Original Paper

Annals of Nutrition & Metabolism

Ann Nutr Metab 2013;63:1–9
DOI: 10.1159/000349922

Received: October 5, 2012
Accepted after revision: February 15, 2013
Published online: July 5, 2013

Effect of Multispecies Probiotic Supplements on Metabolic Profiles, hs-CRP, and Oxidative Stress in Patients with Type 2 Diabetes

Zatollah Asemi[a] Zohreh Zare[b, c] Hossein Shakeri[a] Sima-sadat Sabihi[a] Ahmad Esmaillzadeh[d, e]

[a]Research Center for Biochemistry and Nutrition in Metabolic Diseases, Kashan University of Medical Sciences, Kashan, [b]Department of Research and Development of Zist-Takhmir Company, and [c]Department of Pharmaceutical Incubator, Tehran University of Medical Sciences, Tehran, and [d]Food Security Research Center, Isfahan University of Medical Sciences, and [e]Department of Community Nutrition, School of Nutrition and Food Science, Isfahan University of Medical Sciences, Isfahan, Iran

Key Words

Probiotics · Metabolic profiles · High-sensitivity C-reactive protein · Oxidative stress · Type 2 diabetes

Abstract

Background: We are aware of no study that has indicated the effects of daily consumption of multispecies probiotic supplements on metabolic profiles, high-sensitivity C-reactive protein (hs-CRP), and oxidative stress in diabetic patients. ***Objective:*** This study was designed to determine the effects of multispecies probiotic supplements on metabolic profiles, hs-CRP, and oxidative stress in diabetic patients. ***Methods:*** This randomized double-blind placebo-controlled clinical trial was performed on 54 diabetic patients aged 35–70 years. Subjects were randomly assigned to take either a multispecies probiotic supplement (n = 27) or placebo (n = 27) for 8 weeks. The multispecies probiotic supplement consisted of 7 viable and freeze-dried strains: *Lactobacillus acidophilus* (2×10^9 CFU), *L. casei* (7×10^9 CFU), *L. rhamnosus* (1.5×10^9 CFU), *L. bulgaricus* (2×10^8 CFU), *Bifidobacterium breve* (2×10^{10} CFU), *B. longum* (7×10^9 CFU), *Streptococcus thermophilus* (1.5×10^9 CFU), and 100 mg fructo-oligosaccharide. Fasting blood samples were taken at baseline and after intervention to measure metabolic profiles, hs-CRP, and biomarkers of oxidative stress including plasma total antioxidant capacity and total glutathione (GSH). ***Results:*** Between-group comparisons of fasting plasma glucose (FPG) revealed that consumption of probiotic supplements prevented a rise in FPG (+28.8 ± 8.5 for placebo vs. +1.6 ± 6 mg/dl for probiotic group, p = 0.01). Although a significant within-group increase in serum insulin and low-density lipoprotein cholesterol levels was found in both the probiotic group and the placebo group, the changes were similar between the two groups. We observed a significant increase in HOMA-IR (homeostasis model of assessment-insulin resistance) in both the probiotic group (p = 0.02) and the placebo group (p = 0.001); however, the increase in the placebo group was significantly higher than that in the probiotic group (+2.38 vs. +0.78, p = 0.03). Mean changes in serum hs-CRP were significantly different between the two groups (–777.57 for the probiotic group vs. +878.72 ng/ml for the placebo group, p = 0.02). Probiotic supplementation led to a significant increase in plasma GSH levels compared to placebo (240.63 vs. –33.46 μmol/l, p = 0.03). ***Conclusion:*** In conclusion, multispecies probiotic supplementation, compared with placebo, for 8 weeks in diabetic patients prevented a rise in FPG and resulted in a decrease in serum hs-CRP and an increase in plasma total GSH.

Copyright © 2013 S. Karger AG, Basel

KARGER

© 2013 S. Karger AG, Basel
0250–6807/13/0632–0001$38.00/0

E-Mail karger@karger.com
www.karger.com/anm

Ahmad Esmaillzadeh, PhD
Department of Community Nutrition
School of Nutrition and Food Science, Isfahan University of Medical Sciences
Isfahan PO Box 81745-151 (Iran)
E-Mail Esmaillzadeh@hlth.mui.ac.ir

Downloaded by:
University of Pennsylvania
165.123.34.86 - 9/28/2013 9:49:21 PM

Introduction

Type 2 diabetes (T2D) is a metabolic disorder characterized by high blood glucose arising from a combination of insufficient insulin secretion and resistance to insulin action [1]. The International Diabetes Federation (IDF) estimated that about 258 million people around the world suffered from diabetes mellitus in 2010 [2]. The prevalence of this condition among the Iranian adult population is almost 8% [3]. In addition to an abnormal metabolic profile, elevated levels of proinflammatory biomarkers and oxidative stress have previously been documented in diabetic patients [4], and these in turn make them prone to metabolic disorders [5] and cardiovascular events [6].

In addition to various strategies suggested for metabolic control of diabetes [7, 8], the use of antioxidants, vitamins E, A, and C, and coenzyme Q_{10} [9] as well as oxidative stress-lowering [10] and anti-inflammatory agents including low-dose aspirin and statins [11] have been used in diabetes. Recently, the use of probiotics to decrease metabolic profiles [12], inflammatory factors [13], and biomarkers of oxidative stress [14, 15] has received great attention. However, the beneficial effects have mainly been reported in animal models or nondiabetic patients. It seems that probiotics influence metabolic profiles by improving insulin sensitivity [5], enzymatic deconjugation of bile acids, and conversion of cholesterol into coprostanol in the gut [16]. In addition, decreased inflammation and oxidative stress by probiotics might be due to their effects on increasing glutathione (GSH) levels [17], scavenging superoxide and hydroxyl radicals [18], decreasing expression of interleukin-6 (IL-6) in adipocytes, and decreasing adiposity [19].

Few data are available on the effects of probiotics on the metabolic profile of diabetic patients. It has been shown that a diet supplemented with dahi, a fermented dairy product containing *Lactobacillus acidophilus* and *L. casei*, resulted in a significant delay in the progression of glucose intolerance, hyperglycemia, hyperinsulinemia, dyslipidemia, and oxidative stress in rats [5]. Oral administration of *L. casei* led to decreased plasma glucose levels in KK-Ay mice [20]. Consumption of *L. plantarum* (299v) also resulted in reduced systolic blood pressure, serum insulin levels, leptin, fibrinogen, F2-isoprostanes, and IL-6 [21]. Almost all earlier studies have considered the effect of monospecies probiotics, and to our knowledge no reports are available indicating the effects of multispecies probiotic supplements on metabolic profiles, inflammatory factors, and oxidative stress. Furthermore, previous studies have mostly been done in animal models and limited data on the effects on humans are available. Moreover, the effects of probiotics on several human conditions have been assessed, but evidence of the effects of probiotics, in particular multispecies probiotics, on diabetes is scarce. The aim of the current study was, therefore, to investigate the effects of daily consumption of multispecies probiotic supplements on metabolic profiles, high sensitivity C-reactive protein (hs-CRP), and biomarkers of oxidative stress among type 2 diabetic patients.

Materials and Methods

Participants

This randomized double-blinded controlled clinical trial was carried out in Kashan, Iran, from November 2011 to February 2012. On the basis of the sample size formula suggested for randomized clinical trials [22], we considered a type I error of 5% ($\alpha = 0.05$) and a type II error of 20% ($\beta = 0.2$; power = 80%) and serum hs-CRP levels as a key variable and reached the sample size of 22 patients for each group. The diagnosis of T2D was made based on the criteria of the American Diabetes Association [23]; those with one of the following criteria were considered to have T2D: fasting plasma glucose (FPG) ≥126 mg/dl, blood sugar (2 h postprandial) ≥200 mg/dl, and hemoglobin $A1_C$ ($HbA1_C$) ≥6.5%. Individuals with the above mentioned inclusion criteria were called for participation in the study from those who attended the Golabchi Diabetes Clinic affiliated with 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, or allergies. A total of 60 patients (18 males and 42 females) with T2D were recruited into the study and, after matching for age, sex, BMI, and type and dosage of the oral hypoglycemic medications they were taking, they were randomly assigned to receive either multispecies probiotic supplements (n = 30, i.e. 9 males and 21 females) or the placebo (n = 30, i.e. 9 males and 21 females) for 8 weeks. The 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.

Study Design

To obtain detailed information about the dietary intakes of the study participants, all patients entered a 2-week run-in period during which they had to refrain from ingesting any other probiotic food. During the run-in period, participants were asked to record their dietary intakes for 3 nonconsecutive days. At the end of run-in period, subjects were randomly assigned to receive either the placebo or multispecies probiotic supplements for 8 weeks. All participants were asked to take one supplement each day. Participants were asked not to alter their routine physical activity or usual diets and not to consume any probiotic capsules other than the one provided to them by the investigators. They were also asked to avoid consuming any other probiotic and fermented products.

Downloaded by:
University of Pennsylvania
165.123.34.86 - 9/28/2013 9:49:21 PM

Placebo or multispecies probiotic supplements were provided for participants every month. Compliance with consumption of capsules was monitored once a week through phone interviews. Compliance was also double-checked by the use of 3 day dietary records completed throughout the study. To obtain the nutrient intakes of participants based on these 3-day food diaries, we used Nutritionist IV software (First Databank, San Bruno, Calif., USA) modified for Iranian foods.

Assessment of Variables

Anthropometric measurements were assessed at baseline and after 8 weeks of intervention. Body weight was measured in an overnight fasting status, without shoes and in minimal clothing, using a digital scale (Seca, Hamburg, Germany) to the nearest 0.1 kg. Height was measured using a nonstretched tape measure (Seca) to the nearest 0.1 cm. BMI was calculated as weight in kilograms divided by height in meters squared. Fasting blood samples (10 ml) were taken at baseline and after an 8-week intervention at the Kashan reference laboratory early in the morning after an overnight fast. The serum samples were separated from whole blood by centrifugation at 3,500 rpm for 10 min (Hettich D-78532; Tuttlingen, Germany). Plasma glucose levels were quantified via the glucose oxidase/peroxidase method with commercially available kits (Pars Azmoon Co., Iran). $HbA1_C$ levels in the whole blood were measured using a Glycomat kit (BiocodeHycel, USA) via the exchange chromatography method at the Kashan reference laboratory. Serum insulin levels were assayed using enzyme-linked immunoassay kits (DiaMetra, Italy). Insulin resistance was assessed using the homeostatic model assessment of insulin resistance (HOMA-IR). Serum total cholesterol and triglyceride concentrations were assayed using commercial kits (Pars Azmoon) by enzymatic colorimetric tests with cholesterol oxidase p-aminophenazone and glycerol phosphate oxidase, respectively. Serum high-density lipoprotein cholesterol (HDL-C) was measured after precipitation of the apolipoprotein B containing lipoproteins with phosphotungistic acid. Serum low-density lipoprotein cholesterol (LDL-C) levels were also measured using available kits. Serum hs-CRP was assayed using ELISA and enzyme-linked immunoassay kits (LDN, Nordhorn, Germany). Plasma samples were analyzed for concentrations of total antioxidant capacity (TAC) and total GSH levels. Plasma TAC was assessed via the FRAP method developed by Benzie and Strain [24]. The plasma total GSH was measured using the method of Beutler et al. [25]. The serum uric acid concentration was assayed using a uric acid kit (Pars Azmoon).

Characteristics of Supplements

The multispecies probiotic supplement (ZistTakhmir Co., Tehran, Iran) consisted of 7 viable and freeze-dried strains: *L. acidophilus* (2×10^9 CFU), *L. casei* (7×10^9 CFU), *L. rhamnosus* (1.5×10^9 CFU), *L. bulgaricus* (2×10^8 CFU), *Bifidobacterium breve* (2×10^{10} CFU), *B. longum* (7×10^9 CFU), *Streptococcus thermophilus* (1.5×10^9 CFU), and 100 mg fructo-oligosaccharide with lactose as carrier substances. The placebo (the same substance without bacteria) was packed in identical capsules and coded by the producer to guarantee blinding.

Statistical Analysis

To ensure a normal distribution of variables, histogram and Kolmogrov-Smirnov tests were applied. We used paired-samples t tests to identify within-group differences (before and after intervention). Student's t test was used to detect differences between the two groups (placebo and probiotic supplement). $p < 0.05$ was considered statistically significant. All statistical analyses were performed using the Statistical Package for Social Sciences version 17 (SPSS Inc., Chicago, Ill., USA).

Results

Among individuals in the placebo group, 3 patients (need for antibiotic treatment, n = 1; need for insulin therapy, n = 2) were excluded. Three patients in the multispecies probiotic group were excluded (chronic kidney disease, n = 1; supplement therapy, n = 1; need for insulin therapy, n = 1). Finally, 54 participants (placebo group, n = 27; probiotic group, n = 27) completed the trial (fig. 1).

No serious adverse reactions were reported following the consumption of multispecies probiotic supplements in patients with T2D throughout the study. Mean age and height were not significantly different between the two groups (table 1). Furthermore, patients did not change the type and dosage of the medications they were taking throughout the study. Within-group comparisons revealed no significant changes in weight and BMI after intervention either in the placebo group or in the probiotic group. Baseline weight and BMI as well as their means after intervention were not significantly different between the probiotic and placebo groups.

At the beginning of the study, no significant differences were found between the two groups in terms of dietary intakes. Comparing the dietary intakes in the run-in period and throughout the study separately in each group, we observed no significant within-group differences except for vitamin E in the probiotic group (table 2). Based on the 3-day dietary records throughout the study, no statistically significant difference was seen between the two groups in terms of dietary intakes of energy, fat, saturated fatty acids, polyunsaturated fatty acids, monounsaturated fatty acids, cholesterol, dietary fiber, vitamins E and C, and selenium.

At the study baseline, no significant differences were found between the two groups in terms of biochemical measures except for HbA_1C concentrations, which were significantly higher in the probiotic group compared to placebo (7.71 ± 0.37 vs. $6.35 \pm 0.3\%$, $p = 0.007$). Within-group comparisons of FPG revealed that consumption of probiotic supplements in the intervention group prevented a rise in FPG, while in the placebo group we observed a significant increase in FPG ($p = 0.002$). When the mean changes in FPG were compared between the two groups,

Downloaded by:
University of Pennsylvania
165.123.34.86 - 9/28/2013 9:49:21 PM

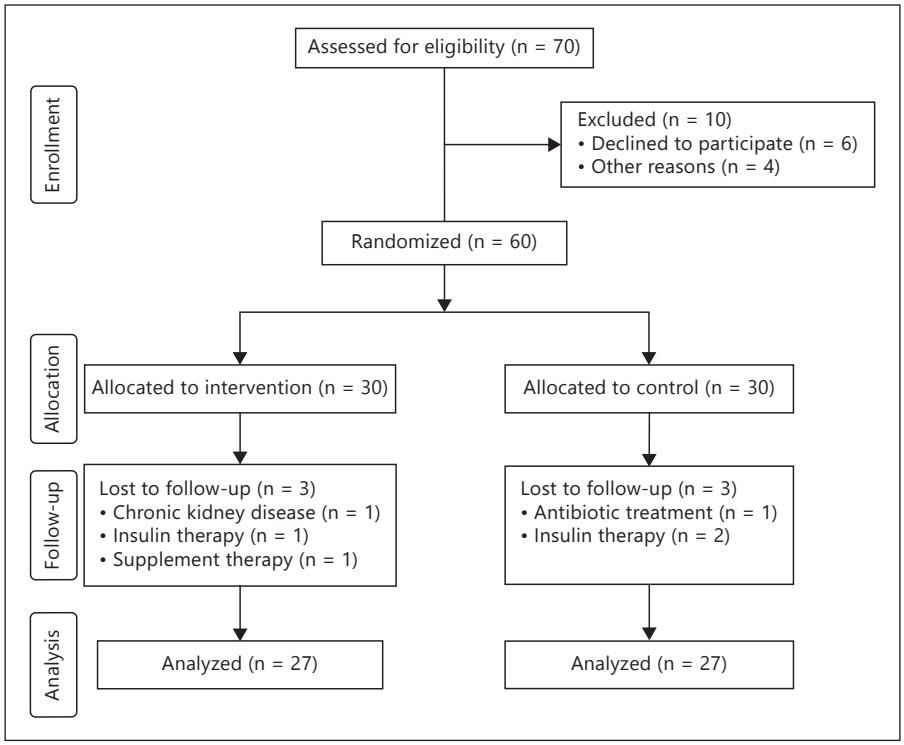

Fig. 1. Summary of patient flow.

Table 1. General characteristics of the study participants

	Placebo (n = 27)	Probiotic supplement (n = 27)	p[a]
Age, years	52.59±7.14	50.51±9.82	0.37
Height, cm	155.59±7.09	154.55±7.64	0.60
Weight at study baseline, kg	73.03±13.3	73.48±13.37	0.55
Weight at the end of the trial, kg	72.42±11.42	73.48±13.37	0.75
BMI at study baseline	30.17±4.23	31.61±6.36	0.33
BMI at the end of the trial	29.91±4.27	30.96±6.39	0.48
Metformin use, n/day	1.9±1	1.8±1.3	0.72
Glibenclamide use, n/day	2.1±1.1	2.2±1	0.70

Data are means ± SD.
[a] Obtained from an independent t test.

there was a statistically significant difference (+28.8 ± 8.5 for placebo vs. +1.6 ± 6 mg/dl for the probiotic group, p = 0.01) (table 3). Despite the significant within-group increase in serum insulin levels in both the probiotic group (p = 0.02) and the placebo group (p < 0.001), we failed to find a significant difference in changes comparing the two groups. For the HOMA-IR score, we observed a significant increase after intervention in both the probiotic group (p = 0.02) and the placebo group (p = 0.001). Comparing the two groups, we found that the increase in HOMA-IR score in the placebo group was significantly greater than that in the probiotic group (+2.38 vs. +0.78, p = 0.03). Although a significant increase in serum LDL-C and a significant reduction in serum HDL-C levels were seen after intervention in both groups (p < 0.05 for both), the changes were similar between the two groups. Within-group differences revealed a trend toward reduction in serum hs-CRP levels in the probiotic group after supplementation (p = 0.09). The mean changes in serum hs-CRP were significantly different between the two groups (–777.57 for probiotic vs. +878.72 ng/ml for the placebo group, p = 0.02). Probiotic supplementation led to a significant increase in plasma GSH levels after intervention (p = 0.02). Comparing the two groups, we found that the increase in plasma GSH levels was significantly different from the changes in the placebo group (240.63 vs. –33.46 μmol/l, p = 0.03). Although within-group differences revealed a significant increase in plasma TAC levels in the placebo group (p = 0.002) and a trend toward elevation in the probiotic group (p = 0.06), we failed to find a significant difference in changes between the two groups.

Downloaded by:
University of Pennsylvania
165.123.34.86 - 9/28/2013 9:49:21 PM

Table 2. Dietary intakes of study participants in the run-in period and throughout the study

	Placebo			Probiotic supplement			p[b]
	run-in (n = 27)	throughout the study (n = 27)	p[a]	run-in (n = 27)	throughout the study (n = 27)	p[a]	
Energy, kcal/day	2,170±268	2,286±299	0.13	2,113±391	2,217±218	0.14	0.39
Fat, g/day	97.9±23.2	101.9±24.4	0.55	103.1±25.6	102.7±18.3	0.93	0.89
SFA, g/day	22.6±4	23.7±5.4	0.45	23.5±3.7	25.1±5.4	0.14	0.39
PUFA, g/day	46±14.8	44.7±14.6	0.74	48.5±13.1	43.6±8.7	0.10	0.75
MUFA, g/day	23.2±6.2	25.6±8.3	0.26	26.7±8.7	28.5±8.1	0.39	0.26
Cholesterol, mg/day	149.9±42.6	165.5±30.4	0.14	150.2±33.4	162.6±31.6	0.11	0.76
Dietary fiber, g/day	15±2.8	16.4±3.1	0.16	14.4±2.6	15.2±3.4	0.18	0.25
Vitamin E, mg/day	14.1±1.7	14.9±2.3	0.14	14.1±1.7	14.9±2.1	0.03	0.93
Vitamin C, mg/day	151.3±76.2	152.6±77	0.95	156.9±64	164.4±63.4	0.58	0.50
Selenium, μg/day	68.5±20.3	72.3±14.7	0.44	69.5±25.2	74.5±16.2	0.28	0.57

Data are means ± SD. SFA = Saturated fatty acid; PUFA = polyunsaturated fatty acid; MUFA = monounsaturated fatty acid. [a] Obtained from a paired t test. [b] Obtained from Student's t test for the comparison of dietary intakes throughout the study between the two groups.

Table 3. Within-group and between-group comparisons of metabolic profiles, hs-CRP, and biomarkers of oxidative stress after supplementation

	Placebo (n = 27)				Probiotic supplement (n = 27)				p[b]
	week 0	week 8	change	p[a]	week 0	week 8	change	p[a]	
FPG, mg/dl	134.5±9.6	163.3±12	28.8±8.5	0.002	143.8±10.7	145.4±9.5	1.6±6	0.80	0.01
$HbA1_C$, %	6.35±0.3	6.53±0.28	0.18±0.31	0.55	7.71±0.37	7.41±0.41	−0.3±0.37	0.42	0.32
Insulin, μIU/ml	5.82±1	9.93±1.51	4.11±0.91	<0.0001	5.7±0.8	7.74±1.11	2.04±0.82	0.02	0.09
HOMA-IR	2.03±0.44	4.41±0.88	2.38±0.65	0.001	1.98±0.33	2.76±0.44	0.78±0.31	0.02	0.03
Total cholesterol, mg/dl	164.6±10.1	177.8±6.6	13.2±8.9	0.15	171.3±9.4	176.2±7.4	4.9±6.9	0.48	0.46
Triglycerides, mg/dl	134±11.7	150.2±11.6	16.2±11.5	0.17	159.5±15.2	160.3±13.7	0.8±10.5	0.93	0.33
LDL-C, mg/dl	84.2±7.5	102.8±5.9	18.6±6.6	0.009	83.4±6.3	97.4±6.4	14±4.6	0.006	0.56
HDL-C, mg/dl	53.6±2.8	44.9±1.7	−8.7±2.2	0.001	56±3	46.8±2	−9.2±2.3	<0.0001	0.83
Total:HDL-C ratio	3.1±0.2	4±0.2	0.9±0.15	<0.0001	3±0.1	3.8±0.2	0.8±0.1	<0.0001	0.30
Hs-CRP, ng/ml	2,107.75±361.79	2,986.47±652.09	878.72±586.44	0.14	2,793.42±617.2	2,015.85±380.16	−777.57±441.7	0.09	0.02
TAC, mmol/l	870.06±30.75	955±28.06	84.94±24.32	0.002	925.3±41.59	1,005.27±40.49	79.97±41.8	0.06	0.91
GSH, μmol/l	750.5±60.72	717.04±40.06	−33.46±69.54	0.63	832.96±68.95	1,073.59±65.76	240.63±101.29	0.02	0.03
Uric acid, mg/dl	4.73±0.27	4.88±0.24	0.15±0.21	0.47	4.71±0.27	5.51±0.28	0.8±0.27	0.008	0.07

Data are means ± SE. [a] Obtained from a paired t test for the within-group comparisons. [b] Obtained from an independent Student's t test for the between-group comparisons.

Discussion

Our study revealed that consumption of multispecies probiotic supplements compared with the placebo for 8 weeks among patients with T2D patients reduced serum hs-CRP levels, increased plasma total GSH, and prevented a rise in FPG. In addition, the HOMA-IR score increased in both groups and the increase in the placebo group was significantly greater than that in the probiotic group. Despite the significant within-group changes in serum insulin, LDL-C, HDL-C, and TAC levels, we found that the effect of probiotic supplements on serum insulin, lipid profiles, uric acid, and TAC levels was similar to that of placebo.

To our knowledge, this study is the first reporting the effect of multispecies probiotic supplements on the meta-

Downloaded by:
University of Pennsylvania
165.123.34.86 - 9/28/2013 9:49:21 PM

bolic profile of diabetic patients. The majority of diabetic patients have dyslipidemia, insulin resistance, increased levels of systemic inflammation and oxidative stress. An abnormal metabolic profile, proinflammatory factors, and oxidative stress in these patients could result in several complications including an increased risk of CVD [6], development and progression of diabetic retinopathy, neuropathy and nephropathy [26], and hypertension [27]. Previously, the effect of probiotics on metabolic profiles in humans has been assessed in hyperlipidemic men [28] and healthy adults [29]. Similar investigations have also been performed in animal models [5, 30]. Although our findings revealed a preventive effect of multispecies probiotics on the rise of FPG, we could not find a prominent beneficial effect of multispecies probiotic supplements on the glycemic control of diabetic patients because it led to a significant increase in serum insulin levels and HOMA-IR; however, compared to the placebo group, these effects were much lower. Earlier studies have reported beneficial effects of probiotics on serum insulin levels and insulin resistance. In a study by Naito et al. [31] on diet-induced obesity mice, oral administration of *L. casei* resulted in an improvement of insulin resistance after 4 weeks. Similar findings have also been reported in several experimental studies [32, 33]. Several strains of bacteria, such as Lactobacilli and Bifidobacterium, have also been shown to improve glucose tolerance and insulin resistance in animal models [32, 34]. In another study by Cani et al. [32], improved glucose tolerance and glucose-induced insulin secretion was seen with the consumption of *Bifidobacterium* species in diabetic mice fed a high-fat diet. The same result was obtained with the consumption of *L. rhamnosus* GG in streptozotocin-induced diabetic rats after 4 weeks [20]. As is clear from the above mentioned studies, most studies have been done on animals and limited data are available among humans. Furthermore, almost all previous studies have used monospecies probiotics. In the current study, increased serum insulin levels and HOMA-IR after intervention in both the probiotic group and the placebo group might be attributed to the medications patients were taking. Although no changes in medication use were seen throughout the study and both groups were matched at study baseline in terms of medication use, the medications were probably insufficient to affect insulin resistance. Furthermore, the dosage of probiotics taken seems inadequate to affect the insulin levels. Low compliance of the participants might also a reason. Beneficial effects of probiotics on FPG might result from alteration of the composition of the gut microbiota [35], their immunomodulatory effects [36], and improvement of intestinal integrity and reduction of the concomitant induction of Toll-like receptor (TLR)-4 signaling [37]. The different findings between our study and others can be explained by the type of probiotics used, the study design, and probably the different nature of the subjects. Further studies are required to determine the effect of multispecies probiotic supplements on the glycemic control of diabetic patients.

Despite a significant within-group increase in serum LDL-C and a decrease in serum HDL-C levels, these changes in lipid profiles were similar between the two groups. In vitro and in vivo studies on probiotics have reported beneficial effects on serum lipid profiles. In a study by Ejtahed et al. [38], consumption of probiotic yogurt containing *L. acidophilus* and *B. lactis* resulted in a nonsignificant decrease in total cholesterol and LDL-C among patients with T2D after 6 weeks. *L. acidophilus*- and *L. casei*-containing food prevented a rise in lipid profiles in diabetic rats [5]. Ingestion of 200 ml/day of a synbiotic shake containing 10^8 CFU/ml *L. acidophilus*, 10^8 CFU/ml *B. bifidum*, and 2 g oligofructose resulted in increased serum HDL-C but did not affect serum total cholesterol and triglycerides among elderly people [39]. However, others failed to find any change in serum lipid profiles with probiotic yogurt consumption in the elderly [40]. Consumption of probiotics also did not affect serum lipid profiles among normo-cholesterolemic [41] and hyperlipidemic men [28]. In our study, increased serum LDL-C and decreased HDL-C levels in the probiotic group might have resulted from the elevation of serum insulin levels and insulin resistance. High insulin levels reduce the normal effects of insulin on lipids and lead to a reduced uptake of circulating lipids and increased hydrolysis of stored triglycerides. Increased mobilization of stored lipids in these cells would result in abnormal lipid profiles [42]. Inconsistencies of our findings with different studies could be explained by differences in the conditions of the studied populations and the discrepancy in probiotic strains and dosages. Furthermore, discrepancies in findings might be attributable to the different cultures used. Some studies that have observed a significant effect on blood lipid profiles have used a dairy product. The beneficial effects of these products on lipid profiles might be explained by the fatty acid distribution and amount of sphingolipids in dairy products versus probiotics [43]. Moreover, the calcium and protein content of dairy products might play a role. Even probiotic-free dairy products have been reported to influence serum lipid profiles. For instance, in a study by Smedman et al. [44], an inverse association between the consumption of

Downloaded by:
University of Pennsylvania
165.123.34.86 - 9/28/2013 9:49:21 PM

milk products and the LDL/HDL ratio was found. Overall, it seems that the effect of probiotics per se on serum lipid profiles might not be significant enough to introduce these microorganisms as lipid-lowering agents.

Our study demonstrated that consumption of probiotic supplements results in a significant reduction of serum hs-CRP levels compared with placebo. Earlier, the effect of probiotics on serum hs-CRP had been reported among immunocompromised patients [45], pregnant women [46], and patients with rheumatoid arthritis [47]. In immunocompromised patients, consumption of a combination of *L. casei*, *B. breve*, and prebiotic galactooligosaccharides [48] as well as *B. longum* [49] has been found to decrease serum hs-CRP levels. The same findings have been reported with the consumption of probiotic yoghurt containing *L. acidophilus* and *B. animalis* among pregnant women after 9 weeks [46], administration of *B.* supplementation among patients undergoing resection for colorectal cancer [22], and probiotic bacteria supplementation among patients with stage 3 and 4 chronic kidney disease after 6 months [50]. In our study, supplementation with multispecies probiotics resulted in a trend toward a decrease in serum hs-CRP levels. A higher dosage of probiotic bacteria and a long period of supplementation might lead to additional benefits. Several mechanisms can explain the effects of probiotics on serum hs-CRP levels. This effect might result from short-chain fatty acids that are produced from probiotics in the colon [51]. Produced short-chain fatty acids can result in decreased enzymatic synthesis of hepatic CRP. Furthermore, decreased serum CRP levels might also result from decreased expression of IL-6. In a study by Hegazy et al. [19], consumption of *L. delbruekii* and *L. fermentum* resulted in decreased expression of IL-6 among patients with ulcerative colitis.

We found that administration of multispecies probiotic supplements significantly increased plasma total GSH levels in diabetic patients; however, it did not affect plasma TAC and serum uric acid levels. Such findings have also been reached with the consumption of *L. fermentum* ME-3 [52] and multispecies probiotics in rats [53]. However, in our previous study in pregnant women [54], consumption of probiotic yogurt containing two strains of *L. acidophilus* LA5 and *B. animalis* BB12 did not lead to a significant effect on GSH compared to conventional yogurt. The difference in findings might be explained by differences in the studied populations. The effects of multispecies probiotics on GSH might result from enhanced glutamate-cysteine-ligase (GCL) activity, increased mRNA expression of GCL subunits, and increased synthesis of GSH [53]. Our findings regarding the effect of probiotics on TAC and serum uric acid are in contrast to those previously reported. Increased TAC levels in healthy volunteers [18] have been reported using different species of probiotics. In relation to serum uric acid, some studies have reported an increased level and others have reported a decreased level following probiotic administration [55]. It seems that the effects of probiotics on biomarkers of oxidative stress are highly strain specific. The lack of a significant effect on plasma TAC and serum uric acid levels in the present study could be explained by the different strains and dosage of bacteria strains we used.

In conclusion, multispecies probiotic supplementation, compared with placebo, for 8 weeks among diabetic patients prevented a rise in FPG and resulted in a decrease in serum hs-CRP and an increase in plasma total GSH.

Acknowledgement

The present study was supported by a grant (No. 8714) from the Vice Chancellor for Research, Kashan University of Medical Sciences, Iran. We are grateful to ZistTakhmir Company, Tehran, Iran, for providing FamiLact® (a multispecies probiotic supplement) for the present study. The authors would like to thank the staff of the Golabchi Diabetes Clinic (Kashan, Iran) for their assistance with this project.

Conflicts of Interest

The authors have no personal or financial conflicts of interest.

References

1 Yan L, Hui G: Subcutaneous implanted system for the treatment of type 2 diabetes. Zhongguo Yi Xue Ke Xue Yuan Xue Bao 2011;33:473–477.
2 Neumann A, Schwarz P, Lindholm L: Estimating the cost-effectiveness of lifestyle intervention programmes to prevent diabetes based on an example from Germany: Markov modelling. Cost Eff Resour Alloc 2011;9:17.
3 Haghdoost AA, Rezazadeh-Kermani M, Sadghirad B, Baradaran HR: Prevalence of type 2 diabetes in the Islamic Republic of Iran: systematic review and meta-analysis. East Mediterr Health J 2009;15:591–599.
4 Ferreira L, Teixeira-de-Lemos E, Pinto F, Parada B, Mega C, Vala H, et al: Effects of sitagliptin treatment on dysmetabolism, inflammation, and oxidative stress in an animal model of type 2 diabetes (ZDF rat). Mediators Inflamm 2010;2010:592760.
5 Yadav H, Jain S, Sinha PR: Antidiabetic effect of probiotic dahi containing *Lactobacillus acidophilus* and *Lactobacillus casei* in high fructose fed rats. Nutrition 2007;23:62–68.

Downloaded by:
University of Pennsylvania
165.123.34.86 - 9/28/2013 9:49:21 PM

6 Shim U, Lee H, Oh JY, Sung YA: Sleep disorder and cardiovascular risk factors among patients with type 2 diabetes mellitus. Korean J Intern Med 2011;26:277–284.
7 Picot J, Jones J, Colquitt JL, Gospodarevskaya E, Loveman E, Baxter L, et al: The clinical effectiveness and cost-effectiveness of bariatric (weight loss) surgery for obesity: a systematic review and economic evaluation. Health Technol Assess 2009;13:1–190, 215–357, iii–iv.
8 Zanuso S, Jimenez A, Pugliese G, Corigliano G, Balducci S: Exercise for the management of type 2 diabetes: a review of the evidence. Acta Diabetol 2010;47:15–22.
9 Huynh K, Kiriazis H, Du XJ, Love JE, Jandeleit-Dahm KA, Forbes JM, et al: Coenzyme Q10 attenuates diastolic dysfunction, cardiomyocyte hypertrophy and cardiac fibrosis in the db/db mouse model of type 2 diabetes. Diabetologia 2012;55:1544–1553.
10 Abdulkadir AA, Thanoon IA: Comparative effects of glibenclamide and metformin on C-reactive protein and oxidant/antioxidant status in patients with type II diabetes mellitus. Sultan Qaboos Univ Med J 2012;12:55–61.
11 Undas A, Ariens RA: Fibrin clot structure and function: a role in the pathophysiology of arterial and venous thromboembolic diseases. Arterioscler Thromb Vasc Biol 2011;31:e88–e99.
12 Lye HS, Kuan CY, Ewe JA, Fung WY, Liong MT: The improvement of hypertension by probiotics: effects on cholesterol, diabetes, renin, and phytoestrogens. Int J Mol Sci 2009;10:3755–3775.
13 Mallappa RH, Rokana N, Duary RK, Panwar H, Batish VK, Grover S: Management of metabolic syndrome through probiotic and prebiotic interventions. Indian J Endocrinol Metab 2012;16:20–27.
14 Kullisaar T, Songisepp E, Mikelsaar M, Zilmer K, Vihalemm T, Zilmer M: Antioxidative probiotic fermented goats' milk decreases oxidative stress-mediated atherogenicity in human subjects. Br J Nutr 2003;90:449–456.
15 Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, Mofid V: Probiotic yogurt improves antioxidant status in type 2 diabetic patients. Nutrition 2012;28:539–543.
16 Lambert JM, Bongers RS, de Vos WM, Kleerebezem M: Functional analysis of four bile salt hydrolase and penicillin acylase family members in *Lactobacillus plantarum* WCFS1. Appl Environ Microbiol 2008;74:4719–4726.
17 Peran L, Camuesco D, Comalada M, Nieto A, Concha A, Adrio JL, et al: *Lactobacillus fermentum,* a probiotic capable to release glutathione, prevents colonic inflammation in the TNBS model of rat colitis. Int J Colorectal Dis 2006;21:737–746.
18 Kullisaar T, Zilmer M, Mikelsaar M, Vihalemm T, Annuk H, Kairane C, et al: Two antioxidative Lactobacilli strains as promising probiotics. Int J Food Microbiol 2002;72:215–224.
19 Hegazy SK, El-Bedewy MM: Effect of probiotics on pro-inflammatory cytokines and NF-kappaB activation in ulcerative colitis. World J Gastroenterol 2010;16:4145–4151.
20 Tabuchi M, Ozaki M, Tamura A, Yamada N, Ishida T, Hosoda M, et al: Antidiabetic effect of *Lactobacillus* GG in streptozotocin-induced diabetic rats. Biosci Biotechnol Biochem 2003;67:1421–1424.
21 Naruszewicz M, Johansson ML, Zapolska-Downar D, Bukowska H: Effect of *Lactobacillus plantarum* 299v on cardiovascular disease risk factors in smokers. Am J Clin Nutr 2002;76:1249–1255.
22 Zhang JW, Du P, Chen DW, Cui L, Ying CM: Effect of viable *Bifidobacterium* supplement on the immune status and inflammatory response in patients undergoing resection for colorectal cancer (in Chinese). Zhonghua Wei Chang Wai Ke Za Zhi 2010;13:40–43.
23 American Diabetes Association: Diagnosis and classification of diabetes mellitus. Diabetes Care 2012;35:S64–S71.
24 Erel O: A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004;37:277–285.
25 Surapaneni KM, Venkataramana G: Status of lipid peroxidation, glutathione, ascorbic acid, vitamin E and antioxidant enzymes in patients with osteoarthritis. Indian J Med Sci 2007;61:9–14.
26 Giacco F, Brownlee M: Oxidative stress and diabetic complications. Circ Res 2010;107:1058–1070.
27 White JR Jr: Economic considerations in treating patients with type 2 diabetes mellitus. Am J Health Syst Pharm 2002;59:S14–S17.
28 St-Onge MP, Farnworth ER, Savard T, Chabot D, Mafu A, Jones PJ: Kefir consumption does not alter plasma lipid levels or cholesterol fractional synthesis rates relative to milk in hyperlipidemic men: a randomized controlled trial (ISRCTN10820810). BMC Complement Altern Med 2002;2:1.
29 Ooi LG, Liong MT: Cholesterol-lowering effects of probiotics and prebiotics: a review of in vivo and in vitro findings. Int J Mol Sci 2010;11:2499–2522.
30 Cavallini DC, Bedani R, Bomdespacho LQ, Vendramini RC, Rossi EA: Effects of probiotic bacteria, isoflavones and simvastatin on lipid profile and atherosclerosis in cholesterol-fed rabbits: a randomized double-blind study. Lipids Health Dis 2009;8:1.
31 Naito E, Yoshida Y, Makino K, Kounoshi Y, Kunihiro S, Takahashi R, et al: Beneficial effect of oral administration of *Lactobacillus casei* strain Shirota on insulin resistance in diet-induced obesity mice. J Appl Microbiol 2011;110:650–657.
32 Cani PD, Neyrinck AM, Fava F, Knauf C, Burcelin RG, Tuohy KM, et al: Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia 2007;50:2374–2383.
33 Li Z, Yang S, Lin H, Huang J, Watkins PA, Moser AB, et al: Probiotics and antibodies to TNF inhibit inflammatory activity and improve nonalcoholic fatty liver disease. Hepatology 2003;37:343–350.
34 Musso G, Gambino R, Cassader M: Obesity, diabetes, and gut microbiota: the hygiene hypothesis expanded? Diabetes Care 2010;33:2277–2284.
35 Membrez M, Blancher F, Jaquet M, Bibiloni R, Cani PD, Burcelin RG, et al: Gut microbiota modulation with norfloxacin and ampicillin enhances glucose tolerance in mice. FASEB J 2008;22:2416–2426.
36 Konstantinov SR, Smidt H, de Vos WM, Bruijns SC, Singh SK, Valence F, et al: S layer protein A of *Lactobacillus acidophilus* NCFM regulates immature dendritic cell and T cell functions. Proc Natl Acad Sci USA 2008;105:19474–19479.
37 Ouwehand AC, Tiihonen K, Saarinen M, Putaala H, Rautonen N: Influence of a combination of *Lactobacillus acidophilus* NCFM and lactitol on healthy elderly: intestinal and immune parameters. Br J Nutr 2009;101:367–375.
38 Ejtahed HS, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, Mofid V, et al: Effect of probiotic yogurt containing *Lactobacillus acidophilus* and *Bifidobacterium lactis* on lipid profile in individuals with type 2 diabetes mellitus. J Dairy Sci 2011;94:3288–3294.
39 Moroti C, Souza Magri LF, de Rezende Costa M, Cavallini DC, Sivieri K: Effect of the consumption of a new symbiotic shake on glycemia and cholesterol levels in elderly people with type 2 diabetes mellitus. Lipids Health Dis 2012;11:29.
40 Lewis S, Burmeister S, Cohen S, Brazier J, Awasthi A: Failure of dietary oligofructose to prevent antibiotic-associated diarrhoea. Aliment Pharmacol Ther 2005;21:469–477.
41 Greany KA, Bonorden MJ, Hamilton-Reeves JM, McMullen MH, Wangen KE, Phipps WR, et al: Probiotic capsules do not lower plasma lipids in young women and men. Eur J Clin Nutr 2008;62:232–237.
42 Ribas V, Nguyen MT, Henstridge DC, Nguyen AK, Beaven SW, Watt MJ, et al: Impaired oxidative metabolism and inflammation are associated with insulin resistance in ERalpha-deficient mice. Am J Physiol Endocrinol Metab 2010;298:E304–E319.
43 Kiessling G, Schneider J, Jahreis G: Long-term consumption of fermented dairy products over 6 months increases HDL cholesterol. Eur J Clin Nutr 2002;56:843–849.
44 Smedman AE, Gustafsson IB, Berglund LG, Vessby BO: Pentadecanoic acid in serum as a marker for intake of milk fat: relations between intake of milk fat and metabolic risk factors. Am J Clin Nutr 1999;69:22–29.
45 Anderson AD, McNaught CE, Jain PK, MacFie J: Randomised clinical trial of synbiotic therapy in elective surgical patients. Gut 2004;53:241–245.

Downloaded by:
University of Pennsylvania
165.123.34.86 - 9/28/2013 9:49:21 PM

46 Asemi Z, Jazayeri S, Najafi M, Samimi M, Mofid V, Shidfar F, et al: Effects of daily consumption of probiotic yoghurt on inflammatory factors in pregnant women: a randomized controlled trial. Pak J Biol Sci 2011;14:476–482.
47 Hatakka K, Martio J, Korpela M, Herranen M, Poussa T, Laasanen T, et al: Effects of probiotic therapy on the activity and activation of mild rheumatoid arthritis – a pilot study. Scand J Rheumatol 2003;32:211–215.
48 Sugawara G, Nagino M, Nishio H, Ebata T, Takagi K, Asahara T, et al: Perioperative symbiotic treatment to prevent postoperative infectious complications in biliary cancer surgery: a randomized controlled trial. Ann Surg 2006;244:706–714.
49 Furrie E, Macfarlane S, Kennedy A, Cummings JH, Walsh SV, O'Neil DA, et al: Synbiotic therapy (*Bifidobacterium longum*/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. Gut 2005;54:242–249.
50 Ranganathan N, Friedman EA, Tam P, Rao V, Ranganathan P, Dheer R: Probiotic dietary supplementation in patients with stage 3 and 4 chronic kidney disease: a 6-month pilot scale trial in Canada. Curr Med Res Opin 2009;25:1919–1930.
51 Sadrzadeh-Yeganeh H, Elmadfa I, Djazayery A, Jalali M, Heshmat R, Chamary M: The effects of probiotic and conventional yoghurt on lipid profile in women. Br J Nutr 2010;103:1778–1783.
52 Kullisaar T, Songisepp E, Aunapuu M, Kilk K, Arend A, Mikelsaar M, et al: Complete glutathione system in probiotic *Lactobacillus fermentum* ME-3. Prikl Biokhim Mikrobiol 2010;46:527–531.
53 Lutgendorff F, Trulsson LM, van Minnen LP, Rijkers GT, Timmerman HM, Franzen LE, et al: Probiotics enhance pancreatic glutathione biosynthesis and reduce oxidative stress in experimental acute pancreatitis. Am J Physiol Gastrointest Liver Physiol 2008;295:G1111–G1121.
54 Asemi Z, Jazayeri S, Najafi M, Samimi M, Mofid V, Shidfar F, et al: Effect of daily consumption of probiotic yogurt on oxidative stress in pregnant women: a randomized controlled clinical trial. Ann Nutr Metab 2012;60:62–68.
55 Hashem MA, Mohamed MH: Haemato-biochemical and pathological studies on aflatoxicosis and treatment of broiler chicks in Egypt. Vet Ital 2009;45:323–337.

Downloaded by:
University of Pennsylvania
165.123.34.86 - 9/28/2013 9:49:21 PM