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Randomized control trials

Effects of synbiotic food consumption on metabolic status of diabetic patients: A double-blind randomized cross-over controlled clinical trial

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SUMMARY

Background & aims: We are aware of no study indicating the effects of synbiotic food consumption on metabolic profiles, inflammation and oxidative stress among diabetic patients. The aim of the current study was to investigate the effects of synbiotic food consumption on metabolic profiles, hs-CRP and biomarkers of oxidative stress in diabetic patients.

Methods: This randomized double-blinded cross-over controlled clinical trial was performed among 62 diabetic patients aged 35–70 y. After a 2-wk run-in period, subjects were randomly assigned to consume either a synbiotic ($n = 62$) or control food ($n = 62$) for 6 weeks. A 3-week washout period was applied following which subjects were crossed over to the alternate treatment arm for an additional 6 weeks. The synbiotic food consisted of a probiotic viable and heat-resistant *Lactobacillus sporogenes* (1×10^7 CFU), 0.04 g inulin (HPX) as prebiotic with 0.38 g isomalt, 0.36 g sorbitol and 0.05 g stevia as sweetener per 1 g. Control food (the same substance without probiotic bacteria and prebiotic inulin) was packed in identical 9-gram packages. Patients were asked to consume the synbiotic and control foods three times a day. Fasting blood samples were taken at baseline and after a 6-wk intervention to measure metabolic profiles, hs-CRP and biomarkers of oxidative stress.

Results: Consumption of a synbiotic food, compared to the control, resulted in a significant decrease in serum insulin levels (changes from baseline: -1.75 ± 0.60 vs. $+0.95 \pm 1.09$ μIU/mL, $P = 0.03$). Although we failed to find a significant effect of synbiotic food consumption on total- and LDL-cholesterol levels and HOMA-IR, the effects on FPG (22.3 vs. 4.2 mg/dL, $P = 0.09$), serum triglycerides (45.9 vs. 20.6 mg/dL, $P = 0.08$) and HDL-cholesterol levels (3.1 vs. −2 mg/dL, $P = 0.06$) tended to be significant. A significant reduction in serum hs-CRP levels (-1057.86 ± 283.74 vs. 95.40 ± 385.38 ng/mL, $P = 0.01$) was found following the consumption of synbiotic food compared with the control group. Supplementation with the synbiotic food led to a significant increase in plasma total GSH (319.98 vs. 19.73 μmol/L, $P < 0.001$) and serum uric acid levels (+0.7 vs. −0.1 mg/dL, $P = 0.04$) compared to the control food. No significant effect of the synbiotic food was observed on plasma TAC levels.

Conclusions: In conclusion, consumption of a synbiotic food for 6 weeks among diabetic patients had significant effects on serum insulin, hs-CRP, uric acid and plasma total GSH levels.

Clinical trial registration number: www.irct.ir: IRCT201201195623N1.

1. Introduction

Type 2 diabetes (T2D) is a metabolic disorder that is characterized by high blood glucose levels, insulin resistance and relative insulin deficiency.[1] Diabetes is highly prevalent in the world with an estimated prevalence of 8.3% in US.[2] In Iran, it has been

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estimated that 8% of adult population are affected.[3] Several studies have reported that increased levels of inflammatory factors and biomarkers of oxidative stress can result in the development of T2D. Elevated inflammation and oxidative stress can also play a key role in the pathogenesis of both macro- and micro-vascular complications of diabetes.[4]

Prior studies have shown that decreasing circulating levels of pro-inflammatory factors and biomarkers of oxidative stress in diabetic patients are associated with better glycemic control. The effectiveness of diet therapy and antioxidant supplementation and high fitness levels on these biomarkers has been reported.[5] Some studies have suggested that consumption of synbiotic foods might help controlling the metabolic profile, inflammatory factors[6] and biomarkers of oxidative stress.[7] However, such effects have mainly been observed in animal models or non-diabetic patients. Liong et al.[8] showed that intake of a synbiotic containing *Lactobacillus acidophilus*, fructooligosaccharide, inulin and mannitol for 8 weeks resulted in a decreased serum triglyceride, total- and LDL-cholesterol levels as well as increased HDL-cholesterol concentrations in hypercholesterolaemic pigs. In addition, increased levels of superoxide dismutase and glutathione, along with reduced levels of nitric oxide have been reported with a consumption of synbiotic containing *L. acidophilus* and inulin in a murine model.[9] Synbiotics influence the production of short chain fatty acid (SCFA), carbon disulfide and methyl acetate,[10] and can increase the lipolytic activity. Their direct immunomodulatory effects[11] as well as down-regulatory influence on genes involved in toll-like receptor (TLR) pathways might explain their favorable actions.[12] Due to their effects on caveolin-1, endothelial NOS and neuronal NOS down-regulation,[7] synbiotics might affect oxidative stress.[13]

We are aware of no study indicating the effects of synbiotic food consumption on metabolic profiles, inflammation and oxidative stress among diabetic patients. The aim of the current study was, therefore, to investigate the effects of a synbiotic food on metabolic profiles, hs-CRP and biomarkers of oxidative stress in diabetic patients.

2. Subjects and methods

2.1. Participants

This randomized double-blinded crossover controlled clinical trial was carried out in Kashan, Iran, during July 2011 to January 2012. On the basis of sample size formula suggested for cross-over clinical trials,[14] we considered the type I error of 5% ($\alpha = 0.05$) and type II error of 20% ($\beta = 0.20$; Power = 80%) and serum hs-CRP levels as a key variable and reached the sample size of 23 patients for each group. Diagnosis of T2D was done based on the criteria of American Diabetes Association[1]: those with one of the following criteria were considered as having T2D: fasting plasma glucose (FPG) $\geq$126 mg/dL, blood sugar (BS) 2-h pp $\geq$200 mg/dL and HbA1C $\geq$6.5%. Individuals with the above-mentioned inclusion criteria were called for participation in the study from those that attended Golabchi Diabetes Clinic affiliated to Kashan University of Medical Sciences, Kashan, Iran. Subjects were not included if they were pregnant, using insulin or vitamin supplements, or had chronic kidney disease, liver, lung and chronic or acute inflammatory disease, heart valve disease, short bowel syndrome and allergies. A total of 70 patients with T2D aged 35 to 70 y were recruited in the study and were randomly assigned to receive either a synbiotic ($n = 35$) or control food ($n = 35$) for 6 weeks. The study was conducted according to the guidelines laid down in the Declaration of Helsinki. The ethical committee of Qom University of Medical Sciences approved the study (91288-91-7-5) and informed written consent was obtained from all participants.

2.2. Study design

To obtain detailed information about the dietary intakes of study participants, all patients entered into a 2-wk run-in period; during which all subjects had to refrain from taking any other synbiotic and probiotic foods. During the run-in period, participants were asked to record their dietary intakes for three non-consecutive days. At the end of run-in period, subjects were randomly assigned to the initial arm of the study to receive either a synbiotic or control food for 6 weeks. A 3-week washout period was applied following which subjects were crossed over to the alternate treatment arm for an additional 6 weeks. Participants were asked not to alter their routine physical activity or usual diets and not to consume any synbiotic, probiotic and fermented products other than the one provided to them by the investigators. Synbiotic or control foods were provided to participants every month. Compliance with the consumption of foods was monitored once a week through phone interviews. The compliance was also double-checked by the use of three-day dietary records completed throughout the study in each phase of intervention. To obtain nutrient intakes of participants based on these three-day food diaries in each phase, we used Nutritionist IV software (First Databank, San Bruno, CA) modified for Iranian foods.

2.3. Synbiotic and control foods

The synbiotic food consisted of a probiotic viable and heat-resistant *Lactobacillus sporogenes* (1×10^7 CFU), 0.04 g inulin (HPX) as prebiotic with 0.38 g isomalt, 0.36 g sorbitol and 0.05 g stevia as sweetener per 1 g. Patients were asked to consume the synbiotic food three times a day in a 9 g package. Therefore, they received 27×10^7 CFU *L. sporogenes* and 1.08 g inulin each day. Control food (the same substance without probiotic bacteria and prebiotic inulin) was packed in identical packages and coded by the producer to guarantee blinding. The synbiotic and control foods were provided by Sekkeh Gaz Company, Isfahan, Iran.

The probiotic effects of *L. sporogenes* have earlier been shown.[15] Although different studies have used different dosages of the probiotics, we used it at the dosage of 10^7 based on prior publications that indicated the efficacy of this dosage of *L. sporogenes* on lipid profiles.[15] The dosage of inulin we used in the current study was comparable to prior studies.[16]

2.4. Assessment of variables

Anthropometric measurements were assessed at baseline and after 6 weeks of intervention in each separate arm. Body weight was measured in an overnight fasting status, without shoes and in a minimal clothing state by the use of a digital scale (Seca, Hamburg, Germany) to the nearest 0.1 kg. Height was measured using a non-stretched tape measure (Seca, Hamburg, Germany) to the nearest 0.1 cm. BMI was calculated as weight in kg divided by height in meters squared. Fasting blood samples (10 mL) were taken at baseline and after 6-wk intervention in each separate arm at Kashan reference laboratory in an early morning after an overnight fast. Plasma glucose levels were quantified by the use of glucose oxidase/peroxidase (GOD-POD) method with commercially available kits (Pars Azmoon Inc, Tehran, Iran). Serum insulin levels were assayed by enzyme-linked immunoassay kits (DiaMetra, Italy). Insulin resistance was assessed using the homeostatic model assessment of insulin resistance (HOMA-IR). Serum total cholesterol and triacylglycerol concentrations were assayed using commercial kits (Pars Azmoon Inc, Tehran, Iran) by enzymatic colorimetric tests with cholesterol oxidase p-aminophenazone and glycerol phosphate oxidase, respectively. Serum HDL-C levels were