Effect of probiotic yogurt containing *Lactobacillus acidophilus* and *Bifidobacterium lactis* on lipid profile in individuals with type 2 diabetes mellitus

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ABSTRACT

The purpose of this study was to investigate the effects of probiotic and conventional yogurt on the lipid profile in type 2 diabetic people. In a randomized double-blind controlled trial, 60 people (23 males and 37 females) with type 2 diabetes and low-density lipoprotein cholesterol (LDL-C) greater than 2.6 mmol/L were assigned to 2 groups. Participants consumed daily 300 g of probiotic yogurt containing *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12 or 300 g of conventional yogurt for 6 wk. Fasting blood samples, anthropometric measurements and 3-d, 24-h dietary recalls were collected at the baseline and at the end of the trial. Probiotic yogurt consumption caused a 4.54% decrease in total cholesterol and a 7.45% decrease in LDL-C compared with the control group. No significant changes from baseline were shown in triglyceride and high-density lipoprotein cholesterol (HDL-C) in the probiotic group. The total cholesterol:HDL-C ratio and LDL-C:HDL-C ratio as atherogenic indices significantly decreased in the probiotic group compared with the control group. Probiotic yogurt improved total cholesterol and LDL-C concentrations in type 2 diabetic people and may contribute to the improvement of cardiovascular disease risk factors.

Key words: probiotic yogurt, lipid profile, type 2 diabetes mellitus

INTRODUCTION

Cardiovascular disease (**CVD**) is the primary cause of death in people with type 2 diabetes mellitus (**T2DM**). Relative risk of CVD is 2- to 4-fold higher in diabetic people compared with nondiabetic people (Ray et al., 2009). Dyslipidemia has been identified as a risk factor for cardiovascular complications in T2DM. The American Diabetes Association (2010) guidelines indicate that, the primary goal of low-density lipoprotein cholesterol (**LDL-C**) concentration is less than 2.6 mmol/L (100 mg/dL) in diabetic people without overt CVD.

Probiotics are defined as living microorganisms, which, when ingested in sufficient amounts, beneficially influence the health of the host by improving the composition of intestinal microflora (Guarner and Schaafsma, 1998; Homayouni et al., 2008; Homayouni, 2009). In addition to improving gut health, probiotics may play a beneficial role in several medical conditions, including lactose intolerance, cancer, allergies, hepatic disease, *Helicobacter pylori* infections, urinary tract infections and hyperlipidemia (Goldin and Gorbach, 2008; Homayouni, 2008; Kaur et al., 2009).

In vitro experiments have shown that probiotics were able to decrease cholesterol concentration via possible mechanisms, which include deconjugation of bile acids by bile salt hydrolase (Begley et al., 2006), assimilation of cholesterol (Pereira and Gibson, 2002b), cholesterol binding to cell walls of probiotics (Liong and Shah, 2005), and production of short-chain fatty acids (**SCFA**), which can inhibit hepatic cholesterol synthesis (Pereira and Gibson, 2002a). The cholesterol-lowering activity of probiotics has been reported in experimental animals (Rao et al., 1981; Kiyosawa et al., 1984; Grunewald, 1992; Nguyen et al., 2007).

The hypocholesterolemic potential of probiotics has also been evaluated in normo- and hypercholesterolemic people. Even though some animal studies have suggested a strain-dependent cholesterol-lowering property of probiotic products, human studies have not been conclusive (Anderson and Gilliland, 1999; Xiao et al., 2003; Lewis and Burmeister, 2005; Fabian and Elmadfa, 2006; Greany et al., 2008; Ataie-Jafari et al., 2009; Sadrzadeh-Yeganeh et al., 2010). These controversial findings may be attributed to factors such as different strains and doses of probiotics, clinical characteristics

of participants, duration of treatment period, sample size and study design (Ooi and Liong, 2010). These inconsistent findings call for more in-depth studies.

Larsen et al. (2010) reported that T2DM was associated with compositional changes in the intestinal microflora and the proportion of phylum *Firmicutes* was significantly reduced in human adults with T2DM compared with nondiabetic people. Therefore, because individuals with T2DM have an increased prevalence of lipid abnormalities, the aim of the present study was to investigate the effect of probiotic yogurt containing *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12 on the lipid profile in type 2 diabetic people compared with the effect of conventional yogurt.

MATERIALS AND METHODS

Subjects

A total of 64 subjects with T2DM, aged between 30 and 60 yr, with a body mass index (**BMI**) of less than 35 kg/m^2 and LDL-C greater or equal to 2.6 mmol/L (100 mg/dL), were recruited for this study from the endocrinology clinic of Sina Hospital in Tabriz, Iran. Recruitment was done via the telephone and advertisements. All participants had been diagnosed with T2DM for at least 1 yr. Exclusion criteria were smoking; having kidney, liver, or inflammatory intestinal disease, thyroid disorders, immunodeficiency diseases, or lactose intolerance; requiring insulin injections; taking nutritional supplements within the previous 3 wk or the 6-wk study period; receiving cholesterol-lowering medication, estrogen, progesterone, or diuretics; being pregnant or breast feeding; and consuming probiotic yogurt or any other probiotic products within the previous 2 mo.

Sample size was determined based on primary information obtained from the study by Sadrzadeh-Yeganeh et al. (2010) for total cholesterol (**TC**). For an expected change of 0.25 mmol/L (9.65 mg/dL) between intervention and control groups and by considering α = 0.05 and a power of 80%, the sample size was computed as 29.765 (≈30) per group (Pocock, 1983). This number was increased to 32 per group to accommodate the anticipated dropout rate.

Study Design and Measurements

The present study was a double-blind, randomized controlled clinical trial. Participants were randomly allocated in 2 groups using a block randomization procedure with matched subjects in each block based on sex and age (Fleiss, 1999). Finally, each group consisted of 32 patients. During the 1-wk run-in period, all patients refrained from eating yogurt or any other fermented food. Over 6 wk, the probiotic (intervention) and conventional (control) groups consumed 300 g daily of probiotic or conventional yogurt, respectively. All participants were instructed to maintain their usual dietary habits and lifestyle and to avoid consuming any yogurt other than that provided to them by the researchers throughout the 6-wk trial. The subjects were also instructed to keep the yogurt under refrigeration and to avoid any changes in medication if possible.

The allocation of intervention or control group was concealed from the researchers and probiotic and conventional yogurt containers were identical looking. Therefore, neither the subjects nor the investigators were aware of treatment assignments in this double-blind study. The probiotic and conventional yogurts were distributed weekly by the researchers. Compliance with the yogurt consumption guidelines was monitored via phone interviews once per week.

Information on food consumption, anthropometric measurements, and fasting blood samples were collected at the beginning and at the end of the trial. Nutrient intakes were estimated using 24-h dietary recall at the beginning and at the end of the study for 3 d. Three-day averages of energy and macronutrient intakes were analyzed by Nutritionist 4 software (First Databank Inc., Hearst Corp., San Bruno, CA). All data entry was performed by trained dietitians. If a participant ate a food that was not in the database, a food with very similar nutrient composition was chosen. Nutrient information was also obtained through food labels or recipes from participants.

Body weight was measured using a scale (Seca, Hamburg, Germany) with 0.1-kg accuracy without shoes and wearing light clothing. Heights were measured using a statiometer (Seca) with 0.1-cm accuracy without shoes. Body mass index was calculated by dividing BW (kg) by height squared (m^2).

The 12-h overnight fasting blood samples were collected between 7 and 10 a.m. The serum samples were separated from whole blood by centrifugation at 2606.8 × g for 10 min (Beckman Avanti J-25; Beckman Coulter, Brea, CA). The serum samples were frozen immediately at −70°C until assay. Blood samples were analyzed at the Drug Applied Research Center (Tabriz University of Medical Sciences, Tabriz, Iran). Serum concentrations of TC, triglyceride (**TG**), and high-density lipoprotein cholesterol (**HDL-C**) were measured using the standard enzymatic methods with commercially available Parsazmun kits (Karaj, Iran; TC was assayed with the cholesterol esterase and cholesterol oxidase method and TG was assayed using glycerol phosphate oxidase; HDL-C concentration was measured after precipitation of the apolipoprotein B-containing lipoproteins). Low-

density lipoprotein cholesterol concentration was determined by the Friedewald formula (Friedewald et al., 1972). The ratios between TC and HDL-C and between LDL-C and HDL-C were calculated as atherogenic indices, which have been suggested that can predict CVD (Kinosian et al., 1994).

This study was approved by the Ethics Committee at Tabriz University of Medical Sciences and was conducted according to the principles laid down in the Declaration of Helsinki. Prior to the trial, a full explanation concerning the purpose and methodology of the study was given to the participants by the researchers and written informed consent was obtained from all participants.

Intervention

Both probiotic and conventional yogurts contained *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. The probiotic yogurt was also enriched with *B. lactis* Bb12 and *L. acidophilus* La5 (Chr. Hansen, Denmark) as direct vat set cultures. The yogurts were produced weekly and distributed to the participants.

de Man, Rogosa, and Sharpe (MRS)-bile agar medium was used for the differential enumeration of mixed probiotic bacteria in the presence of yogurt bacteria (Vinderola and Reinheimer, 1999; Mortazavian et al., 2007). Probiotic yogurts were sampled on d 1 after manufacture (time of distribution) and microbiologically analyzed every week. Samples were refrigerated at 4°C, with subsequent analyzing on d 7 of storage. Serial dilutions of yogurts were made with Ringer solution. The diluted samples were cultivated and enumerated using MRS-bile agar medium, applying pour plate technique. Bile (Sigma Chemical Co., St. Louis, MO) was added to the MRS agar medium (Merck, Darmstadt, Germany) in a concentration of 0.15% and the medium was autoclaved at 121°C for 15 min. All the samples were incubated at 37°C for 72 h under both aerobic and anaerobic conditions. Anaerobic condition was generated by using the GasPak system (Merck). All of the experiments were done in triplicate. Counts of *L. acidophilus* were achieved at aerobic conditions and viable counts of *B. lactis* were selectively achieved using the subtractive enumeration method (Mortazavian et al., 2007).

Microbiological analyses of the probiotic yogurts showed that the average colony counts of *L. acidophilus* La5 and *B. lactis* Bb12 on d 1 were 7.23×10^6 and 6.04×10^6 cfu/g, respectively. Probiotic yogurts contained 1.05×10^6 cfu/g of *L. acidophilus* La5 and 1.19×10^6 cfu/g of *B. lactis* Bb12 on d 7. Both probiotic bacteria showed steady survival rate during a 7-d storage time at the average rate of 4.14×10^6 cfu/g and 3.61×10^6

cfu/g, respectively. The yogurt pH was 4.65 on d 1 and 4.48 on d 7 and the fat content was 2.5%, comparable in both yogurt types. The probiotic and conventional yogurt containers were identical and the yogurts had a similar taste and appearance. Both types of yogurt could be found in Iranian markets, but the yogurts were specially prepared for this study by the Iran Dairy Industries Co.

Statistical Analyses

The experimental data were analyzed by SPSS software (version 11.5; SPSS Inc., Chicago, IL) and the results were expressed as mean ± standard error. The normality of the distribution of variables was determined by the Kolmogorov-Smirnov test. For the duration of diabetes, monounsaturated fatty acids (**MUFA**) and cholesterol intakes, triglyceride, and LDL-C:HDL-C ratio analyses were performed after log transformation. The background characteristics and nutrient intakes of participants in the 2 groups were compared using independent sample *t*-tests and chi-squared test. The diabetes medication use in the 2 groups was compared using the Mann-Whitney U test. Analysis of covariance was used to identify any differences between the 2 groups after intervention, adjusting for baseline measurements and covariates. The changes in anthropometric measurements, nutrient intakes, and blood lipid parameters of the participants between the beginning and end of the trial were compared by paired sample *t*-tests. Differences with $P < 0.05$ were considered to be statistically significant (Zar, 1998).

RESULTS

All participants completed the study, but 4 people were excluded from the statistical analysis because 2 people needed to change their medication during the trial (1 from each group) and 2 people did not consume the yogurts according to the plan (1 from each group). Participants demonstrated good compliance with the yogurt consumption and no adverse effects or symptoms were reported. Table 1 presents the baseline characteristics of the participants in the 2 groups. The 2 groups resembled each other statistically in most baseline characteristics, except that participants in the probiotic group had longer duration of diabetes compared with participants in the conventional group ($P = 0.039$). Weight and BMI remained unchanged during the study in both groups.

The dietary intakes of participants throughout the study are shown in Table 2. Intake of polyunsaturated fatty acids (PUFA) was significantly different between probiotic and conventional groups at the beginning of

Table 1. Baseline characteristics of study participants[1]

Item	Conventional yogurt (n = 30)	Probiotic yogurt (n = 30)
Age (yr)	51.00 ± 1.34	50.87 ± 1.40
Sex[2] (M:F)	12:18	11:19
Weight (kg)	75.42 ± 2.06	76.18 ± 2.00
BMI (kg/m^2)	29.14 ± 0.78	28.95 ± 0.67
Duration of diabetes (yr)	4.08 ± 0.78	5.82 ± 0.90*
Metformin[3] (tablets/d)	2.00 ± 1.25	2.00 ± 1.25
Glibenclamide[3] (tablets/d)	1.00 ± 1.00	2.00 ± 2.00
Glucose (mmol/L)	7.35 ± 0.23	8.06 ± 0.45
HbA1c[4] (%)	6.87 ± 0.15	7.29 ± 0.22

[1]Data are presented as means ± standard error.

[2]Frequency.

[3]Medians and interquartile ranges.

[4]Glycosylated hemoglobin.

*$P < 0.05$ significantly different from conventional yogurt group.

the study ($P = 0.033$). No significant differences in energy and other nutrient intakes were observed between the 2 groups at baseline. No significant changes from baseline were observed within the 2 groups. At the end of the study, no statistically significant differences between the 2 groups were observed for dietary intakes.

No statistically significant differences existed in blood lipid parameters between the probiotic and conventional groups at baseline. Results of analysis of covariance showed statistically significant differences between the 2 groups in TC ($P = 0.008$), LDL-C ($P = 0.004$), TC:HDL-C ratio ($P = 0.027$), and LDL-C:HDL-C ratio ($P = 0.033$) at the end of study, adjusted for duration of diabetes, PUFA intake, and baseline values (Table 3). Probiotic yogurt consumption caused a 4.54% decrease in TC and a 7.45% decrease in LDL-C compared with the control group ($P < 0.01$ for both).

As shown in Table 3, TC and LDL-C concentrations were significantly decreased in the probiotic group compared with the baseline values ($P < 0.001$ for both). Serum TG and HDL-C concentrations remained unchanged in the probiotic group during the study, and no differences were observed between the 2 groups at the end of study. However, HDL-C was significantly decreased in the control group during the study ($P = 0.043$). the TC:HDL-C ratio was significantly decreased by 5.43% and the LDL-C:HDL-C ratio was significantly decreased by 8.6% in the probiotic group during the study ($P = 0.02$ and $P = 0.004$, respectively).

Table 2. Dietary intake of subjects throughout the study[1]

Variable	Measurement period	Conventional yogurt (n = 30)	Probiotic yogurt (n = 30)
Energy (Kcal)	Baseline	1,774.99 ± 88.12	1,775.20 ± 82.03
	After intervention	1,809.67 ± 79.25	1,776.67 ± 71.58
Carbohydrate (g)	Baseline	232.69 ± 13.89	242.88 ± 12.85
	After intervention	241.86 ± 12.42	239.64 ± 10.86
Protein (g)	Baseline	70.08 ± 3.69	68.12 ± 3.65
	After intervention	72.82 ± 3.89	71.84 ± 3.28
Total fat (g)	Baseline	68.01 ± 3.33	65.49 ± 3.51
	After intervention	65.93 ± 3.02	61.10 ± 3.40
Saturated fat (g)	Baseline	20.62 ± 1.38	20.86 ± 1.31
	After intervention	19.21 ± 0.99	18.78 ± 1.09
Monounsaturated fat (g)	Baseline	22.76 ± 1.30	23.61 ± 1.87
	After intervention	21.73 ± 1.15	21.88 ± 1.74
Polyunsaturated fat (g)	Baseline	18.13 ± 1.17	15.03 ± 0.79*
	After intervention	16.06 ± 1.05	15.01 ± 0.95
Cholesterol (mg)	Baseline	168.30 ± 12.13	157.38 ± 12.91
	After intervention	174.12 ± 14.21	165.80 ± 12.32
Dietary fiber (g)	Baseline	14.19 ± 0.81	16.05 ± 1.28
	After intervention	14.86 ± 1.15	15.47 ± 1.01

[1]Data are presented as means ± SE.

*$P < 0.05$ significantly different from conventional yogurt group.

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Table 3. Effects of probiotic and conventional yogurt consumption on blood lipid parameters in diabetic people[1]

Variable	Measurement period	Conventional yogurt (n = 30)	Probiotic yogurt (n = 30)
Total cholesterol (mmol/L)	Baseline	4.89 ± 0.10	5.19 ± 0.12
	After intervention	4.85 ± 0.11	4.95 ± 0.13[a,b]
Triglyceride (mmol/L)	Baseline	1.52 ± 0.10	1.71 ± 0.14
	After intervention	1.61 ± 0.10	1.71 ± 0.17
HDL-C[2] (mmol/L)	Baseline	1.31 ± 0.05	1.25 ± 0.05
	After intervention	1.24 ± 0.05[b]	1.25 ± 0.05
LDL-C[3] (mmol/L)	Baseline	2.89 ± 0.11	3.15 ± 0.11
	After intervention	2.86 ± 0.12	2.90 ± 0.11[a,b]
Total-C/HDL-C	Baseline	3.96 ± 0.20	4.37 ± 0.22
	After intervention	4.12 ± 0.21	4.14 ± 0.20[a,b]
LDL-C/HDL-C	Baseline	2.40 ± 0.17	2.68 ± 0.16
	After intervention	2.49 ± 0.18	2.45 ± 0.15[a,b]

[a]Significant difference between conventional and probiotic yogurt groups after intervention ($P < 0.05$).

[b]Significant difference within groups throughout the study ($P < 0.05$).

[1]All data are expressed as mean ± standard error.

[2]High-density lipoprotein cholesterol.

[3]Low-density lipoprotein cholesterol.

DISCUSSION

Dyslipidemia is the leading cause of CVD in type 2 diabetic people and is common in T2DM (Bertoni et al., 2004). Some studies have reported that probiotics may be able to improve the lipid profile. Hence, the present study was designed to investigate the effects of 6-wk probiotic and conventional yogurt consumption on the lipid profile in type 2 diabetic people with LDL-C greater than 2.6 mmol/L. It was shown that probiotic yogurt consumption significantly decreased TC, LDL-C, TC:HDL-C ratio, and LDL-C:HDL-C ratio compared with the conventional yogurt. However, TG and HDL-C remained unchanged in the intervention group during the study.

In the present study, no statistically significant changes were noted in weight, BMI, energy, and nutrient intakes within any group during the study. Therefore, the observed decrease in serum TC and LDL-C concentrations in the probiotic group could not be because of the changes in weight and dietary intakes.

Chin (2005) reported that T2DM could arise from imbalances of microflora in the gastrointestinal tract. Larsen et al. (2010) documented that the intestinal microbiota of type 2 diabetic people was relatively enriched with gram-negative bacteria, belonging to the phyla *Bacteroidetes* and *Proteobacteria.* The proportion of *Firmicutes* to *Bacteroidetes* was significantly decreased in the people with T2DM compared with the nondiabetic people. According to our knowledge, this study is the first randomized controlled clinical trial investigating the effects of probiotic yogurt fermented with *L. acidophilus* La5 and *B. lactis* Bb12 on the lipid profile in people with T2DM. Our results are in accordance with the findings of an animal study, which has shown that probiotic dahi containing *L. acidophilus* and *L. casei* delayed the onset of dyslipidemia in high fructose-induced diabetic rats (Yadav et al., 2007).

Within all probiotic strains investigated for cholesterol-lowering effect, *L. acidophilus* has been the most widely studied. Anderson and Gilliland (1999) reported that daily consumption of 200 g of yogurt containing *L. acidophilus* L1 contributed to a 2.9% decrease in serum cholesterol concentration in hypercholesterolemic humans. In a study by Ataie-Jafari et al. (2009), a significant decrease in serum TC was seen with the daily consumption of probiotic yogurt containing *L. acidophilus* and *B. lactis* in hypercholesterolemic people. In another study, Schaafsma et al. (1998) showed that consumption of fermented milk containing *L. acidophilus* and fructo-oligosaccharides significantly decreased TC concentration after 3 wk. These results are in agreement with the results of this study. However, Sadrzadeh-Yeganeh et al. (2010) and Fabian and Elmadfa (2006) demonstrated that both probiotic and conventional yogurts had positive effects on the lipid profile of healthy women. On the other hand, other studies documented that probiotics could improve HDL-C concentration (Kawase et al., 2000; Naruszewicz et al., 2002). The observed effect on HDL-C concentration could be the result of sphingolipids in yogurts and in cell membranes of probiotic bacteria. Sphingolipids can be found in lipid-rich structures and have effects on the cholesterol metabolism and transport (Vesper et al., 1999).

These inconsistent findings could be partly because of varying strains and doses of probiotics, different du-

ration of treatment periods, sample size, and clinical characteristics of participants (Ooi and Liong, 2010). Furthermore, results of some studies that used probiotic capsules instead of dairy products for administrating probiotics did not support the cholesterol-lowering potential of probiotics (Lewis and Burmeister, 2005; Greany et al., 2008; Hatakka et al., 2008). It was proposed that dairy products are more effective mediums for administrating probiotics. Lewis and Burmeister (2005) declared that it is possible that sufficient time was not available for the freeze-dried bacteria in the probiotic capsules to become metabolically active in the intestine before being flushed into the colon.

In the present study, the TC:HDL-C ratio and LDL-C:HDL-C ratio, as atherogenic indices were significantly decreased in the probiotic group compared with the conventional group. It has been suggested that these ratios have greater predictive value for CVD than either serum TC or LDL-C (Kinosian et al., 1994).

Several mechanisms for cholesterol concentration decrease by probiotics have been proposed. One of these proposed mechanisms is the assimilation of cholesterol by probiotics (Pereira and Gibson, 2002b). Cholesterol is incorporated into the cell membrane or cell wall of probiotic bacteria. These mechanisms could decrease serum cholesterol concentration by decreasing cholesterol absorption in the intestine (Liong and Shah, 2005; Ooi and Liong, 2010). The cholesterol-lowering effect of probiotics may also be explained by enzymatic deconjugation of bile acids by bile salt hydrolase. It could interfere with the enterohepatic circulation of bile salts. Because cholesterol is the precursor for the de novo synthesis of new bile acids, this interference would lead to a decrease in serum cholesterol concentration (Begley et al., 2006; Ooi and Liong, 2010).

Another mechanism that may have contributed to the cholesterol decrease is the production of short-chain fatty acids by probiotics upon fermentation. Short-chain fatty acids can inhibit hepatic cholesterol synthesis. Hydroxymethyl glutarate CoA (HMG CoA) is another compound that helps probiotics block HMG CoA reductase activity and endogenous production of cholesterol (Pereira and Gibson, 2002b; Homayouni, 2008; Ooi and Liong, 2010).

The limitations of this study included its short duration and the absence of a control group that consumed no yogurt. Further investigations with longer duration and a no-yogurt control group are needed for better confirmation of the positive effects of probiotic yogurt on the lipid profile in type 2 diabetic people.

In conclusion, this trial showed that consumption of probiotic yogurt containing *L. acidophilus* La5 and *B. lactis* Bb12 could decrease serum TC and LDL-C concentrations in type 2 diabetic people. These findings suggest that probiotic yogurt may help decrease CVD risk factors in people with T2DM.

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