ORIGINAL ARTICLE

Effects of probiotic yogurt consumption on lipid profile in type 2 diabetic patients: A randomized controlled clinical trial

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Background: Alteration in plasma lipid and lipoprotein profile has been documented in diabetic patients. The purpose of this study was to compare the effect of probiotic and conventional yogurt on lipid profile in type 2 diabetes mellitus patients. **Materials and Methods:** A total of 44 patients with type 2 diabetes aged 30-60 years old who had low density lipoprotein cholesterol (LDL-c) ≥100 mg/dl enrolled in this randomized, double – blind controlled trial and were assigned to two intervention and control groups. The subjects in the intervention group consumed 300 g/d probiotic yogurt containing *Lactobacillus acidophilus* La-5 and *Bifidobacterium lactis* Bb-12 and subjects in the control group consumed 300 g/d conventional yogurt for 8 weeks. Anthropometric indices, dietary intake, and serum lipid profile were evaluated at the beginning and end of the intervention. Independent-sample *t*-test, paired sample *t*-test, ANCOVA, and repeated measures were used for statistical analysis. **Results:** The consumption of probiotic yogurt caused significant decrease in LDL-c/high density lipoprotein cholesterol (HDL-c) ratio (3.13 ± 1.00-2.07 ± 0.71, $P = 0.016$). The levels of HDL-c were increased significantly (43.66 ± 6.80-50.42 ± 6.64, $P = 0.023$) in the intervention group postintervention. However, there were no significant differences in triglyceride and total cholesterol levels between two groups postintervention ($P < 0.05$). **Conclusion:** It is suggested that probiotic yogurt consumption may be used as an alternative prevention approach and treatment method to improve dyslipidemia in patients with type 2 diabetes.

Key words: Lipid profile, probiotic yogurt, type 2 diabetes

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INTRODUCTION

Diabetes mellitus is one of the main endocrine diseases, which is more prevalent in the worldwide.[1] Cardio-vascular disease, as one of several chronic disorders, is the major complication of type 2 diabetes mellitus (T2DM). Cardio-vascular disease may result from associated abnormalities of plasma lipid and lipoprotein metabolism.[2] Alteration in plasma lipid and lipoprotein profile has been documented in diabetic patients.[3] There are some reports in literature suggesting that the insulin resistance has a central role in the development of dyslipidemia in diabetic patients.[4-6] In insulin resistance status, free fatty acids flux from adipose tissue to liver and subsequently increase synthesis of very low density lipoprotein cholesterol and low density lipoprotein cholesterol (LDL-c) and decrease high density lipoprotein cholesterol (HDL-c) levels.[7] Moreover, hyperglycemia in insulin resistance can lead to increase of advanced glycation end products density. These products may directly promote atherosclerosis through changes in endothelial, macrophage, and smooth muscle cells functions. Therefore, improving dyslipidemia would be effective to prevent complications of diabetic patients.[8] Several treatments including consumption of herbal medicines,[9] soy protein,[10] w-3 fatty acids,[11] and fiber[12] have been suggested to improve dyslipidemia in diabetic patients. It is suggested that consumption of probiotics would be a novel approach to reduce the elevated levels of cholesterol.[13] Probiotics are defined as live microorganisms which have beneficial health effects on their host, when enter the intestine with an adequate amount.[14] Some of these health effects include: lowering hypercholesterolemia,[15] prevention or management of diarrhea, constipation, lactose intolerance, diabetes mellitus,[16] and colon cancer[17] Two main groups of probiotic bacteria, which are most commonly used, involve *Lactobacilli* and *Bifidobacteria*.[18] Some studies indicated that probiotics may be able to prevent increased levels of total cholesterol (TC), LDL-c and balance the ratio of LDL-c/HDL-c by de-conjugating of bile, hydrolysis of bile salts and increase

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cholesterol absorption, which consequently prevent and reduce the prevalence of cardio-vascular disease.[19] Consumption of probiotics in healthy men increased in serum levels of HDL-c and reduced in LDL-c/HDL-c indices.[20] Decreased serum LDL-c levels have been reported in hyper-lipidemic subjects by probiotic.[21] Nevertheless, intake of *Lactobacillus rhamnosus* and *Propionibacterium freudenreichii* in hypercholesterolemia volunteers had no affect on lipid profile.[22] As was mentioned, available evidences about the effects of probiotic bacteria on lipid profile are controversial.[20-22] Therefore, this study with different type of probiotic bacteria was designed to evaluate the hypocholestrolemic effect of probiotic yogurt on lipid profile in patients with type 2 diabetes.

MATERIALS AND METHODS

Subjects

This study was conducted in 44 patients with T2DM were participated in this double-blind, randomized controlled clinical trial. This 8 weeks clinical trial was conducted from December 2011 to February 2012. The inclusion criteria included males and females, body mass index (BMI) >25, serum LDL-c level of ≥100 mg/dl (normal range for men and women <100 mg/dl). Exclusion criteria included: insulin injection; any changes in using medication; smoking; lactose intolerance; thyroid dysfunction; chronic inflammatory diseases; cardio-vascular disease; renal dysfunction; pregnancy; breast feeding; and having any weight loose or weight gain regimes. The sample size was determined based on the primary information obtained from the study by Oze *et al*. for LDL-c.[20] For an α value equal to 0.05 and a power of 80%, the sample size was computed as 15 per group. This number was increased to 22 per group to accommodate the anticipated dropout rate. The study was approved by the Research Ethic Committee of Ahvaz Jundishapur University of Medical Sciences, Iran (NRC - 9008). A written consent was obtained from all subjects.

All subjects were allocated into two groups using blocked randomization. For the subjects in the intervention group 300 g probiotic yogurt containing 3.7×10^6 cfu/mg of both *Lactobacillus acidophilus* La-5 and *Bifidobacterium lactis* Bb-12 (Chr. Hansen, Hoersholm, Denmark) was provided to consume every day for 8 weeks. On the other hand, the control group received 300 g/d conventional yogurt containing *Lactobacillus bulgaricus* and *Streptococcus thermophiles* for 8 weeks. The mean of the components of probiotic and conventional yogurt per 100 g is presented in Table 1. Both types of yogurts had a similar taste, appearance and specially prepared for this study by Pegah Dairy Industries Co. (Ahvaz, Iran). The patients were instructed to eat yogurt twice a day by lunch and dinner.[23]

Table 1: Components of probiotic and conventional yogurt*

Components	Probiotic yogurt	Conventional yogurt
Calories (kcal)	48.8	46.0
Carbohydrate (g)	7.5	4.9
Fat (g)	1.5	1.5
Protein (g)	2.4	3.3
Sodium (mg)	40	50
Potassium (mg)	110	156
Phosphorus (mg)	53	120
Calcium (mg)	100	100
pH	4.3	4.3

*Pegah dairy industries company analysis

All subjects were asked to maintain their normal lifestyle, physical activity and eating habits and avoid consuming other probiotic and fermented products during the study. They also were asked to keep yogurts in the refrigerator at a temperature of below 4°C. Yogurts were freshly produced and provided to subjects weekly. The compliance with the yogurt consumption of all subjects was assessed twice a week by telephone interviews.

Anthropometric indices, dietary intakes and biochemical indices were evaluated in all subjects before and after intervention. Physical activity level was also evaluated and classified into low, moderate and vigorous levels.[24] In this study, dietary intake of subjects was assessed by 3-day diet records and Nutritionist IV program was used to estimate dietary intake of patients. The body fat mass of subjects were also measured by body state set (QuadScan 4000).

A volume of 10 ml blood sample was collected and sera were separated, processed and stored at −80°C until analysis. Serum TC, triglycerides (TG) and HDL-c were measured enzymatically with Parsazmun's kits (DiaSys, Germany). LDL-c level was measured by Friedewald formula as follow:

LDL-c = TC – HDL-c − (triglyceride/5).

Statistical analyses

Statistical analyses were carried out using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA) Program version 17 for windows. All data were expressed as mean ± standard deviation Independent-sample *t*-test, paired sample *t*-test, ANCOVA and repeated measures were used for statistical analysis. $P < 0.05$ were considered to be significant.

RESULT

In the current study, 42 patients attended randomization and blood collection. Thus, 21 subjects were randomly assigned to each group. Patients did not report any adverse effect during the study related to yogurt consumption and both yogurts were well-tolerated.

The mean age of subjects was 51 years. 26.19% (11 patients) of the study population was male and 73.80% (31 patients) was female. Regarding with the classification of physical activity level, 54.76%, 42.85, and 2.38% of the study population had low, moderate and vigorous physical activity level, respectively. However, there were not significant differences between two groups regarding with physical activity ($P > 0.05$).

Anthropometric characteristics of subjects at baseline and postintervention were shown in Table 2. For anthropometric parameters, no significant differences were seen between intervention and control groups at baseline and postintervention ($P > 0.05$).

Regarding with dietary intake analysis, the differences in mean energy and nutrient intake were not significant ($P > 0.05$) between two groups at baseline. Calcium intake was increased in both groups after conventional and probiotic yogurt consumption, but not significantly ($P = 0.061$ and $P = 0.057$, respectively). Protein intake was significantly ($P = 0.008$) elevated in control group at the end of study. However, the intakes of other nutrients did not significantly change from baseline to the postintervention in both groups [Table 3].

Table 2: Anthropometric characteristics of subjects at baseline and postintervention

Variables	Conventional (means ± SD)	Probiotic (means ± SD)	P_a
Weight (kg)			
Baseline	79.33±10.15	74.66±11.11	0.163
Postintervention	78.61±9.04	74.33±10.89	0.173
P_b	0.543	0.516	–
BMI (kg/m^2)			
Baseline	29.22±3.20	28.36±4.14	0.464
Postintervention	29.18±3.57	28.24±4.10	0.434
P_b	0.949	0.525	–
WC (cm)			
Baseline	107.66±14.28	101.90±10.06	0.139
Postintervention	108.00±14.51	102.04±10.24	0.133
P_b	0.495	0.480	–
HC (cm)			
Baseline	115.42±12.55	111.00±9.59	0.206
Postintervention	115.61±12.55	110.85±9.72	0.177
P_b	0.329	0.186	–
WHR			
Baseline	0.93±0.06	0.91±0.03	0.412
Postintervention	0.93±0.64	0.92±0.03	0.448
P_b	0.776	0.202	–
Body fat (%)			
Baseline	37.10±8.25	36.15±9.40	0.733
Postintervention	37.12±8.00	35.99±8.16	0.654
P_b	0.948	0.846	–

SD = Standard deviation; BMI = Body mass index; WC=Waist circumference; HC = Hip circumference; WHR = Waist to hip ratio; P_a = *P* value between two groups at baseline and postintervention (independent-sample *t*-test); P_b = *P* value within groups from baseline to postintervention (paired sample *t*-test)

In this study, the levels of HbA1c were significantly reduced in the intervention group compared with the control group at the end of study (7.09 ± 1.23 vs. 8.09 ± 1.58, $P = 0.038$). After adjusting for confounding factors (age, gender, physical activity, waist to hip ratio, and energy intake), the effects

Table 3: Dietary intakes of subjects at baseline and postintervention

Variables	Conventional (means ± SD)	Probiotic (means ± SD)	P_a
Energy (kcal)			
Baseline	2401.14±516.07	2439.85±454.37	0.798
Postintervention	2655.61±491.30	2265.33±737.45	0.060
P_b	0.080	0.327	–
Protein (g)			
Baseline	136.79±22.82	136.38±22.36	0.957
Postintervention	158.02±26.42	144.64±26.15	0.107
P_b	0.008	0.301	–
Fat (g)			
Baseline	75.60±11.59	77.18±9.63	0.634
Postintervention	81.64±12.15	74.43±12.23	0.062
P_b	0.149	0.395	–
SFA (g)			
Baseline	28.89±2.76	28.08±2.78	0.350
Postintervention	29.66±2.96	28.97±2.98	0.459
P_b	0.433	0.383	–
PUFA (g)			
Baseline	10.35±0.88	10.39±1.00	0.879
Postintervention	10.25±0.65	10.33±0.78	0.738
P_b	0.69	0.81	–
MUFA (g)			
Baseline	11.32±0.60	11.39±0.64	0.717
Postintervention	11.06±0.65	11.10±0.66	0.531
P_b	0.19	0.33	–
Dietary fiber (g)			
Baseline	13.05±3.06	13.09±2.56	0.728
Postintervention	13.29±3.16	12.58±2.97	0.647
P_b	0.698	0.664	–
Calcium (mg)			
Baseline	1022.04±206.48	971.42±158.84	0.379
Postintervention	1292.9±245.97	1253.71±225.69	0.594
P_b	0.061	0.057	–
Vitamin D (μg)			
Baseline	2.65±1.12	2.42±0.91	0.469
Postintervention	3.05±1.76	2.76±1.18	0.531
P_b	0.353	0.305	–
Vitamin K (μg)			
Baseline	53.6±17.2	50.69±8.29	0.489
Postintervention	62.45±34.22	56.27±23.04	0.497
P_b	0.299	0.300	–
Zinc (mg)			
Baseline	13.05±3.06	13.06±2.56	0.965
Postintervention	13.29±3.16	12.58±2.97	0.459
P_b	0.773	0.573	–

SD = Standard deviation; SFA = Saturated fatty acid; MUFA = Mono-unsaturated fatty acid; PUFA = Poly-unsaturated fatty acid; P_a = *P* value between two groups at baseline and postintervention (independent -sample *t*-test); P_b = *P* value within two groups from baseline to postintervention (paired sample *t*-test)

of probiotic and conventional yogurt consumption on lipid profile in patients with T2DM have been shown in Table 4. There were no significant differences found between two groups regarding with serum TC, TG, HDL-c and LDL-c at baseline. Serum levels of LDL-c were decreased in subjects in the intervention group post probiotic consumption, but not significantly (P = 0.059). Moreover, the LDL-c/HDL-c ratio was significantly reduced in the intervention group compared with the control group at the end of study. HDL-c levels were also significantly higher in the intervention group than in the control group postintervention (P = 0.023). However, no significant differences were observed in TC and TG levels between two groups at the end of study (P = 0.104 and P = 0.108 respectively). In the intervention group the levels of TC were significantly lowered postintervention (P = 0.044). Serum levels of TG were also diminished in the intervention group, but not significantly (P = 0.18).

DISCUSSION

Diabetes mellitus is an endocrine disorder that characterized by hyperglycemia[25] and associated with disorders in lipid metabolism.[26] Dyslipidemia is a major risk factor for cardio-vascular disease in patients with diabetes.[27] Some human and animal studies suggested that using probiotic may reduce serum lipid levels.[28,29] Therefore, this study was carried out to evaluate probiotic effects on lipid profile in patients with T2DM. In the present study, none of the subjects reported any adverse effect during the study related to yogurt consumption and it was reported that both kinds of yogurts were well-tolerated, therefore it is suggested that the compliance rate was satisfied. There were no significant differences observed in dietary intake, anthropometric indices and physical activity level between probiotic and conventional consumers during the study. Hence, it is suggested that these possible confounders did not affect on the results of lipid profiles in this study. In another study, Sadrzadeh-Yeganeh *et al*. indicated that use of *L. acidophilus* La-5 and *B. lactis* Bb-12 didn't significant changes anthropometry indices in hypercholestrolemic patients.[30] Results of the study by Fuentes *et al*. also indicated that 12 weeks consumption of *Lactobacillus plantarum* has no effect on weight, BMI, body fat mass in hyper-cholesterolemic subjects.[31] In other experiments shown that body weight and weight of liver were not affected by *B. lactis* Bb-12 in wild type mice.[32]

Table 4: Effect of probiotic and conventional yogurt on lipid profile in the subjects

Variables	Conventional (means ± SD)	Probiotic (means ± SD)	P_a
TC (mg/dl)			
Baseline	220.66±36.97	219.19±42.05	0.904
Postintervention	217.38±58.37	193.47±32.22	0.108
P_b	0.812	0.044	–
LDL-C (mg/dl)			
Baseline	135.81±41.01	133.39±34.85	0.837
Postintervention	128.69±51.45	103.06±31.63	0.059
P_b	0.590	0.013	–
HDL-C (mg/dl)			
Baseline	44.33±6.03	43.66±6.80	0.739
Postintervention	45.19±7.72	50.42±6.64	0.023
P_b	0.697	0.007	–
TG (mg/dl)			
Baseline	202.57±63.08	210.66±108.99	0.770
Postintervention	217.48±92.71	199.90±94.21	0.104
P_b	0.517	0.186	–
LDL-C/HDL-C			
Baseline	3.11±1.11	3.13±1.00	0.954
Postintervention	3.02±1.57	2.07±0.71	0.016
P_b	0.833	0.002	–

SD = Standard deviation; WHR = Waist to hip ratio; TC = Total cholesterol; LDL-C = Low density lipoprotein-cholesterol; HDL-C = High density lipoprotein-cholesterol; TG = Triglyceride; P_a = P value between two groups at baseline and postintervention after adjusting for age, gender, physical activity, WHR and energy intake (ANCOVA); P_b = P value within two groups from baseline to postintervention after adjusting for WHR change and energy intake change (repeated measure ANCOVA)

In this study, it was shown that consumption of probiotic yogurt significantly reduced HbA1c levels. The results also showed that consumption of probiotic yogurt significantly reduced serum levels of LDL-c and LDL-c/HDL-c ratio, in patients with T2DM patients. Moreover, serum HDL-c levels were significantly increased after consumption of probiotic yogurt. These results concur with other studies. Ejtahed *et al*. found that consumption of probiotic yogurt in T2DM patients significantly reduced HbA1c concentration and serum levels of LDL-c and LDL-c/HDL-c ratio.[33] Decreased serum levels of LDL-c by *Lactobacillus reuteri* NCIBM30242 have been also reported by Jones *et al*.[34] In a study by Wang *et al*., it was indicated that *Lactobacillus* LIP-1 and MG9-2 significantly decreased serum level of TG, TC and LDL-c in high-lipid diet fed mice.[35] Baroutkoub *et al*. found that *L. acidophilus* and *B. lactis* significantly reduced LDL-c and TC level in hypercholestrolemic patients.[19] Consumption of a new symbiotic shake containing *L. acidophilus*, *B. bifidum* and fructo-oligosaccharides in older with type 2 diabetic patients significantly increased serum HDL-c, but did not impact on TC and TG in Moroti *et al*. Study.[36] Improvement of LDL-c/HDL-c ratio and increased serum level of HDL-c by probiotic have been reported in Kiessling *et al*. trial.[37] In another study, Fabian and Elmadfa found that daily consumption of probiotic yogurt significantly raised serum HDL-c and improved ratio of LDL-c/HDL-c.[38]

However, there are some other studies in contrast with our study regarding with the above-mentioned results. Asemi *et al*. showed that eating probiotic yogurt did not change lipid profile in pregnant women.[39] Sadrzadeh-Yeganeh *et al*. also reported similar results in women.[29] Moreover, Hattaka *et al*. indicated that intake of *L. rhamnosus* LC705 did not affect blood lipids in hyperlipidemic men.[22]

There are several possible mechanisms suggested about the effects of probiotic bacteria on lipid profile. It is indicated that different bacteria species may have different abilities to affect lipid profile.[29,40]

Lee do *et al*. showed that short chain fatty acids that are produced by lactic acid bacteria could inhibit the enzymatic synthesis of cholesterol.[40] Probiotic bacteria may also facilitate excreting of cholesterol through feces.[36] Furthermore, these bacteria can assimilate cholesterol and lead to its reduction. Moreover, it is suggested that lactic acid bacteria may bind with cholesterol and inhibit its reabsorption in the body.[41] In addition, *Lactobacilli* and *Bifidobacteria* cells are able to hydrolyze conjugated bile acids, excrete them faster and reduce its level to which they can be absorbed.[42,43] The findings of this study also suggested that probiotic yogurt consumption might not affect on serum TC and TG levels in patients with T2DM due to some possibilities that we suggest the type of microorganism used and some limitations of study including the number of subjects and short period of intervention in this study.

CONCLUSION

This study indicated that consuming probiotic yogurt can improve lipid abnormalities in patients with T2DM. Therefore, it is suggested that eating probiotic yogurt may be used as an alternative prevention approach and treatment method to reduce diabetic complications. We also suggest further studies with larger sample size, longer period of intervention and various type of probiotic.

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