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A Randomized Controlled Clinical Trial Investigating the Effect of Synbiotic Administration on Markers of Insulin Metabolism and Lipid Profiles in Overweight Type 2 Diabetic Patients with Coronary Heart Disease

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ABSTRACT

Objective: The current study was performed to evaluate the effects of synbiotic administration on metabolic profiles in overweight diabetic patients with coronary heart disease (CHD).

Methods: This randomized, double-blind, placebo-controlled trial was done among 60 diabetic patients with CHD. Participants were randomly divided into 2 groups: group A (n = 30) received synbiotic supplements containing 3 probiotic bacteria spices Lactobacillus acidophilus 2×10^9, Lactobacillus casei 2×10^9, Bifidobacterium bifidum 2×10^9 CFU/g plus 800 mg inulin and group B (n = 30) received placebo for 12 weeks. Fasting blood samples were taken at baseline and after 12-week intervention to determine metabolic profiles.

Results: After 12 weeks of intervention, patients who consumed synbiotic capsule had significantly decreased fasting plasma glucose (− 19.6 ± 74.6 vs. + 19.2 ± 66.9 mg/dL, P = 0.03), serum insulin concentrations (− 0.7 ± 5.1 vs. + 3.3 ± 6.3 μIU/mL, P = 0.01), the homeostasis model of assessment-estimated b cell function (− 3.4 ± 19.5 vs. + 11.5 ± 21.0, P = 0.006) and increased the quantitative insulin sensitivity check index (+ 0.002 ± 0.01 vs. − 0.01 ± 0.02, P = 0.03) compared with the placebo. In addition, changes in HLDL-cholesterol levels (+ 1.8 ± 5.7 vs. − 2.2 ± 6.0 mg/dL, P = 0.01) in supplemented patients were significantly different from those of patients in the placebo group.

Conclusion: Synbiotic supplementation for 12 weeks among diabetic patients with CHD had beneficial effects on markers of insulin metabolism and HDL-cholesterol levels.

Introduction

The prevalence of type 2 diabetes mellitus (T2DM) has increased dramatically worldwide and this increase accompanies by approximately 2-fold increment in the risk of coronary heart disease (CHD) [1]. Impaired insulin metabolism in patients with T2DM predisposes them to CHD through dyslipidemia [2]. Therefore, tight control of lipid profiles and parameters of glucose homeostasis could effectively decrease the morbidity and mortality rate of CHD in patients with T2DM [3]. Adherence to the therapeutic lifestyle change diet (TLC) and regular physical activity is the important part of the CHD managing [4]. However, therapies with new approaches such as the intestinal microbiota modulation can be considered alongside the available interventions to reduce the risk of CHD [5]. The gut microbiota modulation can be done through oral administration of the alive beneficial bacteria like Lactobacillus and Bifidobacterium in the form of probiotics or through providing die-

tary prebiotic that is indigestible oligosaccharides resulted in the beneficial changes of the composition and activity of the intestinal microbiota [6, 7]. To effectively modulate gut microbiota, dietary synbiotic is used that contains both probiotics and prebiotics [8].

There is a growing body of evidence pointing to the important roles of the probiotics, prebiotics and synbiotics in human metabolic regulation [9]. They could affect glucose metabolism and lipid profiles via mechanisms such as energy storage and expenditure from diet, regulation of lipid and cholesterol synthesis, short chain fatty acids production (SCFA), gut hormone balance, improvement in insulin resistance and immune function [10, 11]. Some previous studies showed that probiotic intake could significantly reduce total- and LDL-cholesterol nearly similar to that of the existing TLC interventions [5]. In addition, a recent meta-analysis study reviewing 13 trials revealed that prebiotic supplementation reduced serum total-, LDL-cholesterol and triglycerides concentrations and also increased HDL-cholesterol levels in adult patients with overweight and obesity [12]. In addition, synbiotic supplementation led to decreased concentrations of plasma fasting insulin and triglycerides [13]. Based on the pervious findings, synbiotic supplementation has greater effects on the intestinal microbiota and immune system than the intake of probiotic and prebiotic, alone [14, 15].

It is confirmed that the gut microflora differs between diabetic and nodiabetic patients and also between lean and obese subjects, both in composition and function [16, 17]. Therefore, it seems that synbiotic supplementation might be beneficial in the prevention and treatment of CHD in T2DM patients with overweight. However, in view of the limited number of clinical trials in this regard, present study was designed to investigate the effects of synbiotic consumption on markers of insulin metabolism and lipid profiles in diabetic patients with CHD in a randomized controlled trial model.

Materials and Methods

Participants

The present study was a randomized double-blind placebo-controlled trial that was prospectively registered at the Iranian registry of clinical trials (http://www.irct.ir: IRCT201503025623N37). Patients with T2DM, overweight (BMI ≥ 25) aged 40–85 years old and with stable CHD condition were recruited from the cardiology clinic of the Kashan University of Medical Sciences (KUMS), Kashan, Iran, between March-June 2015. Based on the criteria of the American Diabetes Association [18], subjects who had one out of 3 of the following criteria were diagnosed with T2DM: fasting plasma glucose (FPG) ≥ 126 mg/dL, blood glucose 2-h pp ≥ 200 mg/dL, and HbA1C ≥ 6.5 %. In addition, stable CHD status was confirmed in patients who had one or more of the following criteria: a history of myocardial infarction, a document of at least 50 % stenosis in one or more coronary vessels under cardiac catheterization assessed by the angiography, having an exercise-induced ischemia by treadmill electrocardiogram or nuclear perfusion stress imaging and a history of coronary revascularization [19]. Exclusion criteria were as follows: intake of probiotic and synbiotic supplements within the last 3 months, an acute myocardial infarction within the past 3 months, a cardiac surgery within the past 3 months and a major renal or liver failure. To calculation of sample size, we used the detail of Asemi et al. [13] study. Totally, 25 subjects were selected for each arm of our trial with 1.96 as SD and 1.57 as mean change for homeostasis model of assessment-estimated insulin resistance (HOMA-IR). We considered 0.05 and 0.2 (80 % power) as type I and type II error, respectively. In order to cover possible dropouts, 30 subjects were added to each group to reach the 30 for final sample size.

Ethics statements

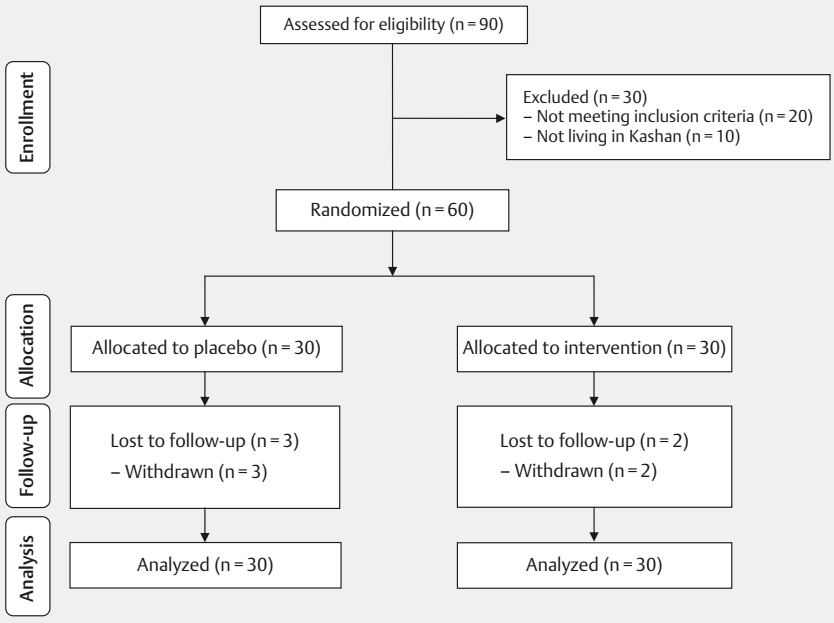
The current study protocol was confirmed to the principals of the Declaration of Helsinki and approved by the ethics committee of KUMS (reference number: 93209). All subjects signed the written informed consent prior to participation.

Study design

At the beginning of the study, participants were first matched one-by-one according to pre-intervention BMI (25–29.9 and ≥ 30 kg/m^2) and age (< 60 and ≥ 60 y), gender and the dosage and kind of medications. Patients were randomly assigned to intervention (n = 30) or placebo group (n = 30) to receive either a synbiotic capsule or placebo for 8 weeks, respectively. Synbiotic capsules were contained 3 probiotic bacteria spices Lactobacillus acidophilus 2×10^9, Lactobacillus casei 2×10^9, Bifidobacterium bifidum 2×10^9 CFU/g plus 800 mg inulin and manufactured by Tak Gen Zist Pharmaceutical Company (Tehran, Iran). Placebos (starch) were similar in color, shape, size and package to the synbiotic capsules and also produced by the same pharmaceutical company. Computer-generated random numbers were used for random assignment. Randomization and allocation were concealed from the researcher and subjects until the statistical analyses were completed. At the cardiology clinic, a trained nutritionist enrolled and assigned the patients to the trial groups based on the randomized sequences. Subjects were advised to maintain their life style habits such as usual diet and levels of physical activity during the study period. Compliance to the trial protocol was assessed by unused containers of the synbiotic and placebo capsules which were returned to the researchers. In addition, we sent a reminder on subjects' cell phones regarding consumption of supplements. 3 dietary records (2 week days and one weekend) at weeks 3, 6 and 9 of the trial were obtained from each participant. We used modified Nutritionist IV software (First Databank, San Bruno, CA) to calculate average daily nutrient intakes of patients. In our study, physical activity was defined as metabolic equivalents (METs) in hours per day. To measure the METs for each subject, we multiplied the times (in hour per day) reported for each physical activity by its related METs coefficient by standard tables [20].

Assessment of anthropometric measures

Weight and height (Seca, Hamburg, Germany) were measured without shoes in light clothing in the cardiology clinic by a trained nutritionist, at baseline and at the end of the study. BMI was calculated as weight (kg) divided by height squared (m^2). All anthropometric measures were done by a trained nutritionist. Furthermore, nutritionist was blinded to the randomization assignments.



▶ **Fig. 1** Summary of patient flow diagram.

Primary and secondary outcomes

In the current study, we considered insulin metabolism parameters as primary outcomes and lipid profiles as secondary outcomes.

Biochemical assessment

10 mL blood samples were collected from each participant after 10–12 h overnight fast, pre- and post-study at Kashan reference laboratory. Then, the samples were centrifuged and stored at − 80 °C until further analyzed. FPG was quantified on the day of blood collection. Fasting insulin levels were measured by enzyme-linked immunosorbent assay (ELISA) method (Monobind, California, USA) with intra- and inter-assay coefficient variances (CVs) of 2.9 and 4.7 %, respectively. HOMA-IR, β-cell function (HOMA-B) and the quantitative insulin sensitivity check index (QUICKI) were calculated based on the suggested formulas [21]. FPG levels were quantified by the glucoseoxidase method (Pars Azmoon Co, Tehran, Iran). Serum total-, LDL-, HDL-, VLDL-cholesterol and triglycerides concentrations were determined using enzymatic kits (Pars Azmoon Co, Tehran, Iran). All inter- and intra-assay CVs for FPG and lipid concentrations were less than 5 %. Measurements of insulin and lipid concentrations were conducted in a blinded fashion, in duplicate, in pairs (pre/post-intervention) at the same time, in the same analytical run, and in random order to reduce systematic error and inter-assay variability.

▶ **Table 1** General characteristics of study participants.

	Placebo group (n = 30)	Synbiotic group (n = 30)	P[1]
Age (y)	64.0 ± 11.7	64.2 ± 12.0	0.94
Height (cm)	158.5 ± 10.8	156.4 ± 6.8	0.39
Weight at study baseline (kg)	74.3 ± 13.7	79.2 ± 15.4	0.20
Weight at end-of-trial (kg)	74.5 ± 13.9	79.1 ± 15.4	0.23
Weight change (kg)	0.2 ± 1.6	− 0.1 ± 1.2	0.52
BMI at study baseline (kg/m^2)	29.6 ± 4.6	32.3 ± 6.0	0.05
BMI at end-of-trial (kg/m^2)	29.7 ± 4.7	32.3 ± 6.1	0.06
BMI change (kg/m^2)	0.1 ± 0.6	− 0.01 ± 0.5	0.51
MET-h/day at study baseline	26.7 ± 1.9	26.4 ± 1.9	0.53
MET-h/day at end-of-trial	26.7 ± 2.1	26.4 ± 1.9	0.65
MET-h/day change	− 0.01 ± 1.0	0.05 ± 0.8	0.76

Data are means ± SDs

[1] Obtained from independent t test. METs, metabolic equivalents

Statistical methods

Normal distribution of the variables was determined by Kolmogrov-Smirnov test. In the current study, all variables had normal distribution. The intention-to-treat (ITT) analysis of the primary study end-point was done for all of the randomly allocated participants. To detect differences in the general characteristics, and dietary nutrient intakes between the 2 groups, independent samples student's t-test was used. To compare within-group differences (before and after treatment), we used paired-samples t-tests. One-way repeated measures ANOVA was used to determine the effects of synbiotic consumptions on glucose homeostasis parameters and lipid profiles. To control for confounders, ANCOVA test was used to compare the mean changes of the outcome variables between the groups while adjusting for baseline values, age and baseline BMI.

▶ **Table 2** Dietary intakes of study participants throughout the study.

	Placebo group (n = 30)	Synbiotic group (n = 30)	P[1]
Energy (kcal/d)	2 159 ± 230	2 171 ± 244	0.85
Carbohydrates (g/d)	291.5 ± 47.0	293.9 ± 60.2	0.86
Protein (g/d)	80.5 ± 15.6	82.6 ± 17.6	0.60
Fat (g/d)	78.0 ± 13.5	77.6 ± 14.4	0.90
SFAs (g/d)	23.7 ± 5.2	23.7 ± 5.9	0.97
PUFAs (g/d)	24.6 ± 5.4	24.2 ± 5.5	0.78
MUFAs (g/d)	20.4 ± 5.3	20.9 ± 6.4	0.72
Cholesterol (mg/d)	230.4 ± 127.2	193.7 ± 115.4	0.24
TDF (g/d)	16.9 ± 4.9	18.3 ± 3.7	0.21
Magnesium (mg/d)	238.2 ± 50.2	259.6 ± 59.8	0.13
Zinc (mg/d)	11.6 ± 5.2	11.9 ± 5.9	0.79

Data are means ± SDs

[1] Obtained from independent t test

MUFAs, monounsaturated fatty acids; PUFAs, polyunsaturated fatty acids; SFAs, saturated fatty acids; TDF, total dietary fiber

P-value < 0.05 was considered statistically significant. All statistical analyses were conducted by the Statistical Package for Social Science version 18 (SPSS Inc., Chicago, Illinois, USA).

Results

In the current study, among patients in synbiotic supplements group, 3 patients and in the placebo group, 2 patients withdrawn due to personal reasons therefore, did not complete the trial (▶ **Fig. 1**). However, all 60 participants were included in the final analysis using ITT principle. Overall, the compliance rate was high, such that higher than 90 % of capsules were consumed throughout the study in both groups.

There were no significant differences between the 2 groups in terms of mean of age, height, baseline weight, baseline BMI, and mean of changes in weight and BMI before and after intervention (▶ **Table 1**).

Based on the 3-day dietary records obtained throughout the intervention, we observed no significant change in dietary macro- and micro-nutrient intakes between the 2 groups (▶ **Table 2**).

After 12 weeks of intervention, patients who consumed synbiotic capsule had significantly decreased FPG (− 19.6 ± 74.6 vs. + 19.2 ± 66.9 mg/dL, P = 0.03), serum insulin concentrations (− 0.7 ± 5.1 vs. + 3.3 ± 6.3 μIU/mL, P = 0.01), HOMA-B (− 3.4 ± 19.5 vs. + 11.5 ± 21.0, P = 0.006) and increased QUICKI (+ 0.002 ± 0.01 vs. − 0.01 ± 0.02, P = 0.03) compared with the placebo (▶ **Table 3**). In addition, changes in serum HLDL-cholesterol levels (+ 1.8 ± 5.7 vs. − 2.2 ± 6.0 mg/dL, P = 0.01) in supplemented patients were significantly different from those of patients in the placebo group. We did not observe any significant changes in other lipid concentrations. A trend toward a significant difference of synbiotic supplementation on decreasing total-/HDL-cholesterol (− 0.01 ± 0.6 vs. + 0.3 ± 0.6, P = 0.05) was observed.

We controlled the analyses for the baseline levels. However, extra adjustment for these variables the results remained the same except for FPG (P = 0.17) and QUICKI (P = 0.05) (▶ **Table 4**). Similarly, additional adjustments for age and BMI at baseline did not affect our findings except for FPG (P = 0.06) and QUICKI (P = 0.07).

Discussion

In the present study, which to the best of our knowledge is the first of its kind, we found that supplementation with synbiotic capsule for 12 weeks had beneficial effects on FBG and serum insulin, HOMA-B, QUICKI and HDL-cholesterol levels in diabetic patients with CHD.

Impaired insulin metabolism leads to hyperglycemia, dyslipidemia, hormonal imbalances, hypertension, inflammation, oxidative stress and many other complications that increase the risk of atherosclerosis and CHD in T2DM patients [22]. Then, improving insulin function might effectively prevent CHD in patients with T2DM. The results of our study showed that synbiotic intake compared with the placebo among diabetic patients with CHD for 12 weeks was associated with a significant decrease in FPG, serum insulin levels, HOMA-B and a significant increase in QUICKI score. Few clinical trials have evaluated the effects of synbiotic on markers of insulin metabolism. In line with our results, Eslamparast et al. [23] reported that the intake of synbiotic capsule, twice a day for 28 weeks significantly decreased fasting blood sugar and insulin resistance in subjects with metabolic syndrome. The findings of another study by Moroti et al. [24] was showed that the consumption of a daily dose of 200 mL synbiotic supplements containing Lactobacillus acidophilus, Bifidobacterium bifidum and oligofructose for 30 days significantly reduced fasting glycemia in elderly patients with T2DM. Moreover, consumption of a synbiotic (Bifidobacterium longum with fructo-oligosaccharides) for 24 weeks led to a significant decrease in HOMA-IR in patients with non alcoholic steatohepatitis [25]. Few underlying mechanisms have been proposed for the effects of synbiotics on parameters of glucose homeostasis. The components of the gram negative bacterial cell walls are associated with insulin resistance and glucose levels [26]. By adding friendly bacteria to the gut microbiota, the synbiotic may prevent the growth of gram negative pathogens in intestinal mucosa. Furthermore, it maintains the gut barrier integrity and reduces the transfers of the pathogens into the blood stream [27]. Synbiotic supplementation may also improve insulin function through the effects on hepatic insulin signaling, reduced phosphorylation of insulin receptor substrate-1 and decreased production of inflammatory cytokines [28].

The current study demonstrated that consumption of synbiotic in diabetic patients with CHD for 12 weeks resulted in a significant rise in serum HDL-cholesterol levels compared with placebo, while did not affect other lipid profiles. Some studies have evaluated the effects of synbiotics and probiotics on lipid profiles [11, 13, 29]. Supporting our findings, in a study by Kiessling et al. [30] was seen a significant increase in HDL-cholesterol levels among healthy women who received 300 g synbiotic yogurt daily containing 10^{6-8} CFU L. acidophilus, 10^{3-5} CFU B. longum, and 1 % oligofructose for 7 weeks. No significant change in serum levels of total-, LDL-, HDL-cholesterol and triglycerides was also observed after 8-week consumption of the synbiotic capsules containing L. acidophilus DDS-1 and B. longum UABL-14 plus fructo-oligosac-

▶ **Table 3** Metabolic profiles at baseline and 12 weeks after the intervention in overweight diabetic patients with coronary heart disease.

	Placebo group (n = 30)				Synbiotic group (n = 30)				P[2]		
	Baseline	End-of-trial	Change	P[1]	Baseline	End-of-trial	Change	P[1]	Time	Group	Time × Group
FPG (mg/dL)	125.3 ± 51.1	144.5 ± 72.3	19.2 ± 66.9	0.12	149.4 ± 60.8	129.7 ± 40.5	− 19.6 ± 74.6	0.15	0.97	0.68	0.03
Insulin (μIU/mL)	16.5 ± 8.9	19.8 ± 10.2	3.3 ± 6.3	0.008	16.2 ± 7.9	15.5 ± 8.0	− 0.7 ± 5.1	0.50	0.07	0.27	0.01
HOMA-IR	5.2 ± 4.0	6.1 ± 4.2	0.9 ± 2.1	0.02	5.6 ± 2.7	5.6 ± 3.4	0.01 ± 1.8	0.95	0.07	0.92	0.08
HOMA-B	51.5 ± 40.9	63.1 ± 47.4	11.5 ± 21.0	0.005	42.5 ± 30.7	39.1 ± 25.6	− 3.4 ± 19.5	0.34	0.12	0.07	0.006
QUICKI	0.31 ± 0.03	0.30 ± 0.02	− 0.01 ± 0.02	0.05	0.30 ± 0.02	0.30 ± 0.02	0.002 ± 0.01	0.56	0.13	0.73	0.03
Triglycerides (mg/dL)	127.9 ± 66.9	150.7 ± 69.9	22.8 ± 53.5	0.02	142.0 ± 66.3	153.6 ± 64.7	11.6 ± 50.6	0.22	0.01	0.59	0.40
VLDL-cholesterol (mg/dL)	25.6 ± 13.4	30.1 ± 14.0	4.5 ± 10.7	0.02	28.4 ± 13.2	30.7 ± 12.9	2.3 ± 10.1	0.22	0.01	0.59	0.40
Total cholesterol (mg/dL)	141.0 ± 29.0	147.7 ± 32.5	6.7 ± 29.6	0.22	144.5 ± 25.0	149.1 ± 25.8	4.6 ± 28.4	0.37	0.13	0.69	0.78
LDL-cholesterol (mg/dL)	68.6 ± 23.0	72.9 ± 23.5	4.3 ± 22.2	0.29	70.2 ± 20.0	70.7 ± 23.0	0.5 ± 23.7	0.90	0.41	0.95	0.52
HDL-cholesterol (mg/dL)	46.8 ± 7.4	44.6 ± 5.9	− 2.2 ± 6.0	0.05	45.9 ± 6.3	47.7 ± 6.9	1.8 ± 5.7	0.09	0.78	0.51	0.01
Total-/HDL-cholesterol ratio	3.0 ± 0.6	3.3 ± 0.7	0.3 ± 0.6	0.01	3.2 ± 0.6	3.2 ± 0.5	− 0.01 ± 0.6	0.85	0.09	0.97	0.05

All values are means ± SDs

[1] P-values represent paired-samples t-test

[2] P-values represent the time × group interaction (computed by analysis of the one-way repeated measures ANOVA)

FPG, fasting plasma glucose; HOMA-IR, homeostasis model of assessment-estimated insulin resistance; HOMA-B, homeostasis model of assessment-estimated b cell function; QUICKI, quantitative insulin sensitivity check index

▶ **Table 4** Adjusted changes in metabolic variables in overweight diabetic patients with coronary heart disease.

	Placebo group (n = 30)	Synbiotic group (n = 30)	P[1]
FPG (mg/dL)	14.5 ± 10.7	− 15.0 ± 10.7	0.06
Insulin (μIU/mL)	3.3 ± 1.1	− 0.6 ± 1.1	0.01
HOMA-IR	0.9 ± 0.4	0.1 ± 0.4	0.12
HOMA-B	11.6 ± 3.9	− 3.6 ± 3.9	0.009
QUICKI	− 0.009 ± 0.004	0.001 ± 0.004	0.07
Triglycerides (mg/dL)	28.9 ± 8.6	10.5 ± 8.6	0.28
VLDL-cholesterol (mg/dL)	4.7 ± 1.7	2.1 ± 1.7	0.28
Total cholesterol (mg/dL)	5.5 ± 4.9	5.7 ± 4.9	0.97
LDL-cholesterol (mg/dL)	3.1 ± 3.8	1.8 ± 3.8	0.82
HDL-cholesterol (mg/dL)	− 2.2 ± 0.9	1.8 ± 0.9	0.004
Total-/HDL-cholesterol ratio	0.3 ± 0.1	− 0.004 ± 0.1	0.07

All values are means ± SEs. Values are adjusted for baseline values, age and BMI at baseline

[1] Obtained from ANCOVA

FPG, fasting plasma glucose; HOMA-IR, homeostasis model of assessment-estimated insulin resistance; HOMA-B, homeostasis model of assessment-estimated b cell function; QUICKI, quantitative insulin sensitivity check index

charide in healthy subjects [31]. In addition, in our previous study which were conducted among T2DM patients without any histories of CHD, consumption of a synbiotic food containing L. sporogenes plus inulin for 6 weeks made no significant change in serum levels of lipid profiles except for serum triglycerides concentrations [13]. Dyslipidmia which is the consequences of T2DM lead to exacerbation and progression of CHD [3]. In contrast, few clinical studies have revealed the beneficial effects of synbiotic supplementation on lipid profiles among different groups of subjects. For instance, a 6-week supplementation of a synbiotic containing of Lactobacillus salivarius UBL S22 plus fructo-oligosaccharide in healthy young subjects led to a significant decrease in triglycerides, total- and LDL-cholesterol and a significant increase in HDL-cholesterol levels [32]. Furthermore, following the intake of a synbiotic food (L. sporogenes 1×10^7 CFU plus 0.04 g inulin/1 g) for 9 weeks by healthy pregnant women has resulted in a significant decrease in serum triglycerides and VLDL-cholesterol levels [29]. The discrepancies between the results from the mentioned studies may be due to the variability in probiotic strains or doses, differences in experimental design, subjects and/or the way that they were administered [5]. Synbiotics and probiotics may influence lipid profiles through their immune-modulatory effects [33], toll-like receptor 4 (TLR4) signaling and pro-inflammatory cytokines [34].

Few of the important strengths of our research include its randomized design and consideration of the confounding variables such as the daily dietary nutrients intake and physical activity. However, as the limitation of our study, we did not measure the levels of the SCFA and fecal bacterial loads.

Conclusions

Overall, synbiotic supplementation for 12 weeks among diabetic patients with CHD had beneficial effects on markers of insulin metabolism and serum HDL-cholesterol levels; however, it did not had any effect on other lipid profiles.

Authors' Contributions

ZA contributed in conception, design, statistical analysis and drafting of the manuscript. MT-E, NSh, AF, FR, FK, RR and ST contributed in data collection and manuscript drafting. All authors approved the final version for submission. ZA supervised the study. All authors confirmed the final version for submission.

Clinical Trial Registration Number

http://www.irct.ir: IRCT201503025623N37.

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Conflict of interest

None declared.

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Note of Concern

Since publication of this article, serious concerns have been raised about the integrity of the reported methods, results and analysis. Responses by the leading author and ethics committees have been unsatisfactory and inconclusive; we advise readers to interpret the information presented in the article with due caution.