

The microbial analysis portion of this study represents an exploratory subgroup analysis of a larger trial. Prior to this work, little data existed to educate a power calculation on the effect of HAM-RS2 in humans with CKD. Data are presented as mean ± SD. Statistical analysis was performed in GraphPad and STAMP. Paired data were compared using the Wilcoxon signed-rank nonparametric test. Microbial analysis was processed using QIIME software.

RESULTS

Clinical, metabolic, and inflammatory characteristics

The demographic characteristics of participants between the treatment groups did not differ in terms of age, gender, body weight, height, body mass index, blood pressure, or duration of hemodialysis. No significant difference was found in baseline serum concentrations of

Table 2 Relative frequency of important short-chain fatty acid producing genera known to be altered in CKD and affected by HAM-RS2

General	Control-Day 0 ± SD (%)	Control-Day 56 ± SD (%)	P	HAM-RS2-Day 0 ± SD (%)	HAM-RS2-Day 56 ± SD (%)	P
Faecalibacterium	0.72 ± 0. 40	0.84 ± 1.57	0.50	0.40 ± 0.50	3.21 ± 4.97	**0.03**
Parabacteroides	0.12 ± 0.21	0.06 ± 0.14	0.36	0.21 ± 0.42	0.28 ± 0.37	0.44
Bifidobacteria	6.85 ± 7.24	6.99 ± 12.94	0.10	2.86 ± 4.55	3.07 ± 5.09	0.72
Ruminococcus	1.49 ± 1.03	1.02 ± 1.22	0.08	5.61 ± 13.40	4.88 ± 10.14	0.48
Prevotella	3.48 ± 7.10	1.07 ± 2.23	0.53	0.55 ± 1.04	0.64 ± 0.88	0.11

P values calculated using the Wilcoxon signed-rank test. Significant values are given in bold.

urea nitrogen, creatinine, phosphate, calcium, iPTH, IL-6, TNFα, or MDA between the two groups. The corresponding values obtained at the end of the study remained unchanged in the placebo-treated group. However, serum urea, IL-6, TNFα, and MDA were significantly reduced in the HAM-RS2-treated group (Table 1).

Fecal bacterial 16s sequencing

Bacterial analysis revealed a significant increase in the abundance of *Faecalibacterium* genus in the HAM-RS2-treated group (HAM-RS2-Day 0: 0.40 ± 0.50 vs. HAM-RS2-Day 56: 3.21 ± 4.97 P = 0.03) group and remained unchanged in the control group (Control-Day 0: 0.72 ± 0.72 vs. Control-Day 56: 0.83 ± 1.57 P = 0.5). The four remaining genera of bacteria examined, namely *Bifidobacteria*, *Prevotella*, *Parabacteroides*, and *Ruminococcus*, were not significantly changed by HAM-RS2 supplementation (Figure 1b–e and Table 2), although there was a trend toward an increase in *Prevotella* (Figure 1a and Table 2).

DISCUSSION

Supplementation of HAM-RS2 in our ESRD patients resulted in a significant increase in the fecal population of *Faecalibacterium*, a highly abundant genus in the healthy human colon, known to be depleted in CKD patients.[11,12] It is an extremely oxygen-sensitive bacteria, and therefore approaches utilizing the prebiotic fiber to increase its prevalence are prudent, rather than a more direct probiotic approach. The anti-inflammatory mechanism of *Faecalibacterium* is not known; however, production of the SCFA butyrate plays a vital role in its protective property by modulating the immune system, upregulating epithelial tight junctions, and preserving the intestinal epithelium.

As shown in our previous study, HAM-RS2-treated patients exhibited a significant reduction in serum concentrations in blood urea nitrogen (BUN).[9] Similarly, in a recent study, Sirich et al. found a reduction of urea levels in their ESRD patients supplemented with amylose for 6 weeks.[13] The reduction in urea level with amylose supplementation may, in part, be due to SCFAs' trapping of ammonia, which was derived from hydrolysis of urea by urease-possessing colonic bacteria. Trapping of ammonia by SCFAs may prevent its absorption and conversion back to urea by the liver.

Caution must be taken in interpreting the results of this project given its limitations. Due to logistic and practical reasons, stool from only 20 patients was sequenced, decreasing the power to detect differences in bacterial populations. Furthermore, our hypothesis focused on five specific genera, which ameliorates the problem of false discovery, but does not present a complete overview of the microbial environment. The complete set of sequencing data will be made available online to address this issue.

In conclusion, the finding that amylose resistant starch supplementation decreased inflammation in CKD patients suggests a role for prebiotic supplementation in this population. Microbial analysis indicates that an elevation in *Faecalibacterium* may be central to the mechanism by which this anti-inflammatory effect is mediated. Future work regarding microbial manipulation in CKD patients should acknowledge and explore the possible role of *Faecalibacterium* in ameliorating the disease process.

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