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Probiotic yogurt improves antioxidant status in type 2 diabetic patients

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ABSTRACT

Objective: Oxidative stress plays a major role in the pathogenesis and progression of diabetes. Among various functional foods with an antioxidant effect, probiotic foods have been reported to repress oxidative stress. The objective of this clinical trial was to assess the effects of probiotic and conventional yogurt on blood glucose and antioxidant status in type 2 diabetic patients.
Methods: Sixty-four patients with type 2 diabetes mellitus, 30 to 60 y old, were assigned to two groups in this randomized, double-blind, controlled clinical trial. The patients in the intervention group consumed 300 g/d of probiotic yogurt containing *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12 and those in the control group consumed 300 g/d of conventional yogurt for 6 wk. Fasting blood samples, 24-h dietary recalls, and anthropometric measurements were collected at the baseline and at the end of the trial.
Results: Probiotic yogurt significantly decreased fasting blood glucose ($P < 0.01$) and hemoglobin A1c ($P < 0.05$) and increased erythrocyte superoxide dismutase and glutathione peroxidase activities and total antioxidant status ($P < 0.05$) compared with the control group. In addition, the serum malondialdehyde concentration significantly decreased compared with the baseline value in both groups ($P < 0.05$). No significant changes from baseline were shown in insulin concentration and erythrocyte catalase activity within either group ($P > 0.05$).
Conclusion: The consumption of probiotic yogurt improved fasting blood glucose and antioxidant status in type 2 diabetic patients. These results suggest that probiotic yogurt is a promising agent for diabetes management.

Introduction

Type 2 diabetes mellitus (T2DM) has rapidly increased in the world during the past few decades. Experimental and clinical evidence has suggested that oxidative stress plays a major role in the pathogenesis and progression of diabetes and its complications [1–3]. Diabetes is usually accompanied by an increased production of free radicals and impaired antioxidant defenses [2,4]. These conditions can lead to cellular organelle damage, the dysfunction of enzymes, an impairment of the binding of paraoxonase-1 to high-density lipoprotein and protection against lipid peroxidation, and the development of insulin resistance and may explain the presence of inflammation in T2DM [1,2,4–6].

Probiotics are live micro-organisms that, when administered in adequate amounts, confer health benefits on the host [7–9]. The consumption of probiotics have been shown to provide measurable health benefits, including the prevention and/or management of diarrhea, constipation, urinary tract infections, lactose intolerance, allergies, hepatic disease, inflammatory bowel disease, and diabetes mellitus. Certain species of bifidobacteria and lactobacilli used as probiotics can help balance intestinal microflora [10–13].

Studies have shown that special strains of lactic acid bacteria have antioxidant properties [14,15]. The antioxidative mechanisms of probiotics could be assigned to reactive oxygen species scavenging, metal ion chelation, enzyme inhibition, and the reduction activity and inhibition of ascorbate autoxidation [14]. In healthy persons, the consumption of goat milk fermented with

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Lactobacillus fermentum ME-3 has been shown to increase total antioxidative status (TAS) and decrease markers of oxidative stress [16,17]. The antioxidative properties of other probiotic strains have also been reported in healthy persons [18,19]. Studies using animal models of diabetes have also shown that *Lactobacillus acidophilus* and *Lactobacillus casei* attenuate oxidative stress and have antidiabetic effects [20,21].

Alterations in gut microbiota composition have recently been documented in patients with T2DM, providing a target for probiotic intervention [22]. Modification of gut microflora by probiotics may be seen as a novel means of regulating glucose metabolism and improving oxidative stress in T2DM. Thus, in this controlled trial, we tested the hypothesis that the consumption of probiotic yogurt containing *L. acidophilus* La5 and *Bifidobacterium lactis* Bb12 would improve blood glucose and antioxidant status in patients with T2DM.

Materials and methods

Subjects

Sixty-four patients with T2DM 30 to 60 y old with a body mass index (BMI) lower than 35 kg/m^2 were recruited for this study from the endocrinology clinic of Sina Hospital in Tabriz, Iran. Recruitment was done by telephone and advertisements. All patients had been diagnosed with T2DM for at least 1 y. Exclusion criteria were smoking; the presence of kidney, liver, or inflammatory intestinal disease, thyroid disorders, immunodeficiency diseases, or lactose intolerance; required insulin injections; use of nutritional supplements within the previous 3 wk of testing; use of cholesterol-lowering medication, estrogen, progesterone, or diuretics; pregnancy or breast-feeding; and consuming probiotic yogurt or any other probiotic products within the previous 2 mo of testing.

The sample size was determined based on the primary information obtained from the study by Chamari et al. [19] for catalase (CAT). For an α value equal to 0.05 and a power of 80%, the sample size was computed as 21.788 ($\approx$22) per group [23]. This number was increased to 32 per group to accommodate the anticipated dropout rate.

Study design and measurements

The present study was a double-blinded, randomized controlled clinical trial in which subjects were randomly assigned to the probiotic (intervention) or conventional (control) yogurt group using a block randomization procedure with matched subjects in each block based on sex and age. The allocation of the intervention or control group was concealed from the researchers and the probiotic and conventional yogurt containers had an identical appearance. The yogurt containers had no labeled information about the type of yogurt inside. Therefore, neither the subjects nor the investigators were aware of the treatment assignments in this double-blinded study. Each group consisted of 32 patients.

One week before the beginning of the trial, all patients refrained from eating yogurt or any other fermented foods. Over 6 wk, the probiotic and conventional groups consumed 300 g/d of probiotic and conventional yogurt, respectively. All patients were asked, throughout the 6-wk trial, to maintain their usual dietary habits and lifestyle and to avoid consuming any yogurt other than that provided to them by the researchers and any other fermented foods. The patients were instructed to keep the yogurt under refrigeration and to avoid any changes in medication, if possible.

Arrangements were made so that the patients would receive a 1-wk supply of their probiotic or conventional yogurts every week. Compliance with the yogurt consumption guidelines was monitored by telephone interviews once a week.

Information on food consumption, anthropometric measurements, and fasting blood samples were collected at the beginning and at the end of the trial. Nutrient intakes during 3 d were estimated using a 24-h dietary recall at the beginning and at the end of the study. Three-day averages of macro- and micronutrient intakes were analyzed by Nutritionist 4 software (First Databank, Hearst Corp, San Bruno, CA, USA).

Anthropometric measurements were recorded by trained personnel. Body weights were measured using a scale (Seca, Hamburg, Germany) with 0.1-kg accuracy without shoes and with minimum clothing. Heights were measured using a stadiometer (Seca) with 0.1-cm accuracy without shoes. BMI was calculated by dividing body weight (kilograms) by height (meters) squared.

A blood sample was drawn for each patient from the antecubital vein in the arm after a 12-h overnight fast. The serum samples were separated from whole blood by centrifugation at 3500 rpm for 10 min (Avanti J-25, Beckman, Brea, CA, USA). The serum and whole blood samples were frozen immediately at −70°C until the assay. Blood samples were analyzed at the Drug Applied Research Center (Tabriz University of Medical Sciences, Tabriz, Iran). Fasting blood glucose was measured using the standard enzymatic method with a Parsazmun kit (Karaj, Iran). Glycated hemoglobin (HbA1c) was measured in the whole blood by cation exchange chromatography with a NycoCard HbA1c kit (Oslo, Norway). Insulin concentration was determined by a chemiluminescent immunoassay using a Liaison analyzer (DiaSorin, Salluggia, Italy). Erythrocyte superoxide dismutase (SOD) activity was measured spectrophotometrically using a Ransod kit (Randox Laboratories, Crumlin, UK) [24]. Erythrocyte glutathione peroxidase (GPx) activity was measured using the spectrophotometric technique and a Ransel kit (Randox Laboratories) according to the method described by Paglia and Valentine [25]. Erythrocyte CAT activity was measured by the method of Aebi [26]. Serum total antioxidant capacity was determined using a Randox TAS kit (Randox Laboratories) [27]. Serum malondialdehyde (MDA) concentration was determined using the thiobarbituric acid method described by Bilici et al. [28].

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki and all procedures involving human persons were approved by the ethics committee at Tabriz University of Medical Sciences (no. 897). Written informed consent was obtained from all patients (the trial has been registered in the Iranian Registry of Clinical Trials, available at: http://www.irct.ir, identifier: IRCT 138903223533N1).

Intervention

The probiotic and conventional yogurts contained *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The probiotic yogurt was also enriched with *B. lactis* Bb12 and *L. acidophilus* La5 (Chr. Hansen, Hoersholm, Denmark) as Direct Vat Set cultures. The yogurts were produced weekly and distributed to the participants.

Probiotic yogurts were sampled 1 d after manufacture (time of distribution) and microbiologically analyzed every week. Samples were refrigerated at 4°, with subsequent analyzing on day 7 of storage. MRS-bile agar medium was used for the differential enumeration of mixed probiotic bacteria in presence of yogurt bacteria [29]. All the samples were incubated at 37°C for 72 h under aerobic and anaerobic conditions. All experiments were performed in triplicate. Counts of *L. acidophilus* were achieved at the aerobic condition and viable counts of *B. lactis* were selectively achieved using the subtractive enumeration method [29].

Microbiological analyses of the probiotic yogurts showed that the average colony counts of *L. acidophilus* La5 and *B. lactis* Bb12 on day 1 were 7.23×10^6 and 6.04×10^6 cfu/g, respectively. Probiotic yogurts contained 1.85×10^6 cfu/g of *L. acidophilus* La5 and 1.79×10^6 cfu/g of *B. lactis* Bb12 on day 7. Both probiotic bacteria showed a steady survival rate during a 7-d storage time. The fat content was 2.5% and was comparable in both yogurt types. The probiotic and conventional yogurt containers were identical and the yogurts had a similar taste and appearance. The yogurts were specially prepared for this study by Iran Dairy Industries Co. (Tehran, Iran).

Statistical analyses

The experimental data were analyzed by SPSS 11.5 (SPSS, Inc., Chicago, IL, USA) and the results were expressed as mean ± standard deviation. The normality of the distribution of variables was tested by the Kolmogorov-Smirnov test. For the duration of diabetes, monounsaturated fatty acid, vitamin A, E, and C intakes, fasting blood glucose, and insulin that did not follow normal distributions, analyses were performed after log transformation. The background characteristics and nutrient intakes of patients in the two groups were compared using independent-samples *t* tests and chi-square tests. The use of diabetes medication in the two groups was compared using the Mann-Whitney U test. Differences between the two groups after the intervention were determined by analysis of covariance, adjusting for baseline measurements and covariates. In this study, duration of diabetes and polyunsaturated fatty acid intake were used as possible covariates. The changes in anthropometric measurements, nutrient intakes, fasting blood glucose, HbA1c, insulin, and oxidative stress markers of the patients between the beginning and the end of the trial were compared by paired-samples *t* test [30]. Results with $P < 0.05$ were considered statistically significant.

Results

In this study, four patients were excluded from the statistical analysis because they needed to change their medication during the trial or they did not consume the yogurt according to the plan. Thus, data for 60 patients (23 male and 37 female) were analyzed ($n = 30$ for each group). The patients demonstrated good compliance with the yogurt consumption and no adverse effects or symptoms were reported. The baseline characteristics of the patients in the two groups are listed in Table 1. The