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Randomized control trials

Effects of beta-carotene fortified synbiotic food on metabolic control of patients with type 2 diabetes mellitus: A double-blind randomized cross-over controlled clinical trial

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SUMMARY

Background & aims: The aim of the present study was to determine the beneficial effects of beta-carotene fortified synbiotic food intake on metabolic status in patients with type 2 diabetes mellitus (T2DM).
Methods: This randomized double-blinded placebo-controlled crossover clinical trial was conducted among 51 patients with T2DM. Individuals were randomly assigned to take either a beta-carotene fortified synbiotic (n = 51) or control food (n = 51) for 6 weeks. The beta-carotene fortified synbiotic was containing *Lactobacillus sporogenes* (1×10^7 CFU), 0.1 g inulin and 0.05 g beta-carotene. Control food (the same substance without probiotic, inulin and beta-carotene) was packed in identical 9-g packages. Patients were requested to use the beta-carotene fortified synbiotic and control foods three times a day.
Results: Beta-carotene fortified synbiotic food consumption resulted in a significant decrease in insulin (−1.00 ± 7.90 vs. +3.68 ± 6.91 μIU/mL, P = 0.002), HOMA-IR (−0.73 ± 3.96 vs. +1.82 ± vbnm4.09, P = 0.002), HOMA-B (−0.52 ± 19.75 vs. +8.71 ± 17.15, P = 0.01), triglycerides (−2.86 ± 49.53 vs. +20.14 ± 50.10 mg/dL, P = 0.02), VLDL-cholesterol levels (−0.57 ± 9.90 vs. +4.03 ± 10.02 mg/dL, P = 0.02) and total-/HDL-cholesterol ratio (−0.01 ± 1.08 vs. +0.64 ± 0.81, P = 0.001) compared to the control food. In addition, beta-carotene fortified synbiotic food consumption led to elevated plasma nitric oxide (NO) (+6.83 ± 16.14 vs. -3.76 ± 16.47 μmol/L, P = 0.001) and glutathione (GSH) (+36.58 ± 296.71 vs. -92.04 ± 243.05 μmol/L, P = 0.01).
Conclusions: Beta-carotene fortified synbiotic food intake in patients with T2DM for 6 weeks had favorable effects on insulin, HOMA-IR, HOMA-B, triglycerides, VLDL-cholesterol, total-/HDL-cholesterol ratio, NO and GSH levels.

1. Introduction

Type 2 diabetes mellitus (T2DM), which accounts for 90–95% of cases with diabetes [1], is a chronic disorder characterized by impaired metabolism of glucose and lipids due to impaired insulin secretion (beta cell dysfunction) or action (insulin resistance) [2]. The prevalence of T2DM is 8.3% among adult population in the world [3]. Main complications of T2DM are micro-as well as macrovascular pathologies that influence more than 17.5 million deaths worldwide [4,5]. It has been reported that glycemic variability and oxidative stress are the underlying causes of both macro- and microvascular complications of T2DM [6]. The main strategy for management of T2DM is the integrated control of hyperglycaemia, dyslipidaemia and blood pressure [7,8]. Recently, some clinical trials have reported that administration of synbiotic foods might have beneficial effects on metabolic parameters, biomarkers of inflammation and oxidative stress in these patients [9,10]. Our

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previous study also showed the favorable effects of a synbiotic food on metabolic status of patients with T2DM [9], without any major significant impact on biomarkers of oxidative stress. Due to the antioxidant and anti-inflammatory effects [11], beta-carotene might reinforce effects of the synbiotic food on metabolic profiles in patients with diabetes. Taking antioxidant supplements containing vitamin E, vitamin C, and beta-carotene for 8 weeks led to decreased HOMA-IR among overweight young adults [12]. Furthermore, another study [13] reported that consumption of beta-carotene plus D-galactose compared with D-galactose alone resulted in increased glutathione (GSH), superoxide dismutase (SOD) levels and glutathione peroxidase (GSH-Px) activities in rats at weaning. However, supplementation with fruit and vegetable juice concentrate (FVJC) for 6 months resulted in increased beta-carotene levels but did not affect lipid profiles in overweight boys [14].

The beneficial effects of synbiotics on metabolic profiles may be attributed to decreased expression of the enzymes involved in fatty acid synthesis [15]. It has also been suggested that synbiotics might modulate gut microbiota-short chain fatty acid (SCFA)-hormone axis [16]. Increased glutamate cysteine ligase (GCL) activity and enhanced mRNA expression of both of the GCL subunits following synbiotic and probiotic intake [17] and blocking the activities of caspase-3 and attenuating lipid peroxidation after intake beta-carotene [13] might also lead to anti-inflammatory and reduced oxidative stress. As oxidative stress is a well known factor underlying diabetes related complications of diabetes, we hypothesized that the administration of a beta-carotene fortified synbiotic food might further influence the metabolic status, biomarkers of inflammation and oxidative stress in patients with diabetes. To the best of our knowledge, we are aware of no study indicating the effects of beta-carotene fortified synbiotic food on metabolic status, biomarkers of inflammation and oxidative stress in patients with T2DM. The aim of the present study was, therefore, to assess the favorable effects of a beta-carotene fortified synbiotic food on metabolic status, biomarkers of inflammation and oxidative stress in patients with T2DM.

2. Subjects and methods

2.1. Participants

The present study was a double-blinded controlled crossover clinical trial that was carried out in Kashan, Iran, during January 2013 to May 2013. Patients aged 35–70 years and with a diagnosis of T2DM were selected from a public clinic of Kashan University of Medical Sciences, Kashan, Iran. Individuals with a fasting blood sugar (FBS) of ≥126 mg/dL or 2-h postprandial glucose concentrations of ≥200 mg/dL were defined as having diabetes according to the guidelines of the American Diabetes Association [18]. In this study, we excluded subjects who were pregnant, using insulin or vitamin supplements, and those with liver, inflammatory diseases, coronary heart disease and allergies. Sample size was determined according to serum insulin levels as a key variable in a previous study in subjects with T2DM [9]. Considering the mean difference of 2.2, the standard deviation of 4.0 and the type 2 error of 0.20, we needed 46 patients per group. However we recruited 51 patients per group to account for the possible dropouts. A total of 102 patients with T2DM were randomly allocated to receive either beta-carotene fortified synbiotic (n = 51) or control food (n = 51) for 6 weeks. The current study was conducted according to the guidelines laid down in the Declaration of Helsinki. The ethical committee of Kashan University of Medical Sciences approved the study 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. All patients were matched based on age, sex, BMI, type and dosage of oral hypoglycemic agents (OHAs) they were taking. At the end of run-in period, patients were randomly allocated to the initial arm of the study to receive either a beta-carotene fortified synbiotic or control food for 6 weeks. After this time period, subjects were entered into a 3-week washout period. Then the participants were crossed over to the alternate group for an additional 6 weeks. Subjects were requested not to change their routine physical activity or usual diets and not to use any synbiotic, probiotic and fermented products other than the one provided to them by the investigators. Participants were not asked to provide home blood monitoring (BM) checks to the investigators. In addition, as the duration of intervention was short (6 weeks), they were requested not to change the dosage and type of OHAs they were taking to avoid their possible effect on the study findings. Beta-carotene fortified synbiotic or control foods were provided to participants every 3 weeks. Compliance with the consumption of foods was monitored once a week through phone interviews. To increase the compliance, all individuals were receiving short messages on their cell phones to receive beta-carotene fortified synbiotic or control foods each day. Subjects' nutritional status was evaluated by a three-day food records at 3 time points (baseline, week 3 and 6 of intervention) during the course of the study. Then, data on food records were analyzed using Nutritionist IV software (First Databank, San Bruno, CA, USA) modified for Iranian foods.

2.3. Beta-carotene fortified synbiotic and control foods

A beta-carotene fortified synbiotic food contained a probiotic bacteria of *Lactobacillus sporogenes* (1×10^7 CFU), 0.1 g inulin (HPX) as prebiotic, 0.05 g beta-carotene with 0.38 g isomalt, 0.36 g sorbitol and 0.05 g stevia per 1 g as sweetener. Control food contained the same substance without *L. sporogenes*, inulin and beta-carotene. Individuals were requested to take the synbiotic and control foods in 9-g packages three times a day.

2.4. Assessment of variables

Height and weight were measured at the beginning of the study and the end-of-trial in a standing position by using a digital scale (Seca, Hamburg, Germany). BMI was calculated for each patient by dividing weight in kg to the height in m^2. All the measurements were obtained by the same person (a nutritionist). Patients were visited every week by the same physician (an endocrinologist). After a 12 h fast, 10 mL blood samples were obtain from patients to determine lipid concentrations, insulin, hs-CRP, liver enzymatic, calcium, iron and magnesium concentrations. Serum lipid concentrations were also determined on the day of blood collection. Then, the samples were stored at −70 °C before analysis at the KUMS reference laboratory. Commercial kits were used to measure fasting plasma glucose (FPG), serum levels of cholesterol, triglycerides, VLDL-, LDL-, HDL-cholesterol, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkalin phosphatase (ALP), calcium, iron and magnesium (Pars Azmun, Tehran, Iran). All inter- and intra-assay CVs for FPG, lipid profiles, liver enzymes, calcium, iron and magnesium measurements were less than 5%. Serum levels of insulin were quantified by using ELISA kit (Monobind, California, USA). HOMA-IR and β-cell function (HOMA-B) and