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The Therapeutic Potential of Resistant Starch in Modulation of Insulin Resistance, Endotoxemia, Oxidative Stress and Antioxidant Biomarkers in Women with Type 2 Diabetes: A Randomized Controlled Clinical Trial

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Key Words
Resistant starch · Insulin · Endotoxemia · Malondialdehde · Antioxidant · Type 2 diabetes

Abstract
Aims: This trial aims to determine the effects of resistant starch (RS) subtype 2 (RS2) on glycemic status, metabolic endotoxemia and markers of oxidative stress. ***Methods:*** A randomized, controlled, parallel-group clinical trial group of 56 females with type 2 diabetes mellitus (T2DM) was divided to 2 groups. The intervention group (n = 28) and control group (n = 28) received 10 g/day RS2 or placebo for 8 weeks, respectively. Fasting blood samples were taken to determine glycemic status, endotoxin, high sensitivity C-reactive protein (hs-CRP), malondialdehyde (MDA), total antioxidant capacity (TAC), antioxidant enzymes concentrations as well as uric acid at baseline and after the intervention. ***Results:*** After 8 weeks, RS2 caused a significant decrease in the levels of MDA (–34.10%), glycosylated hemoglobin (–9.40%), insulin (–29.36%), homeostasis model of insulin resistance (–32.85%) and endotoxin (–25.00%), a significant increase in TAC (18.10%) and glutathione peroxidase (11.60%) as compared with control. No significant changes were observed in fasting plasma glucose, quantitative insulin sensitivity check index, hs-CRP, superoxide dismutase, catalase and uric acid in the RS2 group as compared with the control group. ***Conclusion:*** Supplementation with RS2 may be improved glycemic status, endotoxemia and markers of oxidative stress in patients with T2DM.

Introduction

Type 2 diabetes mellitus (T2DM) is a health problem in developing and developed countries [1]. In Iran, the prevalence of known T2DM and impaired fasting glu-

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cose was reported 16.3 and 11.9%, respectively [2]. T2DM is a metabolic disease that is characterized by hyperglycemia together with insulin resistance, oxidative stress [3] and alterations of gut microbiota [4]. Recently, altered gut microbiota has attracted researchers' attention. Reportedly, the Firmicutes/Bacteroidetes ratio increases whereas Bifidobacterium and *Faecalibacterium prausnitzii* decrease in patients with T2DM, resulting in an increase in serum lipopolysaccharides derived from outer membranes of the cell wall of gram-negative in the blood known as metabolic endotoxemia. Metabolic endotoxemia is related to the insulin resistance, oxidative stress and inflammatory pathways [4]. Moreover, elevated oxidative stress in these patients promotes the growth of endotoxin producer by suppressing the signal transduction of gut-associated lymphocytes [5]. Regarding the role of metabolic endotoxemia, in pathogenesis of T2DM and low fiber intake (16.7 g/day) in Iranian diabetic patients [6], it seems that dietary intervention with prebiotic fibers such as resistant starch (RS) improves the potential consequence of diabetes complications by elevating dietary fiber intake and lowering the metabolic endotoxemia [7, 8].

RS is a homo-polysaccharide including numbers of monosaccharide linked together with α-glucose (1 → 4) and (1 → 6) bonds. RS is classified into 5 subtypes (RS1–RS5) based on their structures and sources. RS1 is physically inaccessible to digestive enzymes because it is trapped within the food matrix such as partly milled grains and seeds. RS2 comprises native, uncooked granules like raw potato or banana starch RS2 that is unique because it is stable in most cooking operations. RS3 is retrograded starch. Finally, RS4 is chemically modified starch while RS5 is the amylose-lipid complex starch. RS is defined as the starch fraction that escapes digestion in the small gut and can be fermented in the colon by selective microbiota promoting the growth of Lactobacilli and Bifidobacteria, increasing the viability of probiotics [9] and exerting their health-related benefits by producing short chain fatty acids (SCFAs) such as butyrate (mainly), propionate and acetate [10]. It has been estimated that mean RS intake in developed societies is far below [11] than the recommended contents to confer health benefits (>15 g/day) [9] that can be modified with RS supplementation.

Results from studies on supplementation with RS have shown contrary effects of consumption on glycemic status [12–14] and biomarkers of oxidative stress [12, 15]. Moreover, limited studies have assessed the effects of RS on metabolic endotoxemia [16]. Consumption of rice containing RS (6.5 g/day for 4 weeks) in prediabetic patients improved the glycemic status and oxidative markers [12]. Bodinham et al. [14] indicated that supplementation with large doses (40 g/day for 12 weeks) of RS in patients with T2DM decreased postprandial glucose concentrations with no effect on insulin sensitivity. Due to limited number of studies on metabolic endotoxemia and contrary results on the effects of different doses and types of RS on metabolic parameters, this trial was aimed to assess the clinical efficacy of RS2 on metabolic parameters such as glycemic status, endotoxin, high sensitivity C-reactive protein (hs-CRP), malondialdehyde (MDA), total antioxidant capacity (TAC) and antioxidant enzymes concentrations in women with T2DM.

Materials and Methods

Patients

In this randomized, triple-blind, placebo-controlled trial, 70 females with T2DM who were between 30 and 65 years were voluntarily recruited from the Iran Diabetes Society and the Endocrinology and Metabolism Clinics of Tabriz University of Medical Sciences. Demographic information was collected by a questionnaire at the beginning of the trial. Patients were enrolled if they had T2DM for >6 months, a body mass index (BMI) of >25 kg/m^2 for the past 3 months and had a stable diet, physical activity level and oral anti-diabetic medication use during the trial. The participants were excluded if they had a history of cardiovascular disease, gastrointestinal, pancreatic; thyroid, renal, or liver disturbance; if they were smokers, pregnant or lactating; if they were currently taking prebiotics, probiotics, antibiotics, antacids, alcohol, antidiarrheal, anti-inflammatory or laxative medicines; if they had taken lipid-lowering medications within 2 weeks before the intervention or during the intervention and if they had a daily fiber intake of >30 g.

Intervention

Patients were randomly divided to two groups. The randomization was performed based on block randomization procedure of size 4. To match the groups, each block had to involve the subjects with the same categorizes of BMI and age. The allocation sequence was randomly generated by Random Allocation Software. The intervention group received 10 g/day RS supplement (Hi-maize 260, National Starch LLC), and the control group received similar amounts of maltodextrin as a placebo (Jiujiang-Hurirong Trade Co., China) for 8 weeks. Both the RS and the maltodextrin were powdered and were given to the volunteers in similar packages. The study was approved by the Ethical Committee of the Tabriz University of Medical Sciences and registered on the Iranian Registry of Clinical Trials website (www.irct.ir/, IRCT201110293253N4).

Sample size was determined on the basis of a primary outcome of a change in MDA level [17]. By aiming for a confidence level of 95 and 90% power and 20% drop out, the total number of 28 pa-

tients in each group was assigned. The primary outcomes of the study were fasting plasma glucose (FPG), glycosylated hemoglobin (HbA1c), insulin, homeostasis model of insulin resistance (HOMA-IR), quantitative insulin sensitivity check index (QUICKI), endotoxin, hs-CRP, MDA, TAC, superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase and uric acid while the secondary outcomes were weight and energy changes.

Measurements of Body Weight and Dietary Intake

Body weight and height were measured at baseline and at the end of the trial. BMI was calculated as the weight (kg) divided by the square of the height (m). Nutrient intake was collected using 3-day dietary records questionnaire at baseline and at the end of the trial and was analyzed using Nutritionist 4 software (First Databank Inc., Hearst Corp., San Bruno, Calif., USA).

Biochemical Measurements

Venous blood samples (10 ml) were collected at baseline and at the end of the trial after an overnight fast and put into different Vacutainer tubes. One of the tubes contained ethylenediaminetetraacetic acid for the measurement of HbA1c, and the other tube contained heparin for the measurement of the levels of SOD, GSH-Px and catalase. Plasma was used for determination of FPG, insulin, hs-CRP, endotoxin, uric acid, MDA and TAC. FPG was measured via the enzymatic method with the use of an Abbot Model Aclyon 300 USA autoanalyzer with kits from Pars-Azmone (Tehran, Iran). Uric acid concentrations were assayed using uric acid kit (Pars Azmoon Inc., Tehran, Iran). HbA1c was determined in whole blood using an automated high-performance liquid chromatography analyzer with commercially available kits (Bio-Rad D-10 Q1Laboratories, Schiltigheim, France). Plasma endotoxin concentration was measured with a commercial kit based on a limulus amebocyte lysate extract (LAL kit endpoint-QCL1000; Cambrex BioScience, Walkersville, Md., USA). Plasma hs-CRP concentration was determined using an immune turbidimetric assay (Pars Azmoon Co., Tehran, Iran). Plasma insulin was measured using a chemiluminescent immunoassay method (LIAISON analyzer (310360) Diasorin S.P.A., Verecelli, Italy). HOMA-IR and QUICKI were calculated according to the following formula: HOMA-IR = (fasting insulin (U/ml) × FPG (mg/dl))/405 and QUICKI = 1/(log (insulin, U/ml) + log (FPG, mg/dl)). The concentrations of GSH-Px, SOD and TAC were measured by colorimetric method (TAS: RANDOX kits, SOD: RANSOD kits and GSH-Px: RANSEL kits; RANDOX Laboratory, UK), on an automatic analyzer (Abbott model Alcyon 300, USA). MDA concentrations were measured through reaction with thiobarbituric acid (TBA) as a TBARS to produce a pink colored complex. Then, its fluorescence intensity was measured at 547 nm with excitation at 525 nm using a spectrofluorometer (Kontron, model SFM 25A, Italy) [18]. The levels of catalase were estimated by the method of Aebi [19].

Statistical Analyses

Data were analyzed using SPSS version 13.0 software. The normality of the distribution of data was evaluated by a one-sample Kolmogorov–Smirnov test. An unpaired Student t test (for baseline measurements) and analysis of covariance (ANCOVA) were used to compare quantitative variables. Before–after comparison of quantitative data was performed by paired sample t test. The drugs that were used in the 2 groups were compared with the Mann–Whitney U test. The percentage of mean changes of markers was calculated as: ((8 weeks values – 8 baseline values)/baseline values) × 100. Mean changes of biomarkers between the groups were calculated via the following formula: ((intervention values – control values)/control values) × 100. Values of $p < 0.05$ were considered to be statistically significant. The results are expressed as mean (SD).

Results

Patients

Of the 62 patients that were assessed for trial eligibility, 56 patients completed the trial (intervention group, n = 28; control group, n = 28; fig. 1). Patients did not report any adverse effects with RS2 supplementation. Table 1 shows the baseline characteristics of patients in the 2 groups. At the beginning of the trial, both groups had similar initial characteristics.

Anthropometric Indices and Nutrient Intake

Comparisons between the 2 groups showed that there were no significant differences with regard to baseline body weight, BMI, energy and macro and micronutrients intake (tables 1 and 2). After 8 weeks of supplementation, the body weight and BMI of the patients in the 2 groups were not significantly different. However, decreases in body weight and BMI were noticed in the intervention group (RS2) (74.50 (12.30) to 73.20 (12.20) kg, 31.65 (4.30) to 31.00 (4.2) kg/m^2, respectively). The patients' macronutrient and antioxidant micronutrient (vitamin E, C, β-Carotene, Cu, Zn, Mn and selenium) intakes remained unchanged in the intervention group (RS2) as compared with the control group. The patients' body weight, BMI and dietary compositions did not significantly change in the control group as compared with baseline ($p < 0.05$, paired Student t test). Therefore, the effects of these confounders on trial outcomes have been controlled.

Glycemic Status, Endotoxin and hs-CRP

At the beginning of the study, we did not observe any significant difference in FPG, HbA1c, insulin, HOMA-IR and QUICKI but differences in endotoxin and hs-CRP were significant between the intervention (RS2) and the control group. After 8 weeks, there was a significant decrease in levels of HbA1c (–0.80, –9.40%), insulin (–4.17 μU/ml, 29.36%), HOMA-IR (–1.84, 32.85%) and endotoxin (–6.40 μU/ml, –25.00%) in the intervention group (RS2) compared with the control group. At the end of the study, however, changes in levels of FPG (–7.45

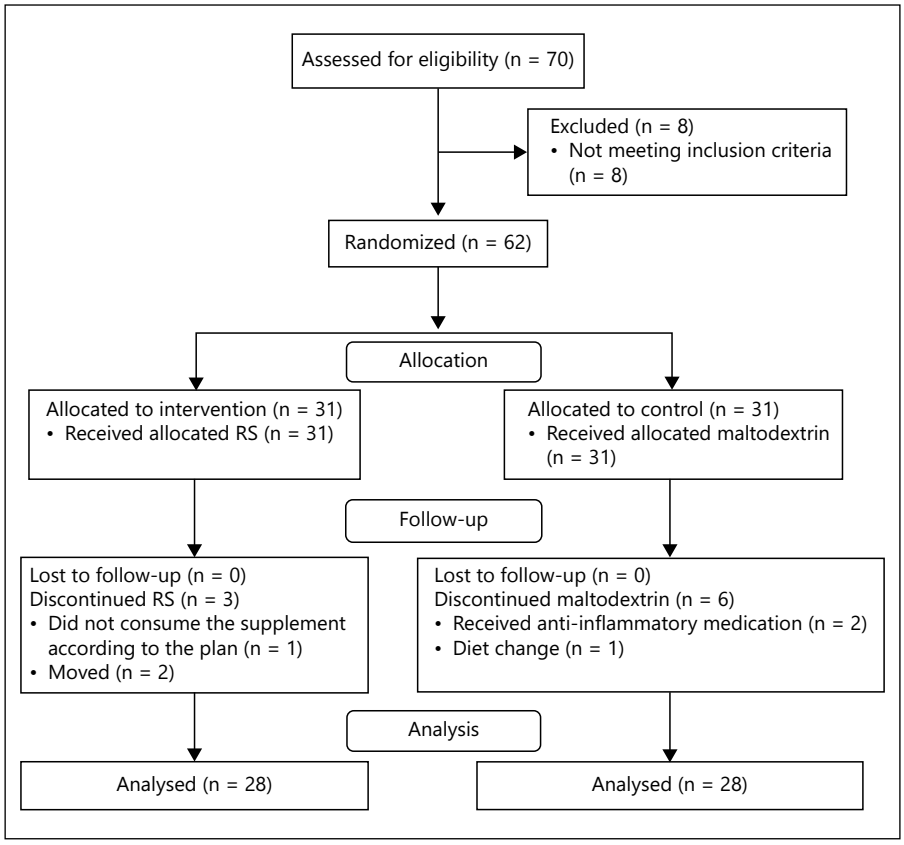

Fig. 1. Diagram of study.

Table 1. Baseline characteristics and dietary intakes of the study participants

Variables	Maltodextrin group (n = 28)	RS group (n = 28)
Age, years	48.6 (7.9)	49.5 (8.0)
Range	32–59	33–65
Menopausal status, n (%)		
Pre	7 (25)	8 (28.5)
Post	21 (75)	20 (71.5)
Weight, kg	73.9 (5.5)	74.2 (4.3)
Height, cm	154.3 (5.2)	153.3 (5.2)
BMI, kg/m^2	31.0 (4.9)	31.5 (4.5)
Diabetes duration, years	5.8 (3.2)	7.5 (5.9)
Metformin, 500 mg (tablets/day)	3.1 (1.2)	2.8 (1.1)
Glibenclamide, 5 mg (tablets/day)	2.5 (1.5)	2.3 (1.4)

Data are presented as mean (SD), with the exception of menopausal status, which is presented as n (%).

mg/dl, –4.70%), QUICKI (–0.17, 5.07%) and hs-CRP (–7.60 ng/ml, –61.20%) were not significant (ANCOVA adjusted for hs-CRP, endotoxin, weight changes and baseline values) in the intervention group (RS2) compared with the control group. The reductions in FPG, HOMA-IR, hs-CRP and endotoxin were significant in the intervention group (RS2) group as compared with baseline ($p < 0.05$) while in control group, they remained unchanged as compared with baseline (paired Student t test; table 3).

Table 2. Dietary intakes of patients at baseline and at the end of the study

Variables	Period	Maltodextrin group (n = 28)	RS group (n = 28)
Energy, kcal/day	Initial	1,678.2 (220.7)	1,539.0 (405.6)
	End	1,650.4 (248.0)	1,500.0 (326.5)
Carbohydrate, g/day	Initial	202.7 (54.8)	211.4 (74.6)
	End	198.7 (45.4)	211.6 (46.7)
Protein, g/day	Initial	49.9 (10.6)	50.9 (12.4)
	End	51.3 (13.6)	51.7 (14.9)
Total fat, g/day	Initial	55.4 (10.3)	52.1 (18.4)
	End	53.4 (12.8)	57.9 (18.4)
Dietary fiber, g/day	Initial	12.1 (3.2)	12.3 (5.0)
	End	13.8 (3.8)	13.7 (4.1)
Vitamin C, mg/day	Initial	102.7 (21.8)	108.9 (31.4)
	End	107.3 (25.6)	127.4 (42.4)
Vitamin E, mg/day	Initial	5.9 (1.4)	4.4 (1.4)
	End	5.2 (1.8)	4.1 (1.9)
Selenium, μg/day	Initial	50.9 (17.4)	55.5 (23.2)
	End	49.4 (17.3)	54.7 (20.6)
Cu, mg/day	Initial	1.9 (0.5)	2.1 (0.5)
	End	1.7 (0.4)	1.0 (0.3)
Zn, mg/day	Initial	7.9 (3.4)	5.8 (2.3)
	End	7.1 (3.2)	5.4 (1.2)
β-Carotene, mg/day	Initial	1.4 (4.2)	4.2 (1.2)
	End	3.4 (2.0)	4.3 (1.3)
Mn, mg/day	Initial	4.3 (1.1)	3.9 (1.1)
	End	4.4 (1.8)	4.3 (1.6)

Data are presented as mean (SD).

MDA, TAC, Antioxidant Enzyme Concentrations and Uric Acid

Comparison between the 2 groups indicated that there were no significant differences in MDA and antioxidant markers including TAC, SOD, GSH-Px, catalase and uric acid at the baseline (table 4). After 8 weeks, a significant decrease in MDA (–1.50 nmol/ml, –34.10%) and a significant increase in levels of TAC (0.15 mmol/l, 18.10.00%) and GSH-Px (3.75 U/g Hb, 11.60%) were observed in the intervention group (RS2) compared with the control group. No significant changes were observed in SOD (74.65 U/mg Hb, 4.70%), catalase (–3.50 U/g Hb, –5.50%) and uric acid (0.20 mg/dl, 3.70%) in the intervention group (RS2) as compared with the control group ($p < 0.05$, ANCOVA for hs-CRP, endotoxin, weight changes and baseline values). The reductions in MDA were significant in the intervention group (RS2) as compared with baseline. However, SOD, catalase and uric acid (with the exception of TAC and GSH-Px which increased in the intervention group (RS2)) did not significantly change in the intervention group (RS2) as compared with baseline. All the mentioned parameters remained unchanged in the control group as compared with baseline with the exception of GSH-Px, which significantly decreased ($p < 0.05$, paired Student t test).

Discussion

According of the results of this trial, 8 weeks supplementation with RS2 in patients with T2DM significantly decreased the levels of HbA1c, insulin, MDA and endotoxin and HOMA-IR. Also, we observed significant increases in TAC and GSH-Px in the intervention group (RS2).

Table 3. Changes in glycemic status, endotoxin and hs-CRP biomarkers of patients at baseline and at the end of the study

Variables	Period	Maltodextrin group (n = 28)	RS group (n = 28)	MD (95% CI) between groups
FPG, mg/dl	Initial	163.00 (23.50)	170.55 (45.90)	7.55 (–8.10 to 33.55)
	End	159.40 (14.30)	151.95 (36.30)	–7.45 (–23.40 to 5.85)[$]
	MD (95% CI) within groups	–3.60 (–5.20 to 3.00)	–18.60 (–33.90 to –6.75)[b]	
HbA1c, %	Initial	8.10 (1.05)	7.90 (1.15)	–0.20 (–0.75 to 0.20)
	End	8.50 (1.15)	7.70 (1.15)[c]	–0.80 (–0.75 to –0.30)[$]
	MD (95% CI) within groups	0.40 (–0.2 to 0.5)	–0.20 (–0.5 to 0.5)	
Fasting insulin, μU/ml	Initial	12.60 (3.45)	11.51 (4.95)	1.09 (–3.40 to 1.30)
	End	14.20 (4.70)	10.03 (1.81)[c]	–4.17 (–7.60 to –2.57)[$]
	MD (95% CI) within groups	1.60 (–1.70 to 0.70)	–1.48 (1.30 to –3.84)	
HOMA-IR	Initial	5.07 (2.30)	4.84 (2.20)	–0.23 (–1.14 to 1.08)
	End	5.60 (2.50)	3.76 (1.72)[b, c]	–1.84 (–3.11 to –0.40)[$]
	MD (95% CI) within groups	0.53 (–2.34 to 0.62)	–1.08 (–0.73 to –2.20)	
QUICKI	Initial	3.31 (0.16)	3.29 (0.19)	–0.02 (–0.07 to 0.13)
	End	3.35 (0.15)	3.18 (0.16)	–0.17 (–0.01 to 0.33)[$]
	MD (95% CI) within groups	0.04 (–0.05 to 0.09)	–0.11 (–0.21 to 0.15)	
hs-CRP, ng/ml	Initial	11.25 (6.45)	5.55 (2.70)[a]	–5.70 (–11. 35 to 3.75)
	End	12.75 (5.40)	4.95 (2.60)[b]	–7.80 (–4.05 to 1.80)[$]
	MD (95% CI) within groups	1.30 (–0.30 to 9.90)	–0.6 (–2.0 to –0.9)	
Endotoxin, EU/ml	Initial	23.00 (9.7)	24.75 (8.7)[a]	1.75 (–6.70 to 5.70)
	End	25.60 (5.8)	19.20 (7.9)[b, c]	–6.40 (–7.50 to –3.80)[$]
	MD (95% CI) within groups	2.60 (–1.8 to 1.3)	–5.5 (–6.7 to –4.3)	

Data are presented as mean (SD).

[a] $p < 0.05$, independent Student t test for baseline values; [b] $p < 0.05$, paired Student t test for comparison of data between the beginning and end of the study; [c] $p < 0.05$, ANCOVA for comparison of data between the intervention and placebo group adjusted after adjusting for hs-CRP, endotoxin and baseline values.

[$] Adjusted for hs-CRP, endotoxin and baselines values with ANCOVA.

Body weight and BMI of patients in this study did not significantly differ between the groups. Reportedly, RS intake (25 g/day for 3 weeks) in subjects with metabolic syndrome [20] and patients with T2DM (40 g/day for 12 weeks) [14] did not decrease the body weight significantly. Our results are in agreement with these results.

In the current study, consumption of RS for 8 weeks among diabetic patients significantly decreased HbA1c, insulin and HOMA-IR. However, decreases in FPG and QUICKI were not significant in the intervention group. Lobley et al. [20] reported that RS intake (25 g/day for 3 weeks) improved plasma insulin and C-peptide, insulin sensitivity and HOMA-IR in obese men, but fasting glycemia remained unchanged. It was reported that in patients with prediabetes or newly diagnosed T2DM, dietary treatment with rice containing RS reduces fasting insulin and insulin resistance, postprandial glucose and insulin levels as well as glucose and insulin areas under the response curve after the standard meal [12]. In Maki et al. [11], consumption of 15–30 g/day of HAM-RS2 for 4 weeks improved insulin sensitivity in men. Limited studies have evaluated the impact of RS on the inflammatory biomarkers and metabolic endotoxemia in humans [14, 16]. Bodinham et al. [14] showed that supplementation with RS2 (40 g/day for 12 weeks) decreased fasting TNF-α but did not significantly change the levels of fasting IL-6 in patients with T2DM. It was reported that 40 g/day RS2 did not change inflammatory markers (IL-6, C-reactive protein and hs-CRP) and endotoxemia after 12 weeks in patients with metabolic syndrome [16]. The difference in results may be due to basal levels of glycemic indices, dosage and type of supplementation, as well as pathologic state of the patients. Several other reports also reported similar findings [21, 22].

Table 4. Changes in the levels of MDA and antioxidant biomarkers of patients at baseline and at the end of the study

Variables	Period	Maltodextrin group (n = 28)	RS group (n = 28)	MD (95% CI) between groups
TAC, mmol/l	Initial	0.89 (0.30)	0.87 (0.14)	−0.05 (−0.11 to 0.03)
	End	0.83 (0.21)	0.98 (0.16)[b, c]	0.15 (0.2 to 0.4)[$]
	MD (95% CI) within groups	−0.06 (−0.08 to 0.03)	0.11 (0.06 to 0.15)	
SOD, U/mg Hb	Initial	1,610.25 (175.90)	1,661.65 (250.65)	51.40 (−75.80 to 199.85)
	End	1,598.65 (198.25)	1,673.30 (226.40)	74.65 (−29.9 to 104.40)[$]
	MD (95% CI) within groups	−11.60 (−83.50 to 49.30)	11.65 (−46.60 to 69.85)	
GSH-Px, U/g Hb	Initial	34.40 (2.00)	35.65 (4.60)	2.25 (−0.30 to 3.80)
	End	32.30 (2.60)[b]	36.05 (3.90)[c]	3.75 (0.50 to 5.00)[$]
	MD (95% CI) within groups	−2.10 (−3.2 to −1.0)	0.4 (−0.35 to 1.10)	
Catalase, U/g Hb	Initial	65.35 (15.15)	64.30 (18.40)	2.45 (−10.8 to 23.50)
	End	63.50 (15.7)	60.00 (21.40)	−3.5 (−20.20 to 29.40)[$]
	MD (95% CI) within groups	−1.85 (−9.90 to 8.45)	−4.30 (−18.05 to 8.30)	
MDA, nmol/ml	Initial	3.65 (1.35)	3.30 (1.95)	0.55 (−0.5 to 1.5)
	End	4.40 (1.90)	2.90 (1.30)[b, c]	−1.50 (−2.05 to −0.55)[$]
	MD (95% CI) within groups	0.75 (−0.25 to 1.05)	−0.40 (−1.20 to −0.2)	
Uric acid, mg/dl	Initial	5.40 (0.70)	5.20 (0.90)	−0.20 (−1.00 to 0.70)
	End	5.50 (0.40)	5.70 (1.00)	0.20 (−1.85 to 0.55)[$]
	MD (95% CI) within groups	0.10 (−0.70 to 1.00)	0.10 (−0.15 to 0.60)	

Data are presented as mean (SD).

[a] $p < 0.05$, independent Student t test for baseline values; [b] $p < 0.05$, paired Student t test for comparison of data between the beginning and end of the study; [c] $p < 0.05$, ANCOVA for comparison of data between the intervention and placebo group adjusted after adjusting for hs-CRP, endotoxin and baseline values.

[$] Adjusted for hs-CRP, endotoxin and baselines values with ANCOVA.

Prebiotics via several mechanisms can improve glycemic status and inflammation. Suppression of the levels of free fatty acids (FFAs) through short-chain fatty acids (acetate, propionate and butyrate) [23] returns to the basal expression levels of transcription factors involved in lipogenesis (sterol regulatory element-binding protein [SREBP-1c]), cholesterol metabolism (SREBP-2, liver X receptors) and fatty acid oxidation (peroxisome proliferator-activated receptor alpha [24]), reductions of endotoxin levels [25] and decreased oxidative stress [26], which could improve insulinemic effects. Non-significant changes in FPG may be attributed to lack of RS effect, as a prebiotic, on basal hepatic glucose production [27]. Mechanisms for the effect of RS2 on inflammation and metabolic endotoxemia are not yet clear. SCFAs mainly butyrate can control macrophage activity and expression of Nuclear Factor kappa B (NF-κB) that is a main regulator of inflammatory and immune responses raises the expression of cytokine signaling 3 suppressor and secretion of IL-10 [28]. Moreover, RS increases the levels of Bifidobacterium and *Faecalibacterium prausnitzii* that have anti-inflammatory effects [4].

Another outcome of RS in patients with T2DM was an improvement in oxidative stress and some antioxidant enzyme concentrations. Kwak et al. [12] reported that rice including 6.5 g/day RS decreased urinary 8-epi-PGF2α and MDA and increased the reactive hyperemia peripheral arterial tone index and total nitric oxide after 4 weeks in patients with prediabetes. Aigster [15] reported that cereal food products supplemented with RS (~18 g of RS) did not change oxidative stress parameters (cellular GSH-Px, F2-isoprostanes and oxygen radical absorbance capacity) in Hispanic women. Research showed that feeding rats with potato (rich source of RS) increased SCFA pools and improved antioxidant status including FRAP, urine TBARS and liver lipid oxidation [29]. Supplementation with RS2 in Wistar rats increased the levels of GSH [30]. It is reported that green dwarf banana flour, as a rich source of RS, prevented the glutathione depletion and inhibited myeloperoxidase activity as well as lipid peroxidation in a model of rat colitis [31]. Different gen-

otype and pathologic states, the dosage, kind and duration of supplementation, background diet, basal antioxidant and glycemic status in T2DM patients as well as the lack of suitable control group can explain the inconsistent findings. The underlying mechanisms of the effect of prebiotics on oxidative stress are not yet known. This study was not designed to investigate the biochemical mechanism. However, other studies have informed researchers about the mechanisms underlying the effects of RS on oxidative stress. Some proposed mechanisms for prebiotics antioxidant effects include the scavenging ability of reactive oxygen species (ROS) [32], SCFAs [33], reduction of inflammation and endotoxin [34], a change in microflora toward Lactobacilli which contain antioxidants [35], suppression of CD11c expression in adipose tissue [36] and reduction of the levels of FFAs [37].

Our study had some limitations. We did not assess glucose clamp, SCFAs, FFAs, or other antioxidant/oxidative stress biomarkers, antioxidant micronutrients and did not evaluate gut and fecal microbial compositions. Also, the duration of the intervention was short.

Based on the results of this trial, RS may improve metabolic endotoxemia and some of the glycemic and oxidative stress/antioxidant biomarkers including HbA1c, insulin, HOMA-IR, TAC, GSH-Px and MDA for patients with T2DM. Further investigations are needed to confirm the positive effects of RS2 in patients with T2DM.

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Disclosure Statement

The authors declare no conflict of interest.

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