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The online version of this article can be found at
http://ebcj.mums.ac.ir/article_۷۹۸۴.html

Evidence Based Care Journal 201۷ 06:26 originally published online 01 January 2017
DOI: 10.22038/ebcj.2016.7984

Online ISSN: 2008-370X

Address: Mashhad Nursing and Midwifery School, Ebn-e-Sina St., Mashhad, Iran
P.O.Box: 9137913199
Tel.: (098 51) 38591511-294
Fax: (098 51) 38539775
Email: EBCJ@mums.ac.ir

Evidence Based Care Journal

Original Article

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The Effect of Probiotic Yogurt on Blood Glucose and cardiovascular Biomarkers in Patients with Type II Diabetes: A Randomized Controlled Trial

Mahin Rezaei[1], Akram Sanagoo[2*], Leila Jouybari[3], Naser Behnampoo[4], Ali Kavosi[5]

Received: 15/10/2016
Accepted: 11/12/2016

Evidence Based Care Journal, 6 (4): 23-30

Abstract

Background: Given the high prevalence of type II diabetes and its complications, the evidence regarding the beneficial effects of probiotic yogurt on some cardiovascular biomarkers in diabetic patients is worthy of investigation.

Aim: To investigate the effect of probiotic yogurt on blood glucose level and cardiovascular biomarkers in patients with type II diabetes.

Method: This randomized, clinical trial was conducted on 90 patients with type II diabetes who visited the 5 Azar diabetes clinic in Gorgan, Iran, in 2014. The intervention group consumed three 100 g packages of probiotic yogurt per day for four weeks, while the control group used an equal amount of plain yogurt. Dietary intake, as well as anthropometric and biochemical parameters were measured before and after the trial. To analyze the data, independent t-test, paired t-test, and analysis of covariance were performed, using SPSS version 18.

Results: The mean ages of the intervention and control groups were 50.49±10.92 and 50.13±9.20 years, respectively. In the intervention group, paired t-test showed significant differences between mean levels of blood glucose, cholesterol, low-density lipoprotein (LDL), triglycerides, diastolic blood pressure, and glycated hemoglobin before and after four weeks of daily intake of probiotic yogurt ($P<0.05$). Conversely, none of these parameters showed any significant change in the control group ($P>0.05$). At the end of trial, the independent t-test showed a significant difference between the two groups in terms of mean levels of blood glucose, LDL, triglycerides, blood pressure, and glycated hemoglobin ($P<0.05$).

Implications for Practice: Consumption of probiotic yogurt showed beneficial effects on blood glucose, glycated hemoglobin, blood pressure, and serum lipid levels in the intervention group. However, it had no significant effect on cholesterol, high-density lipoprotein, and C-reactive protein as compared with the control group. The authors recommend further longitudinal studies to draw a definitive conclusion in this regard. Overall, consumption of probiotic yoghurt can be recommended as an adjunctive therapy for type II diabetic patients.

Keywords: Biomarkers, Blood glucose, Diabetes mellitus type II, Probiotics

1. MSc Student of Critical Care Nursing, School of Nursing & Midwifery, Golestan University of Medical Sciences, Gorgan, Iran
2. PhD of Nursing, Associate Professor, Nursing Research Center, Golestan University of Medical Sciences, Gorgan, Iran
3. PhD of Nursing, Associate Professor, Nursing Research Center, Golestan University of Medical Sciences, Gorgan, Iran
4. PhD of Biostatistics, Assistant Professor, School of Health, Golestan University of Medical Sciences, Gorgan, Iran
5. Msc of Nursing, Instructur, Department of Operating Room, Neyshabur University of Medical Sciences, Neyshabur, Iran
* Corresponding author, Email: sanagoo@goums.ac.ir

Introduction

Type II diabetes is a common metabolic disorder, the prevalence rate of which is on a growing trend; this rate is estimated to reach 366 million by 2030 (1). Currently, 4% of the world population are suffering from diabetes (2), and the prevalence of this disease among 25-64 year-old Iranians is estimated at 7.7% (3). Diabetes can cause chronic and acute complications such as hypoglycemia, infections, and pathological changes in nerves, retina, as well as kidney. Consequently, it can have adverse effects on overall well-being and could possibly lead to patient death (4).

Cardiovascular diseases are the leading causes of mortality in patients with type II diabetes, as the relative risk of developing these diseases is two to four times higher in diabetic patients than in healthy individuals (5). Hypertension and dyslipidemia, which are highly prevalent, are the known risk factors for cardiovascular complications in diabetics (6). Characteristics of dyslipidemia in patients with type II diabetes include reduced level of high-density lipoprotein (HDL), as well as elevated low-density lipoprotein (LDL) and total triglyceride levels (7). Each 1% reduction in serum cholesterol concentration results in two to three times lower risk of coronary heart diseases (CHD), thus, measures to reduce cholesterol can significantly contribute to the reduction of CHD-induced mortality in diabetic patients (8).

Diabetes is incurable but can be controlled (9). Today, therapeutic regimens or nutritional interventions are among the primary recommendations for dealing with diabetes. Probiotic foods are among dietary ingredients that have shown significant health benefits. They may also lower serum cholesterol level and improve insulin sensitivity (10). Probiotics are non-pathogenic microorganisms that when consumed alive and in sufficient amounts, can provide health benefits by contributing to microbial balance in the host intestine. Probiotics can therefore be regarded as functional food. Bacteria producing lactic acid, particularly *Lactobacillus* and *Bifidobacterium* that are normally a part of gastrointestinal ecosystem, are considered probiotics (11).

Probiotics provide many health benefits by contributing to the treatment of lactose intolerance symptoms, diarrhea, constipation, allergies, inflammatory bowel disease, irritable bowel syndrome, and gastric ulcers, as well as stimulating the immune system, preventing autoimmune diseases, and reducing cholesterol; they are also known to have cancer preventive properties (12).

Animal studies have proven the effects of probiotics in terms of reducing blood glucose level, delaying the onset of hyperglycemia, and diminishing insulin resistance in diabetic rats (13). The effect of probiotics on glucose metabolism is most likely due to their immune modulating properties. Some specific strains of probiotics can improve the composition of intestinal microflora and intestinal function. This effect inhibits the transfer of bacterial endotoxins into the bloodstream and reduces the lipopolysaccharide and pro-inflammatory cytokines in circulation, which in turn, leads to a reduction in inflammation. Therefore, probiotics decrease insulin resistance and preserve pancreatic beta cells more efficiently (14).

The mechanisms hypothesized to control cholesterol by probiotics include conjugation of bile acids with hydrolase and inhibition of enterohepatic circulation of bile salt (15). Cholesterol binds to the cell walls of probiotics and assimilates cholesterol into membranes of probiotics during growth. These mechanisms undermine the absorption of dietary cholesterol and hepatic synthesis by short-chain fatty acids resulting from fermentation by probiotics in the presence of prebiotics (16).

A clinical trial conducted by Ejtahedet et al. (2013) on patients with type II diabetes suggested that six weeks of consuming probiotic yogurt reduced blood glucose and serum concentrations of C-reactive protein (CRP) in the intervention group (17). The study of Sharafedtinov et al. (2013) also showed that three weeks of probiotic diet reduced blood pressure, glucose, and fat levels in the intervention group (18). Contrarily, Andreasen et al. (2010) reported that probiotic supplementation showed no significant effect on blood glucose (19). Ataie Jafari et al. (2009) and Sadrizadeh et al. (2010) confirmed the aforementioned findings that probiotic products showed no effect on the subjects' cholesterol level (20, 21). It is noteworthy that the mentioned studies are mostly preliminary and some used probiotic yogurt, while others used probiotic supplements.

Considering the contradictory results on the impact of probiotic supplements and products on blood glucose and cholesterol, this study aimed to determine the effect of probiotic and plain yogurt on blood glucose and cardiovascular biomarkers in patients with type II diabetes to manage blood glucose level and prevent cardiovascular diseases in these patients.

Methods

The double-blind, randomized clinical trial was carried out on diabetic patients who visited the diabetes clinic of 5-Azar Medical Education Center in Gorgan, Iran, 2014. The participants were chosen through reviewing medical records filed in the diabetes clinic. The eligible patients were contacted and invited to participate in the study.

Following the approach of Bayat et al. (22), the standard sample size was calculated using the formula for comparing two independent means (mean blood glucose level was 165.50±41.34 in the control group and 126.25±34.01 in the probiotic group) with 80% power and 95% confidence level. After taking into account the risk of sample loss, the standard size of each group was calculated at 45.

The subjects were selected by non-probability convenient sampling technique and were assigned randomly into two groups of intervention and control (n=45 people). For random assignment, 45 pieces of paper with the header "Probiotic Diet" and another 45 with the header "Routine Diet" were placed in a box and were randomly drawn by clinic staff to place each patient in one group of the trial (it should be noted subjects were matched by age and gender).

The inclusion criteria were 1) diagnosed with type II diabetes for more than one year; 2) consumption of glibenclamide/metformin; 3) receiving insulin; 4) residence in Gorgan; 5) LDL of higher than 100 Mg/dl ; 6) ability to follow the treatment regimen and collaborate with the researchers, and 7) aged between 30 and 70 years. The exclusion criteria included death, heart, renal, hepatic, pulmonary, and inflammatory diseases, chronic diseases of the gastrointestinal tract, impaired thyroid function, lactose intolerance, insulin injection, consumption of estrogen, progesterone, corticosteroids, cholesterol-lowering, and diuretic drugs, body mass index (BMI) of more than 35, smoking, breastfeeding, pregnancy, consumption of vitamin, mineral, and omega-3 supplements during the three weeks before the start of study, as well as consuming antibiotics during and a month before the start of study. At the outset of the study, two participants were excluded because of intolerance and sensitivity to yogurt and were immediately replaced by two other patients.

Before beginning the study, patients' demographics and general information, including gender, age, diabetes duration, diagnosis, or development of other diseases, and type and dose of drugs being consumed to control blood glucose along with pressure, were collected. Systolic and diastolic blood pressure (DBP) were measured by clinic staff using an Alpk2 Mercury Sphygmomanometer (Japan). This measurement was made in the right arm with the patient in a sitting position and was repeated after 15 minutes of rest and the average of the two measurements was recorded. Weight was measured using a Seca scale (Germany) with 100 grams precision and minimal clothing and without shoes. Height was measured using a Seca stadiometer (Germany) with 0.5 cm precision and without shoes. BMI was calculated by dividing weight in kilograms by the square of height in meters.

In the initial meeting, the proper method of storing and consuming yogurt was explained, and the importance of following the usual diet during the intervention period was emphasized. The participants were asked to refrain from consuming yogurt or doogh (diluted yogurt; a popular beverage in the Middle-East) for a week before the start of the study and to replace it with a glass of milk. They were also asked to maintain their usual physical activity and diet and avoid any dietary supplements during the trial. During the trial, changes in the dose and type of medications were kept to a minimum.

During the four-week trial period, daily diet of the control group included 300 grams (three 100 g packages) of plain 2.5% fat yogurt (containing typical yogurt starter cultures, *Streptococcus thermophilus* and *Lactobacillus bulgaricus*). Meanwhile, daily diet of the intervention group included 300 grams (three 100 g packages) of 2.5% fat probiotic yogurt (containing typical yogurt starter cultures, plus *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12) and sans any other doogh or yogurt. The participants received yogurt on a weekly basis by visiting a designated dairy store, where staff was blinded to the type of delivered yogurt. Probiotic yoghurt was similar to plain yogurt in all aspects except for the probiotic bacteria. The patients were also blinded to the type of delivered yogurt. All yogurts were prepared in 100 g packages, exceedingly similar in appearance and without any labels indicating the type of yogurt. To separate the two types, packages were coded in the factory by staff and deliverymen were blinded regarding the association of codes with types.

The participants were contacted weekly to ensure their commitment to the arranged diet and to record their opinions on yogurt and any possible change in medication. Dietary intake of the participants was recorded by a 24-hour dietary recall questionnaire, which was filled out three days at the beginning and three days at end of the trial. Dietary intake information was analyzed with the Nutritionist software, version 4, to determine the intake of energy and macronutrients.

Before and after the trial, a laboratory specialist drew 10 cc of venous blood samples from each patient. For this purpose, the patients were asked to fast for 10 to 12 hours before venipuncture. After 15 minutes of incubation at room temperature and clotting of the blood samples, they were centrifuged at 3500 rpm for 10 min. The separated serum was poured in 1 ml micro-tubes and was stored in a freezer at -70°C to prevent deterioration before tests and analyses. Fasting blood sugar (FBS) and glycated hemoglobin (HBA_{1C}) were measured by an autoanalyzer. The serum concentration of CRP was measured by immunoturbidimetric assay using a commercial test kit (Pars Azmoon, Iran). Cholesterol, triglyceride, and HDL-cholesterol (HDL-C) were also measured using a standard enzymatic assay kit (Pars Azmoon, Iran). LDL-cholesterol (LDL-C) was calculated using the Friedewald formula (23).

During this study, all the research ethics principles were respected. The study commenced after gaining approval from the Ethics Committee of Golestan University of Medical Sciences, and after registering in the Iranian Registry of Clinical Trials. Before the trial, the purpose and method of the study was explained to the participants, and informed consent was obtained from them. The participants entered the study voluntarily and could withdraw from the study at any time. The patients were assured of the confidentiality of the data. The information was handled using numerical codes instead of names and surnames. At the end of the trial, the clinic authorities and patients were given access to the results upon request.

The collected data was imported into a computer and analyzed in SPSS, version 18. Before the initiation of the analysis, normal distribution of quantitative data was checked with Shapiro-Wilk and Kolmogorov-Smirnov tests. In the first step of analysis, demographic variables were evaluated using descriptive statistics (frequency, mean and standard deviation). Independent t-test was used to compare the means of the two groups and paired t-test was run to compare the mean values of the parameters within each group pre- and post-intervention. Analysis of covariance was carried out to account for the difference between mean HbA_{1c} of the two groups before the intervention. P-value less than 0.05 was considered statistically significant.

Results

The mean ages of the participants in the intervention and control groups were 50.49±10.92 and 50.13±9.20 years, respectively, regarding which Fisher's exact test showed no significant difference (P=0.43). The intervention group consisted of 23 males (64.4%) and 22 females (35.6%), and the control group comprised of 21 males (57.8%) and 24 females (42.2%), for which the Chi-square test reflected no significant difference (P=0.76). The mean BMIs were 28.9±3.5 and 29.5±1.6 kg/m^2 in the intervention and control groups, respectively, and t-test showed no significant difference between these two groups in this regard (P=0.62). In conclusion, the participants in the two groups were matched in terms of age, gender, and BMI (Table 1).

Table 1. Distribution of demographic characteristics in the intervention and control groups

Variable		Group		
		Intervention	Control	P-value
Gender		Frequency	Frequency	
	Male	23(51%)	21(47%)	*P=0.76
	Female	22(49%)	24(53%)	
Age group	35-45	16(35%)	12(27%)	**P= 0.43
	45-50	12(27%)	12(27%)	
	50-55	9(20%)	5(12%)	
	55-69	8(18%)	16(34%)	
Mean BMI (kg/cm^2)		28.9±3.5	29.5±1.6	***P=0.62

*Chi-square test **Fisher's exact test *** Independent t-test

Table 2. Inter- and intra-group comparison of mean cardiovascular biomarkers (before and after the intervention)

Variable	Group				Intra-group test (Independent t-test)
	Probiotic yogurt		Ordinary yogurt		
	Before	After	Before	After	
Fasting blood sugar Paired t-test / intragroup	145.5±34.4 P=0.01	129.6±41.3	194.8±66.8 P=0.08	200.9±67.2	P=0.004
Cholesterol (mg/di) Paired t-test / intragroup	213.3±42.4 P<0.001	195.04±40.5	210.7±39.4 P=0/17	212.9±38.6	P=0.56
High-density lipoprotein-cholosterol Paired t-test / intragroup	46.4±7.5 P=0.59	47.02±6.6	47.4±9.3 P=0.93	47.5±11.3	P=0.64
Low-density lipoprotein-cholosterol Paired t-test / intragroup	124.1±17.2 P<0.001	106.8±21.9	124.4±17.5 P=0/05	128.3±20.6	P=0.04
Triglyceride Paired t-test / intragroup	161.4±78.7 P<0.001	146±83.5	211.3±11.5 P=0.54	213.9±10.1	P=0.02
Systolic blood pressure Paired t-test / intragroup	152.9±15.3 P=0.08	140.1±14.2	143.5±15.03 P=0.11	139±13.8	P=0.83
Diastolic blood pressure Paired t-test / intragroup	77.1±12.5 P=0.02	73.7±4.1	74.8±12.3 P=0.82	75.6±6.06	P=0.01
C-reactive protein Paired t-test / intragroup	4.2±4.1 P=0.07	4.1±4.2	3.8±2.6 P=0.08	4.04±2.9	P=0.05
Glycated hemoglobin Paired t-test / intragroup	7.2±1.4 P<0.001	6.8±1.7	7.9±1.1 P=0.28	8.1±0.9	P=0.01

According to the results outlined in Table 2, the mean FBS of the intervention group (consuming probiotic yogurt) decreased from 145.5±34.4 to 129.6±41.3; paired t-test revealed that this reduction was significant (P=0.01). However, in the control group (consuming plain yogurt), mean FBS elevated non-significantly from 194.8±66.8 to 200.9±67.2 (P=0.08). Overall, the independent t-test demonstrated a significant difference between the mean FBS values of the intervention and control groups after four weeks of trial (P=0.004).

In the intervention group, mean cholesterol level diminished from 213.3±42.4 to 195.04±40.5, which was significant according to paired t-test (P<0.001). In the control group, however, mean cholesterol raised from 210.7±39.4 to 212.9±38.6, which was not significant based on paired t-test (P=0.17). The independent t-test showed no significant difference between the mean cholesterol values of the intervention and control groups after the trial (P=0.56).

Paired t-test showed no statistical significance in the increase of mean HDL-C in the intervention group from 46.4±7.5 to 47.02±6.6 (P=0.59), or its slight increase in the control group from 47.4±9.3 to 47.5±11.3 (P=0.93). The independent t-test also found no significant difference between the mean HDL-C values of the intervention and control groups at the end of trial (P=0.64).

The paired t-test reflected a statistically significant reduction in mean LDL-C of the intervention group (from 124.1±17.2 to 106.8±21.9; P=0.001) and a non-significant change in the mean HDL-C of the control group (from 124.4±17.5 to 128.3±20.6; P=0.05). Overall, the independent t-test showed a significant difference between the mean LDL-C values of the intervention and control groups at the end of week four (P=0.004).

In the intervention group, the paired t-test presented a statistically significant decrease in the mean triglyceride level (from 161.4±78.7 to 146 ±83.5; P<0.001). Nevertheless, in the control group, the change in mean triglyceride (from 211.3±11.5 to 213.9±10.1) was non-significant (P=0.54). The independent t-test showed a significant difference between the mean triglyceride levels of the intervention and control groups after four weeks of trial (P=0.02).

The paired t-test manifested no significant change in mean systolic blood pressure (SBP) in the intervention and control groups (from 152.9±15.3 to 140.1±14.2 and from 143.5±15.3 to 139±13.8; P=0.08, P=0.11, respectively). Moreover, no significant difference was noted between the mean SBP of the intervention and control groups at the end of the trial (P=0.83).

The paired t-test indicated that the change in mean DBP of the intervention group (from 77.1±12.5 to 73.7±4.1) was significant (P=0.02), but not the change in mean DBP of the control group (from

74.8±12.3 to 75.6±6.06; P=0.82). The independent t-test also showed a significant difference between the mean DBP of the two groups at the end of fourth week (P=0.01).
The changes of mean CRP from 4.2±4.1 to 4.1±4.2 in the intervention group and from 3.8±2.6 to 4.04±2.9 in the control group were both found to be non-significant (P=0.07 and P=0.08, respectively). The independent t-test also found no significant difference between the mean CRP values of these two groups at the end of the trial (P=0.05).
The paired t-test showed the decrease in mean Glycated hemoglobin of the intervention group from 7.2±1.4 to 6.8±1.7 was significant (P<0.001), but in the control group, the change in this value (from 7.9±1.1 to 8.1±0.9) was non-significant (P=0.28). At the end of trial, the independent t-test also showed a significant difference (P=0.01) between the intervention and control groups in terms of mean Glycated hemoglobin (Table 2).

Discussion

In this study, we investigated the effect of probiotic yogurt and plain yogurt on blood glucose and cardiovascular biomarkers in patients with type II diabetes. Our results showed the positive effect of probiotic yogurt on blood glucose, that is, mean FBS of the intervention group decreased when compared with the control group. The study of Yadav et al. (2006) on laboratory rats showed that the fermented milk by *Lactobacillus acidophilus* and *Lactobacillus casei* can have an anti-diabetic effect, such that it delays the onset of impaired glucose metabolism in mice fed with a high-fructose diet. Intake of this product was reported to delay the onset of glucose intolerance, increased hyperglycemia and insulin in the mice's blood and to decrease the oxidative stress (24). Sharafedtinov et al. (2013) conducted a clinical trial in Russia to evaluate the effect of three weeks of low-calorie and probiotic (cheese) diet on weight loss, blood pressure, blood lipids, and blood glucose, and they found a significant decrease in blood glucose of the intervention group when compared with the control group (18). A systematic review and meta-analysis carried out by Zhang (2016) also reported the consumption of probiotic yogurt as a contributing factor for lowering blood sugar in diabetic patients (25). The study of Sanaie et al. (2013) performed on Iranian patients also supports the positive effects of probiotic yogurt on blood glucose. Thus, the results of animal and human studies suggest that probiotic yogurt can lower blood glucose levels of diabetic patients. Although we found a significant decrease in the mean glycated hemoglobin of the intervention group, this decrease is not definitively attributed to consumption of probiotic yogurt. According to the American Diabetes Association, glycosylation takes place gradually over a period of 60 to 120 days (26), but our glycated hemoglobin measurements were made after four weeks and could possibly be affected by variables such as preexisting anemia (especially due to iron deficiency) and alleviation of this condition during the intervention period. This argument is supported by the American Diabetes Association suggesting that iron deficiency anemia and its medications can affect glycated hemoglobin results, meaning that they cannot truly reflect the glycemic status and parameters of these patients (27).
In this study, we found a significant decline in the mean triglyceride and LDL-C levels of the intervention group (consuming probiotic yogurt) when compared with the control group (consuming plain yogurt). We also found a significant decrease in the mean cholesterol level of the intervention group after four weeks of consuming probiotic yogurt. Our results are consistent with the results of some animal and human studies. For instance, the study of Nabavi et al. (2015) also reported that the daily intake of 300 g of probiotic yogurt containing *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12 for eight weeks resulted in lower cholesterol and LDL-C levels (28). A study by Anderson et al. (1999) also reported that five weeks of probiotic diet reduced the plasma levels of cholesterol and triglycerides (29). Ejtahed et al. (2011) reported that consumption of 300 g of probiotic yogurt containing *Lactobacillus acidophilus* La5 and *Bifidobacterium lactis* Bb12 by patients with type II diabetes on a daily basis resulted in 7.45% and 4.54% reduction in serum LDL-C and cholesterol levels, respectively (30). In a study by Baroutkoub et al. (2010), daily consumption of probiotic yogurt containing *Lactobacillus acidophilus* and *Bifidobacterium* for six weeks was found to cause a significant reduction in plasma LDL-C and cholesterol levels (31). A study by Ataie et al. (2009) on hypercholesterolemic patients also found a significant reduction in serum cholesterol after daily intake of 300 g of probiotic yogurt (20).
There are several hypotheses regarding the mechanisms through which probiotics can lower cholesterol. Laboratory studies have shown that intestinal lactic acid bacteria can not only bind to bile

acids, but also to cholesterol, and thereby decrease the dietary cholesterol available for absorption in the intestine, and consequently, the total cholesterol. Deconjugation of bile acids with hydrolase inhibits the enterohepatic circulation of bile salts. Bile is a water-soluble composition made in the liver by cholesterol and stored in the gallbladder, and it is secreted when food enters the duodenum. Bile is made of cholesterol, phospholipids, conjugated bile acids, bile pigments, and electrolytes. Deconjugated bile acids have a low solubility and absorbability in the intestine and are more likely to be excreted in the feces. As a result, the body may use cholesterol to make new bile acids, which may lead to lower serum concentration of cholesterol. The other hypothesized mechanisms are the binding of cholesterol to cell walls of probiotics and assimilation of cholesterol with cellular membrane of bacteria, all leading to lower absorption of dietary cholesterol. Living and growing bacteria show better cholesterol excretion ability (15, 32). Short-chain fatty acids produced by probiotics in the course of fermentation can also inhibit the hepatic synthesis of cholesterol (15). Excretion of coprostanol produced from cholesterol can also reduce cholesterol absorption and lower blood cholesterol levels. Furthermore, coprostanol production reportedly improved in the presence of probiotics (32).

On the other hand, our results in this regard are inconsistent with some other studies, particularly those that have used probiotic capsules or tablets instead of yogurt (12). Lewis and Burmeister (2005) stated that a lack of sufficient time for metabolic activation of bacteria delivered in capsules could be the cause of their inadequacy on serum cholesterol. Thus, they have suggested that probiotics of dairy products may be more effective than probiotic supplements (33).

Herein, four weeks of consuming probiotic yogurt showed no significant effect on serum HDL-C level of the intervention group when compared with the control group. This result is inconsistent with the results of previous studies reporting such effect in diabetic (30), healthy women (21) and in hypercholesterolemic patients (31,33). This discrepancy may be due to differences in species and doses of probiotics, intervention period, sample size, clinical characteristics of participants, or different study design (32).

Although SBP of the intervention group showed a decrease compared to the control group, independent t-test found the difference of the two groups to be statistically insignificant. For BPD, however, the difference observed after four weeks of intervention was found to be significant. In the study of Bayat et al. (2014) on diabetic patients, probiotic yogurt showed a statistically significant effect on both systolic and diastolic blood pressures (22). Agerholm-Larsen et al. (2000) also reported that consumption of probiotic dairy products for eight weeks led to a significant reduction in SBP and DBP of the obese and overweight (34). The systematic review and meta-analysis of Khalesi (2014) also reported that probiotic products can reduce SBP and DBP (35). The effect of probiotics on blood pressure was hypothesized to be realized through the production of angiotensin-converting enzymeinhibitory peptides (36).

In the current study, we found no statistically significant difference between the two groups in terms of CRP before and after consumption of plain and probiotic yogurt. The study of Mohamadshahi et al. (2014) reported that eight weeks of consuming probiotic yogurt had no effect on inflammatory markers of type II diabetic patients (37). A clinical study by Andreasen on type II diabetic patients also reported that a four-week treatment with probiotic supplements (*Lactobacillus acidophilus*) had no effect on the systemic inflammatory response and serum CRP level (19). Our results, which also suggest the inefficacy of probiotics on inflammatory factors, are in line with these findings, but are inconsistent with the study of Ejtahed et al. (2013). That study reported that consuming probiotic yogurt for six weeks leads to a significant reduction in CRP concentration (17). Similarly, the results of a clinical trial by Asemi et al. (2011). on pregnant women showed that consuming 200 g of probiotic yogurt for nine weeks reduces serum CRP level in these women (38). The study of Kekkonen et al. (2008) on healthy individuals also found that daily intake of probiotic products for three weeks decreased the serum concentrations of CRP and interleukin-2 (39). As mentioned previously, all these reports are inconsistent with our results. The cause of this inconsistency could be the differences in intervention period, study design, quantity, species and strain of probiotics, and difference in probiotic carriers. Note that probiotics may reduce inflammation by improving the intestinal micro-flora, reducing the permeability of intestinal mucosal barrier to lipopolysaccharides, and protecting blood circulation against bacteria-derived endotoxins. Probiotics may control systemic inflammation by increasing the number of natural killer T cells (40).

Limitations of our study included the absence of an initial assay to identify patients with iron deficiency anemia and the short duration of the trial. Thus, future studies with similar objectives are recommended to take the necessary steps to avoid these limitations.

Conclusion

In this study, daily consumption of probiotic yogurt containing *Lactobacillus acidophilus* La5 *Bifidobacterium lactis* Bb12 for four weeks showed beneficial effects on blood glucose, glycated hemoglobin, diastolic blood pressure, and serum lipid levels in the intervention group, but had no significant effect on cholesterol, HDL, and CRP as compared with the control group. The authors recommend further studies with longer intervention periods to draw a definite conclusion in this regard. Overall, consumption of probiotic yogurt can be recommended as an adjunctive therapy for type II diabetic patients.

Acknowledgments

The authors would like to thank the Research Deputy of Golestan University of Medical Sciences for approving the research. They would also like to thank all the participants and staff of diabetes clinic of Gorgan Health Center for their sincere cooperation. This article is a part of thesis prepared for acquiring MSc degree in Intensive Care Nursing from Golestan University of Medical Sciences (authorization code 13930318445), and has been registered at Iranian Registry of Clinical Trials (IRCT201404185866N18).

Conflict of interest

The authors declare no conflict of interest in this study.

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