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Original Article

The effect of probiotic supplementation on glycemic control and lipid profile in patients with type 2 diabetes: A randomized placebo controlled trial

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ABSTRACT

Aims: The role of gut microbiota in the pathogenesis of diabetes is increasing; this study investigates the effect of multi-strain probiotics on fasting plasma glucose (FPG), plasma insulin and lipid profile among patients.

Methods: This randomized double blind controlled trial was performed among 60 patients; individuals were randomly assigned into 2 groups of 30 participants in order to take either probiotic supplements or placebo for 6 weeks. The probiotic supplement consisted of 7 viable strains Lactobacillus, Bifidobacterium and Streptococcus. Nutrient intakes were estimated using a 3-day and 24 hour-dietary recall at the beginning and end of study. Fasting blood samples were taken before and after intervention to measure the levels of FPG, plasma insulin and lipid profiles.

Results: Within group comparisons showed significant decrease and increase in the levels of FPG ($P = 0.001$) and HDL-C ($P = 0.002$) in probiotic group, respectively. No significant alterations were observed for within and between group comparisons in the levels of insulin, triglycerides, total cholesterol, insulin resistance and anthropometric measurements, including weight, waist circumference and body mass index (all $P > 0.05$).

Conclusions: This study showed a significant decrease in FPG level by multi-strain probiotic supplements in within group comparison; though, further studies are needed to confirm results. (IRCT Code: IRCT2013100714925N1).

1. Introduction

The prevalence of diabetes mellitus as one of the main endocrine diseases, has increased worldwide [1]; according to the International Diabetes Federation (IDF), more than 415 million adults had diabetes around the world in 2015 which will rise to 649 million by 2040 [2]. Dyslipidemia is one of the main complications of diabetes mellitus with major abnormalities such as elevated triglycerides (TG) and lower levels of high density of lipoprotein cholesterol (HDL-C), which reported to be particularly important for the development of coronary heart disease and neuropathy [3].

Recent evidence suggests that there is an association between metabolic diseases and gut microbiota; it has been shown that types of bacteria in the gut of patients with diabetes were significantly associated with their glucose concentrations [4]. Based on previous findings, altered intestinal microbiota interacts with

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environmental and genetic factors leading to increased intestinal permeability as well as changing the mucosal immune responses which all result in the development of diabetes [5]; that is to say, the administration of probiotics may improve the prognosis and prevention of diabetes through modulation of gut microbiota [5–7].

Probiotics are live microorganisms that confer health benefits on the host when administered in adequate amounts [8]; a review article reported that probiotic may play a role in the improvement of intestinal health, controlling the gastrointestinal disorders such as constipation, diarrhea or irritable bowel syndrome, enhancement of the immune response, cancer prevention and reduction of serum cholesterol which could possibly contribute to the prevention of coronary heart disease [7]. Meanwhile, the two main strains of probiotics used for health advantages include Lactobacillus and Bifidobacterium; the beneficial role of them in the improvement of glycemic control and diabetes-related conditions including body weight, inflammatory markers and blood pressure has been discussed in many animal and few human studies [9–11]; indeed, animal studies confirmed that Lactobacillus and Bifidobacterium would improve insulin-binding potential and inhibits β-cells destruction in the islets of Langerhans [12]; on the other hand, concerning human trials, few articles with conflicting findings have investigated the effects of multi-strain probiotics on diabetes mellitus using probiotic capsules [13,14]. Despite previous studies that assessed the effects of probiotics containing foods or synbiotics on glycemic disorders [15–17], which were usually followed by other metabolic implications such as dyslipidemia [18] or gestational diabetes mellitus [19], there are limited studies that investigated the efficacies of probiotic alone, on type 2 diabetes [20]; on the other hand, it has been observed that administration of prebiotics, as the food ingredients of probiotic, might retard or reduce the concentrations of advanced glycation end-products in individuals with impaired glucose tolerance [21]. In fact, these previous published healthful efficacies of synbiotics [17,18] were the cooperative effects of probiotics and prebiotics, simultaneously and not the pure effects of probiotics. Nevertheless, more trials are needed to confirm the efficacy of probiotics or synbiotic on glycemic control; thus, the present study was designed to assess the effects of multispecies of probiotic supplements alone, on biochemical markers of patients with type 2 diabetes.

2. Subjects, materials and methods

2.1. Study design and participants

This randomized double blind clinical trial was registered at the Iranian Registry of Clinical trials (NO. IRCT2013100714925N1). A total of 68 patients who were diagnosed with type 2 diabetes mellitus were recruited from endocrinology clinic of Taleghani hospital, Tehran.

Sample size was defined based on primary information in the study by Sadrzadeh-Yeganeh et al. [22]; glycemic and lipid profile variables were used to define the sample size, individually and finally total cholesterol (TC) level was found to be as the key variable which resulted in a maximum determined sample size. For an expected change of 0.25 mmol/L (9.65 mg/dL) between intervention and control groups and by considering $\alpha = 0.05$ and a power of 80%, the sample size was computed to be 29.7 (≈ 30) per group which was increased to 34 to accommodate the expected dropout rate.

Eligible participants consisted of individuals with type 2 diabetes mellitus according to the criteria of American Diabetes Association [23] for at least 10 months prior to the study initiation, aged 30–75 years and without any antibiotic or hormone replacement therapy such as insulin; controlled glucose and lipid profile levels of participants were considered; moreover, taking routine drugs prescribed by doctors were permitted until their dosages were unchanged during the study.

Exclusion criteria were current smokers, individuals on non-steroidal anti-inflammatory drugs and multivitamin or use of any nutritional supplements within the past 3 weeks before the study initiation as well as the presence of liver, kidney, inflammatory or immunodeficiency diseases; thyroid disorders or lactose intolerance; required insulin therapy; use of any kinds of estrogen, progesterone, cholesterol-lowering drugs or diuretics; pregnancy or breast-feeding and consuming any kinds of probiotic products in the past 2 months of testing. Participants, who decided to join the investigation, were asked to sign a consent form, after describing the study procedure by the main researcher.

The present study was approved by the committee of ethics at Qazvin University of Medical Sciences and Shahid Beheshti Endocrinology Research Center; all experiments on participants were conducted in accordance with the Declaration of Helsinki.

68 participants were randomly allocated into 2 groups of 34, using a computer block randomization procedure with block size of 2; the main researchers and all participants were blinded to the contents of the capsules throughout the study procedure and the final analysis. Participants were asked to receive either multispecies probiotic supplements (n = 34, i.e. 14 males and 18 females) or the placebo (n = 34, i.e. 16 males and 16 females). Patients in probiotic or placebo groups received 2 probiotic capsules, one after lunch and one after the evening meal for 6 weeks; they were also asked to keep the capsules in refrigerator after each consumption.

Both probiotic and placebo capsules and packs were identical in terms of appearance, weight, texture and smell and were only differentiated by a code ("A" or "B") that was placed on them; the probiotic supplements (Familact probiotics kindly provided by ZistTakhmir Co., Tehran, Iran) consisted of 7 viable and freeze-dried strains: Lactobacillus acidophilus [2×10^9 colony forming units (CFU)], Lactobacillus casei (7×10^9 CFU), Lactobacillus rhamnosus (1.5×10^9 CFU), Lactobacillus bulgaricus (2×10^8 CFU), Bifidobacterium breve (3×10^{10} CFU), Bifidobacterium longum (7×10^9 CFU), Streptococcus thermophilus (1.5×10^9 CFU), and 100 mg fructo-oligosaccharide with lactose as carrier substances. The placebo capsules consisted of fructo-oligosaccharide and magnesium stearate; magnesium stearate is generally considered safe for human consumption at levels below 2500 mg/kg/day [24]. The most range of bacteria strains used in probiotic products are 10^9–10^{11} CFU/g; it should be note that in healthy humans, Lactobacillus and Bifidobacterium are normally present in the ileum in the range of 10^3–10^7 CFU/g and in the colon in the range of 10^4–10^8 CFU/g of each of strain [25].

Blood samples were taken before and after the 6 weeks of intervention period. Same recommendations were given to the participants by the main researcher at baseline to homogenize their food intake; they were asked not to alter their routine physical activity or their fiber intakes and usual diets; they were also refrained from consuming any other probiotic capsules or probiotic containing foods and fermented products in14 days before and throughout the study.

Compliance with consumption of capsules was monitored every other day through phone interviews that was followed by face to face interviews for once a week; individuals were asked to bring the remaining packs of probiotic or placebo capsules to confirm the compliance of supplement consumption. Nutrient intakes were estimated using a 3-day food diaries and 24-h dietary recall at the beginning and at the end of the study; they were also asked to record their daily intake before each of the two visits to make sure that their food intakes were homogenous during the study time.

For obtaining 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.

2.2. Assessment of variables

Participants were weighted without shoes and heavy clothing using digital scale, with a precision of 0.1 kg and height was measured without shoes to the nearest tenth of a centimeter using a portable stadiometer (Seca model 207 Germany); body mass index (BMI) was calculated by dividing weight (kg) by height square (m^2); waist circumference was measured using a flexible inch tape over the unclothed abdomen, in the standing position from midway between the lowest rib and the iliac crest of participants; fasting Blood samples (10 ml) were taken before and after the 6 weeks of intervention after an overnight 10–12 h fasting between 8 and 9 a.m. The serum samples were separated from whole blood by centrifugation at 3500 rpm for 10 min (Hettich D-78532; Tuttlingen, Germany) and were frozen at −70 °C, immediately until assay. Fasting plasma glucose (FPG) levels were quantified via the glucose oxidase/peroxidase method with commercially available kits (Pars Azmoon Co., Iran). Serum insulin levels were assessed using enzyme-linked immune-assay kits (DiaMetra, Italy).

Serum concentrations of TC, TG, and HDL-C were measured using the standard enzymatic methods with available ParsAzmun kits (Karaj, Iran); TC was assessed using cholesterol oxidase and cholesterol esterase method and TG levels were estimated using glycerol phosphate oxidase; HDL-C concentrations were measured after the precipitation of lipoproteins with apo-lipoprotein B. Finally, the levels of low density of lipoprotein cholesterol (LDL-C) were determined by the Friedewald formula and for more detail information related to calculating the LDL levels according to Friedwald formula, the formula is : LDL-c (mg/dL) = TC (mg/dL) − HDL-c (mg/dL) − TG (mg/dL)/5.

Homeostatic model assessment of insulin resistance (HOMA-IR) was calculated using Matthews et al.'s equation [26].

2.3. Statistical analysis

SPSS software (version 16; SPSS Inc., Chicago, IL) was used to analyze the data and the results were expressed as mean ± standard deviation (SD). Shapiro-Wilk test was used to determine the normality of the distribution of variables. The background characteristics including age, height, sex, drug intakes, diabetes duration and physical activity level as well as nutrient intakes of participants in the 2 groups were compared using independent sample *t*-test and chi-squared test. Per-protocol (PP) analysis was performed for the sensitivity analysis on those individuals who had successfully completed the 6-week intervention with more than 85% of compliance rate and P value of results are presented related to PP analysis. The differences between the 2 groups after the intervention period were determined by analysis of covariance, adjusting for baseline measurements and covariates and dietary intakes including sodium and polyunsaturated fatty acids (PUFA), which might be the confounding factors. Differences in anthropometric measurements, nutrient intakes and biochemical parameters were compared by paired sample *t*-test and Wilcoxon Signed Ranks Test between the beginning and the end of the study; differences with $P < 0.05$ were considered to be statistically significant.

3. Results

A total of 68 patients (32 males and 36 females) with type 2 diabetes were recruited in the study; 8 of the participants were withdrawn during the study (4 individuals in each placebo and probiotic group) as they either needed to have insulin (n = 3) or specific supplement therapies (n = 1) or they did not complete their interventions in the expected time (n = 4), and thus a total of 60 patients remained for the final analysis (Fig. 1). None of the participants reported adverse effects of probiotic supplements; three participants reported higher sexual desire at the end of the trial. Comparing demographic data, there were no statistical differences between probiotic and placebo groups at the beginning of study. Table 1 demonstrates the baseline characteristics of individuals in the 2 groups. The analysis of dietary intakes is also shown in Table 2; as it is demonstrated, there were no statistical differences in the means of nutrient intakes between the 2 groups except for the levels of PUFA ($P = 0.011$) and sodium ($P = 0.02$) in the run in period which were significantly higher and lower in probiotic group than the placebo, respectively. Final analyzes were adjusted for the levels of sodium and PUFA. After the intervention, the levels of energy intake ($P = 0.01$), saturated fatty acid (SFA) ($P = 0.004$) and fiber ($P = 0.04$) were significantly higher in the placebo group than the probiotic group. Final results showed that there were no significant differences either in biochemical measurements including FPG, insulin levels and lipid profiles or in anthropometric measurements including weight, BMI, chest, waist and hip circumferences at the beginning of the study (all $P > 0.05$).

As shown in Table 3, no significant alterations were reported for BMI and weight parameters after the intervention period either in probiotic or in placebo group. Between-group comparisons also showed no significant changes in BMI and weight after the intervention (All $P > 0.05$) (Table 3).

According to Table 4, within group comparisons reported that consumption of probiotic supplement caused a powerful significant decrease in FPG concentrations in probiotic group (13.8 ± 9.6 mg/dL) after the 6 weeks of intervention period ($P = 0.001$), but results of analysis of covariance showed no statistical significant differences in FPG levels in between group comparisons at the end of the study ($P = 0.12$). Similarly, a remarkable significant increase in HDL-C levels was observed in probiotic group (2.1 ± 0.9) after the intervention period ($P = 0.002$), while this increase was not significant in between group comparisons ($P = 0.47$); Table 4 shows that although there was an increase in insulin levels (1 ± 1.7 μU/ml) and a decrease in the levels of TG (6.5 ± 1 mg/dL) and LDL-C (3.6 ± 1.3 mg/dL) in the probiotic group, these results were not statistically significant (all $P > 0.05$).

4. Discussion

We sought to assess the effects of multi-strains probiotic supplement on biochemical factors including FPG, insulin parameters and lipid profile among patients with type 2 diabetes; our study revealed that consumption of probiotic supplement, which consisted of seven kinds of bacteria strains, caused a significant decrease and increase in FPG and HDL-C concentrations, respectively.

Previous inconsistent human investigations have assessed the effects of either probiotic products such as yogurt [16,27] or synbiotic supplements [18] or products such as sourdough bread on diabetes mellitus [18,28,29] or gestational diabetes mellitus (GDM) [30–32]. To our knowledge, only 3 previous human studies assessed the effects of probiotic supplements, in the forms of tablet or capsule, on glucose parameters among patients with type 2 diabetes [14,20].

Contrary to our findings, Lindsay et al. in their recent trial showed that using a single strain probiotic capsule containing Lactobacillus salivarius in a daily dose of 10^9 CFU among 149 pregnant women with abnormal glucose tolerance, had no significant effects on their FPG levels, while the related rise in their TC

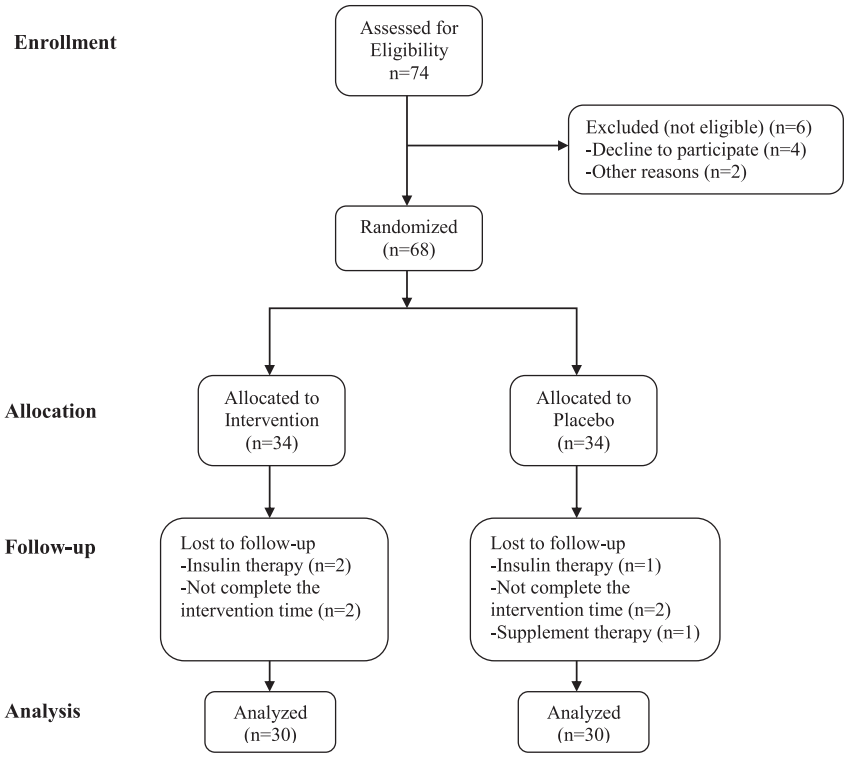

Fig. 1. CONSORT flowchart of trial.

Table 1
Baseline characteristics of study participants.

	Probiotic (no = 30)	Placebo (no = 30)	P-Value
Age (Year)	58.6 ± 6.5	61.3 ± 5.2	0.09
Sex: Female/Male [n (%)]	13 (43.3%)/17 (56.7%)	14 (46.7%)/16 (53.3%)	0.44
Height (Cm)	164.3 ± 9.2	165.4 ± 9.1	0.64
Diabetes duration (Year)	6.2 ± 3.1	5.9 ± 2.9	0.69
Physical Activity (%)			0.86
High	3 (10%)	3 (10%)	
Moderate	16 (53.3%)	13 (43.3%)	
Low	11 (36.7%)	14 (46.7%)	
Drug Intakes (%)			0.27
Metformin	14 (46.7%)	11 (36.7%)	
Glibenclamide	0	3 (10%)	
Others	16 (53.3%)	16 (53.3%)	

Data are presented as Mean + SD and Frequency (Percent).

and LDL-C levels was attenuated after the 6 weeks of intervention period [30]. Similarly, in another study by Asemi et al. the effects of daily consumption of probiotic yoghurt were assessed on insulin resistance and serum insulin levels among Iranian healthy pregnant women; they reported that consumption of 200 g/day of probiotic yogurt enriched with Lactobacillus acidophilus LA5 and Bifidobacterium animalis BB12 in a daily dose of 1×10^7 CFU/day, did not alter the FPG levels, however, it caused significant increase and decrease in fasting insulin levels and insulin resistance, respectively, which may prevent the development of insulin resistance and GDM [28]. One might say that as GDM has been proved to be caused by the hormonal changes and metabolic demands of pregnancy, along with genetic and environmental factors [33], it would be different from other types of diabetes and the related results of investigations.

With respect to studies using probiotic carriers [9,15,27,28,34], Ejtahed et al. assessed the effects of probiotic yogurt enriched with Lactobacillus acidophilus La5 and Bifidobacterium lactis BB12 in a daily dose of 300 g of 600×10^6 CFU/g for 6 weeks [34]; in contrast to our findings, they reported significant reductions in FPG, LDL-C and TG levels in within and between group comparisons [34], while they did not observe any significant augmentation in HDL-C levels [15]; similarly to what Ejtahed et al. have done, Mohammadshahi et al. exactly investigated the same probiotic yogurt with the same dose but for 8 weeks of intervention period; Mohammadshahi et al. were not able to show any significant changes in FPG levels, while, similar to our results, they reported significant increase in HDL-C levels [9,16]; moreover, in line with our findings, these two studies were not able to show any significant alterations in the levels of insulin [16] or HOMA-IR [34].

Importantly, according to previous studies [14,35], dairy products enriched with probiotics have distinct efficacies due to their protein, calcium and sphingolipid contents that lead to a decrease in biochemical markers, indicating that the effect of probiotics alone on lipid profile might not be significant enough to introduce them as lipid-lowering agents [35]; contrary to these researches,

Table 2
Dietary intakes of participants throughout the study.

	Probiotic			Placebo			P-Value Between groups	
	Before	After	P-Value (within group)	Before	After	P-Value (within group)	Before	After
Energy (Kcal)	1970.5 ± 448.1	1830.7 ± 252.4	0.05	2014.6 ± 408.5	2047.4 ± 369.2	0.55	0.69	0.01*
Carbohydrate (gr)	250.7 ± 64.4	228.8 ± 45.7	0.14	242.1 ± 65.2	256.1 ± 72.9	0.18	0.61	0.09
Protein (gr)	79.5 ± 36.9	74.7 ± 25.2	0.53	74.2 ± 25.4	81.6 ± 29.9	0.25	0.52	0.34
Lipid (gr)	74.4 ± 35.1	72.1 ± 27.3	0.68	85.3 ± 25.1	81.1 ± 21.8	0.44	0.17	0.16
SFA (gr)	20.7 ± 7.7	18.4 ± 4.8	0.12	20.5 ± 6.6	22.5 ± 5.7	0.14	0.93	0.004*
PUFA (gr)	24.8 ± 22.7	29.9 ± 20.4	0.28	38.9 ± 18.7	32.6 ± 13.8	0.11	0.011*	0.55
MUFA (gr)	18.2 ± 8.9	15.9 ± 6.4	0.17	20.3 ± 8.3	20.0 ± 6.9	0.89	0.36	0.02
Fiber (gr)	9.6 ± 5.4	7.5 ± 4.9	0.003	10.6 ± 5.6	10.6 ± 5.6	0.59	0.49	0.008*
Serum Cholesterol[a] (mg)	188.8 ± 127.9	198.4 ± 140.1	0.74	191.9 ± 88.6	199.4 ± 101.5	0.79	0.68	0.14
Vitamin A (μg)	523.3 ± 322.6	595.4 ± 353.1	0.41	636.5 ± 397.1	621.7 ± 410.9	0.84	0.23	0.79
Vitamin E (α-tocopherol) (IU)[a]	15.1 ± 15.0	17.9 ± 15.6	0.10	16.8 ± 12.9	17.0 ± 7.6	0.48	0.29	0.79
Vitamin D (μg)[a]	2.0 ± 2.5	2.5 ± 3.9	0.75	2.9 ± 2.1	2.6 ± 1.5	0.06	0.11	0.58
Vitamin K (μg)[a]	123.1 ± 120.9	124.6 ± 100	0.96	125.1 ± 121	127.8 ± 105	0.91	0.92	0.81
Vitamin C (mg)[a]	66.5 ± 65.2	91.5 ± 71.4	0.002	68.7 ± 54.3	91.7 ± 75.6	0.03	0.77	0.98
Vitamin B12 (μg)	2.8 ± 1.8	2.9 ± 1.0	0.56	2.9 ± 1.7	3.2 ± 1.2	0.54	0.75	0.55
Iron (mg)	14.6 ± 4.1	14.2 ± 3.0	0.62	14.4 ± 5.3	14.7 ± 3.9	0.76	0.91	0.56
Zinc (mg)	8.1 ± 2.1	8.7 ± 3.0	0.44	9.1 ± 3.1	8.5 ± 2.3	0.41	0.18	0.76
Magnesium (mg)	248.2 ± 62.7	250.1 ± 58.0	0.9	240.7 ± 66.3	251.6 ± 61.1	0.42	0.66	0.92
Manganese (mg)	9.3 ± 4.6	9.4 ± 3.5	0.98	8.6 ± 3.4	7.9 ± 3.4	0.5	0.48	0.13
Calcium (mg)	774.9 ± 246.4	729.8 ± 267.9	0.42	740.1 ± 313.8	875.6 ± 342.5	0.013	0.64	0.07
Sodium (mg)	2407.3 ± 998.7	2049.7 ± 782.2	0.036	1834.2 ± 868.9	2314.5 ± 794.7	0.001	0.02*	0.19
Selenium (mg)[a]	0.03 ± 0.02	0.04 ± 0.04	0.85	0.03 ± 0.01	0.04 ± 0.02	0.08	0.18	0.47

Abbreviation: SFA: Saturated Fatty Acids, PUFA: Poly unsaturated Fatty Acids, MUFA: Mono unsaturated Fatty Acids.
*Significant difference ($p < 0.05$).
[a] Data are presented as mean ± SD and Median ± IQR.

Table 3
Effects of a 6-week intervention period of probiotic and placebo supplementation on anthropometric measurements.

	Probiotic			Placebo			P-Value Between group	
	Before	After	P-Value (within group)	Before	After	P-Value (within group)	Before	After
Weight (kg)[a]	75.2 ± 15.6	74.6 ± 15.7	0.14	74.1 ± 9.2	73.8 ± 8.9	0.38	0.73	0.79
Chest circumference (cm)[a]	92.4 ± 11.1	91.1 ± 10.2	0.73	95.2 ± 8.9	94.1 ± 8.3	0.81	0.29	0.23
Hip circumference (cm)[a]	102.7 ± 10.9	102.7 ± 10.5	0.92	105.7 ± 10.5	104.8 ± 10.3	0.92	0.27	0.43
Waist circumference (cm)[a]	102.5 ± 12.2	101.5 ± 11.8	0.43	104.9 ± 10.3	104.1 ± 10.6	0.13	0.42	0.37
BMI(kg/cm^2)[a]	27.7 ± 4.2	27.4 ± 4.2	0.10	27.2 ± 4.2	27.1 ± 4.2	0.44	0.68	0.76

Abbreviation: BMI: Body Mass Index.
[a] Data are presented as mean ± SD.

Table 4
Effects of a 6-week intervention period of probiotic and placebo supplementation on blood biochemical factors.

	Probiotic			Placebo			P-Value (Between group)	
	Before	After	P-Value (Within group)	Before	After	P-Value (Within group)	Before	After
FPG (mg/dl)[a]	145.5 ± 40.7	131.7 ± 31.1	0.001*	146.5 ± 34.2	146.1 ± 34.6	0.94	0.97	0.12
TC (mg/dl)[a]	149.3 ± 36.3	151.4 ± 35.2	0.52	155.2 ± 33.9	153.9 ± 38.5	0.75	0.52	0.80
TG (mg/dl) [a]	141.8 ± 62.3	135.3 ± 61.3	0.13	133.6 ± 41.3	138.9 ± 42.4	0.44	0.55	0.79
HDL-C (mg/dl) [a]	44.2 ± 11.7	46.3 ± 10.8	0.002*	44.6 ± 6.0	44.5 ± 6.9	0.83	0.85	0.47
LDL-C (mg/dl) [a]	78.8 ± 25.1	75.2 ± 23.8	0.19	79.8 ± 23.9	78.1 ± 27.3	0.51	0.74	0.61
Insulin (μU/ml) [b]	7.4 ± 6.3	9.4 ± 5.6	0.53	9.2 ± 8.1	9.9 ± 9.7	0.47	0.52	0.98
Insulin resistance[b] **(HOMA-IR)**	2.6 ± 2.9	3.0 ± 2.3	0.47	3.4 ± 2.2	3.9 ± 2.7	0.49	0.63	0.43

Abbreviation: FPG: Fasting Plasma Glucose, TC: Total Cholesterol, TG: Triglyceride, HDL: High Density of Lipoprotein, LDL: Low Density of Lipoprotein, HOMA-IR: Homeostatic Model Assessment of Insulin Resistance.
*Significant difference ($p < 0.05$).
[a] Data are presented as mean ± SD.
[b] Data are presented as Median ± IQR.

we did not use any carrier food for bacteria strains in our study in order to assess the exact effect of probiotics.

Several previous studies assessed the efficacies of synbiotics on type 2 diabetes mellitus, as well [14,18,36,37]. Contrary to our findings, in another study by Asemi et al. who used synbiotic food consisted of viable and heat-resistant Lactobacillus sporogenes (1×10^7 CFU) and 0.04 g of inulin as prebiotic, no significant changes were reported in glucose or lipid profile parameters except

for an increase in insulin levels [17], while other human [38] or animal [37] studies mentioned no significant alteration in any of these parameters. It has been observed that prebiotics per se, would selectively stimulate the growth of beneficial bacteria in the human colon and thus might offer protection against the pathology related to the advanced glycation end-products in individuals who are at risk of developing type 2 diabetes [21]; as a result, these synbiotic related studies might have differences in the outcomes compared to the related probiotic investigations.

Regarding studies that assessed the effects of probiotic supplements on type 2 diabetes, Asemi et al. used a dosage of 10^{10} CFU/day of probiotic supplement, which contained the same strains in our study [14]; contrary to our result, they only reported a significant prevention in an increase in FPG levels after the intervention time, while they could not find any reductions in this parameter [14]. Notably, compared to Asemi et al. who used 1 capsule per day for 8 weeks of treatment period [14], we used 2 capsules of probiotics per day and for 6 weeks of intervention duration.

Furthermore, Mazloom et al. who used a daily dosage of 3000 mg of probiotic capsules containing L. acidophilus, L. bulgaricus, L. bifidum, and L. casei for 6 weeks, could not report any significant alteration in glucose measurements [20]. Compared to the probiotic supplement used in our study, Mazloom et al. used a lower dosage and limited strains of bacteria, which might cause their insignificant results.

On the other hand, in contrasts to our trial, Andreasen et al. used lower dosage and shorter treatment period of single strain probiotic capsules and were not able to report any significant results in glucose parameters except for the levels of insulin sensitivity which was calculated by M/I ratio; they also failed to reach any significant results in HOMA-IR levels. Astonishingly, in a recent trial by Firouzi et al. that was taken for 12 weeks of intervention among 136 patients with diabetes, it was shown that administration of 2 strains (Lactobacillus and Bifidobacterium) of probiotic supplements caused no significant changes in FPG or lipid profiles; although they had a longer period of treatment, they used a lower dose of probiotics compared to ours and in sachet form of intake which needed to get mixed with water before consumption [39]; contrary to our results, Firouzi et al. were able to report significant decrease in HOMA-IR levels in between group comparisons, which was due to the difference of increased and decreased measures of HOMA-IR in placebo and probiotic group, respectively, while this decrease was not significant in probiotic group alone [39].

Thus, to our knowledge our study is the first one that reveals the reducing effect of probiotic supplements on FPG levels, compared to previous published results. It can be deduced that using higher dosages of probiotics might cause better effects on FPG levels than using longer duration; however according to our recent systematic review article, main gut bacteria strains which are lactobacillus sub-strains, have healthy effects on FPG levels if they are administered in long-term or in high but healthy dosages; on the other hand, administrating a moderate-to-high doses of a mixture of probiotics can have better effects on lipid profiles than their very long term periods, with or without dairy products [40].

The exact mechanisms by which probiotics have effects on glucose parameters and lipid profiles are unknown. Honda et al. conducted an animal study and they believed that the effects of probiotic on rats with diabetes, differ depending on whether the bacterium is viable when it arrives in the intestine; they eventually found that only viable L. rhamnosus GG suppressed the elevated FPG compared to the heat treated L. rhamnosus GG [41]; thus, one advantage of our study was that we used active bacteria capsules which needed to get kept in the refrigerator for their proper function. Concerning other mechanism, Yoshida et al. concluded that a reduction in hepatic glycogen storage and a Bacteroides fragilis-mediated vitamin K2-dependent pathway in high fructose fed rats might be possible mechanisms for the reduction in FPG levels, since B. fragilis produces vitamin K2 and vitamin K has been proved to play a beneficial role in humans with insulin resistance and type 2 diabetes [42].

Concerning the effects of probiotics on lipid profiles, inconvenience results have been published; similarly to our findings, Ejtahed et al. reported no significant changes in the levels of TC [15]; however, Tonucci et al. who used fermented milk for a daily dose of 120 mg/day, reported a significant decrease in TC and LDL-C levels in between group comparisons which was not significant in within group analysis [43]; moreover, contrary to our findings, Asemi et al. reported significant reduction in HDL-C levels via administrating the probiotic capsules [14]. In the synbiotic study by Firouzi et al. a significant increment in HDL-C levels was observed [39]. HDL-C is termed as the beneficial cholesterol because of its role of transporting cholesterol in the form of cholesteryl esters to the liver for further hydrolysis; thus, the it has been hypothesized that probiotics or synbiotics could induce a hypocholesterolemic effect via altering the pathways of cholesteryl esters and lipoprotein transporters [44].

The lipid lowering mechanisms of probiotics were reported to be the enzymatic deconjugation of bile acids, the assimilation of cholesterol by the cell membrane of probiotics and more importantly the production of short chain fatty acids by probiotics upon fermentation which could decrease the cholesterol synthesis.

Concerning other factors in the management of hyperglycemia, it has been proved that inflammation and oxidative stress are major mechanisms for the development of complications of diabetes mellitus [45]; previous studies revealed that inhibition and reduction of pro-inflammatory factors including TNF-α, IL-6 and IL-1^9 as long as the augmentation in antioxidant biomarkers including superoxide dismutase and glutathione peroxidase through probiotic interventions [27], are substantial factors for beneficial effects of probiotics on type 2 diabetes.

One advantage of our study is that we used high dose of probiotic-containing capsules, without any food carriers or prebiotics, to assess the exact effects of probiotics on diabetes mellitus; however, we did not perform the stool analysis to determine the precise alterations in the amount of bacteria in the gut; measuring microbial profile in the stool of participants, before and after the intervention, will provide assurance in determining that the observed improvements in glycemic controls were mediated by probiotics. Length of the study is another area of concern; many complications of diabetes mellitus usually develop in a longer period of time and our 6-week intervention study, which was determined based on previous studies, may not be that enough to improve all of the conditions. Hence, further human trials with gut micro-biota examinations are needed to investigate the precise mechanisms among patients with type 2 diabetes; furthermore, unlike previous meta-analysis [46], a very precise and powerful meta-analysis of probiotic supplements, which does not include any prebiotic or probiotic carrier, is needed to determine the proper role of probiotics on glycemic parameters.

5. Conclusion

Our study revealed that consumption of probiotic supplement for 6 weeks, which consisted of seven kinds of bacteria strains, caused significant decrease and increase in FPG and HDL-C concentrations in patients with type 2 diabetes compared to the baseline levels. Despite the increased fasting plasma insulin in probiotic group in within group comparison, the effects of probiotic on insulin levels and insulin resistance in the intervention group were similar to that of placebo group; to our knowledge, our study

is the first one reporting the reducing effects of probiotic supplements on FPG levels in within group comparisons.

Consequently, as the incidence of diabetes mellitus and its comorbidities are increasing at an alarming rate, focusing on such beneficial natural factors, alongside with drug therapy, may lead to promises for alleviating the development of non-communicable diseases. Yet, further studies are needed to confirm our findings and determine possible underlying mechanisms.

Conflicts of interest

The authors declare that they have no conflict of interests.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.dsx.2018.08.008.

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