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Effects of probiotic soy milk on lipid panel in type 2 diabetic patients with nephropathy: A double-blind randomized clinical trial

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Summary. *Background:* Cardiovascular diseases (CVD) are among the most common complications of diabetes. Lipid abnormalities in diabetic patients are not only related to higher risk of CVD, but also accelerate the progression of diabetic nephropathy. To the best of our knowledge, there is no study that has assessed the effects of probiotic soy milk on lipid profile in type 2 diabetic patients with nephropathy. **Objective:** The current study was designed to examine the effects of consumption of soy milk containing *lactobacillus plantarum A7* compared with conventional soy milk on lipid panel in type 2 diabetic patients with nephropathy. *Methods:* A total of 44 type 2 diabetic patients with nephropathy were randomly assigned to receive 200 ml/day of either probiotic soy milk (n=22) or conventional soy milk (n=22) for eight weeks, in this randomized double-blind clinical trial. Fasting blood samples were taken at the beginning and after eight weeks of the intervention for analysis of lipid profile and other relevant variables. P values < 0.05 were considered as statistically significant. *Results:* Consumption of probiotic soy milk for 8 weeks led to an increase in serum genistein (17.6±15.3 vs. 4.5±2.3, p=0.002) and eGFR (15.9±10.8 vs. 3.2±8.4, p<0.001) compared with conventional soy milk. Additionally probiotic soy milk resulted in decreased LDL-cholesterol (-9.2±10.4 vs. -2.2±5.2, p=0.01), total cholesterol (-12.4±4.8 vs. -4.87±14.7, p=0.04), non-HDL cholesterol (-15.3±4.5 vs. -5.9±14.7, p=0.01) and serum TG (-14.6±12.5 vs. -3.9±9.3, p=0.007) compared with control group. We did not detect any significant effect of probiotic soy milk on serum HDL-cholestrol (1.11±3.38 vs. 0.90±2.7, p=0.8) and serum phosphorus (-0.14±0.10 vs. 0.05±0.5, p=0.1). *Conclusion:* Administration of soy milk containing *lactobacillus plantarum A7* in type 2 diabetic patients with nephropathy had beneficial effects on lipid profile and glomerular function, but did not affect HDL-cholesterol. In addition probiotic soy milk did not result in a significant elevation in the serum phosphorus concentration. This trial was registered at http://www.irct.ir as IRCT201601027479N2.

Keywords. Probiotics, Type 2 diabetes mellitus, Diabetic nephropathies, Lipid profile, Soy milk.

Introduction

Type 2 diabetes mellitus (T2DM) is the main form of diabetes and comprises more than 90% of people living with diabetes mellitus, worldwide. As the 21st century, has the most diabetogenic environment in human history, it is estimated that the current prevalence of 415 million, will reach 642 million people in 2040 (1). Diabetes and its complications are the leading causes of death globally (1), and they are responsible for 5 to 20 percent of healthcare budget in most countries and even some developing countries spend about 40% of their total health expenditure on diabetes and its morbid complications (2).

Diabetic nephropathy (DN), which is characterized by constant albuminuria and/or relentless impairment of glomerular filtration rate (GFR) is one of the most prevalent complications of diabetes and represents the major cause of end-stage renal disease (ESRD) (3). About 20 to 30 percent of individuals with diabetes, develop DN which is responsible for 40% of new cases of ESRD (4) and while the worldwide prevalence of diabetes increases, health and economic burden of its renal complications, tends to rise.

T2DM is represented by abnormalities in the composition and levels of HDL cholesterol (HDL-C), triglycerides (TG) and LDL cholesterol (LDL-C) (5) which according to recent studies, are strongly associated with atherosclerosis and mortality rate in diabetic patients (6, 7). Additionally, presence of DN in T2DM is associated with two or three-fold increased risk of cardiovascular death (8) and lipid abnormalities are strong independent predictors of progression of ESRD (9). Hence, interventions that can interfere with the pathological events induced by excess amounts of lipids and lipoproteins may lead to major life and cost savings (10).

Current strategies to treat lipid abnormalities and cardiovascular complications of diabetic nephropathy include: intensive glycemic control, lipid lowering agents and lifestyle interventions such as dietary interventions and physical activity (11). Although the aforementioned therapeutic interventions hinder the progression of DN, the prevalence of DN remains very high and most of patients with diabetic nephropathy continue to progress to ESRD (12).

Medicinal effects of probiotics have been gaining more attention over the last years. Recent studies have reported several beneficial effects of probiotics on gastrointestinal disorders, diabetes and infectious diseases (13, 14). However, a limited number of human studies have assessed the effects of probiotic or synbiotic supplementation on diabetes or its complications and showed some beneficial effects (15, 16).

Furthermore, previous studies reported beneficial effects of soy-based foods on cardio-renal risk factors in diabetic patients with nephropathy (17, 18). Considering possible advantages of the utilization of a functional food as a medium for probiotics and low bioavailability of soy bioactive compounds as well as other serious considerations including the allergenicity of dairy products, lactose intolerance and the need for restriction of cholesterol, phosphorus and protein intake in patients with DN (19), using soy milk as a medium for probiotics may have additional beneficial effects due to the synergistic effect of soy milk and probiotics. To the best of our knowledge, there is no report regarding the effect of probiotic soy milk on lipid profile in type 2 diabetic patients with nephropathy. Hence, the current study was conducted to compare the effect of soy milk containing *lactobacillus plantarum A7* with conventional soy milk on lipid profile of diabetic patients with nephropathy to investigate whether this soy-based probiotic product could have a greater positive effect, to be used as a dietary supplement to reduce the cardio-renal risk factor in diabetic nephropathy.

Materials and methods

CONSORT (Consolidated Standards of Reporting Trials) guidelines were followed for this parallel design double-blind randomized clinical trial (RCT). On the basis of the sample size formula suggested for RCTs, considering a type I error of 5% (α=0.05) and type II error of 20% (β=0.20, power=80%) and C-reactive protein concentration as a key variable (20), we determined a sample size of 20 persons for each group. To adjust for an expected dropout rate of 10%, 44 participants (22 per group) were needed to be enrolled in the study. Convenient sampling method was used to recruit participants and finally a total of 44 participants were randomly allocated to the soy milk group (control group) or probiotic soy milk (intervention group) in which they received 200 ml/day of soy milk or probiotic soy milk for eight weeks. Randomization was done by the use of computer-generated random numbers. At each study visit, participants received sufficient bottles for a 3-day period until the next visit, in a double-blinded design. Randomization and allocation were concealed from the researcher and volunteers till the analyses were finalized. The bottles and soy milk were provided by the same soy milk factory. Bottles were identical in shape and probiotic soy milk was indistinguishable in color, smell and taste from conventional soy milk. Participants were advised to keep the bottles refrigerated at 2-4°C. They were required to return unused or empty bottles at the next study visit to assess study intervention compliance. Our protocol required a minimum intervention adherence of greater than 90% (more than 50 out of 56 bottles). Participants were refrained from consumption of any probiotic product two weeks before beginning of the study. The subjects eligible for our study fulfilled the following inclusion criteria: 25 years of age or older, proven type II diabetes for more than a year with fasting blood glucose higher than 126 mg/dl, 2h postprandial blood glucose higher than 200 mg/dl, microalbuminuria and GFR higher than 60 ml/min. Subjects with prior history of inflammatory bowel disease (IBD), infection, liver disease, rheumatoid arthritis, smoking, alcoholism, recent antibiotic therapy, and consuming multivitamin and mineral or omega-3 supplements 1 months prior to beginning of the intervention were excluded from the study. Anthropometric measurements were done by trained colleagues according to World Health Organization (WHO) standards. Light cloth worn patients were weighted to the nearest 0.1 kg by the use of a digital scale (Seca, Germany) and their height were measured without shoes with a 0.1 cm accuracy wall meter (Seca, Germany). Body mass index (BMI) was calculated as following: the weight (in kilograms) divided by height (in meters) square formula, and the obtained information was recorded in general information questionnaire of patients. These measurements were repeated again at the end of the 8th week of the study. All of the participants were instructed to consume a diet containing 0.8 g/kg protein, 2000 mg sodium, 2000 mg potassium and 1500 mg of phosphorus. The dietary intakes were obtained using 24-hour recall for 3 days (covered two weekdays and a weekend day). Participants were asked to recall all foods and beverages that they consumed during the preceding 24 hours. To assist respondents to report portion size accurately, household measuring instruments such as measuring cups and measuring spoons were used. Portion size of consumed food was converted into grams using the standard reference tables. For mixed dishes, food groups were calculated according to their ingredients. The energy and macronutrients (carbohydrate, protein and fat) content of each food were calculated using the modified version of Nutritionist-4 software. Data on physical activity were obtained using the Persian version of International Physical Activity Questionnaire (IPAQ) (21). Total physical activity lower than 600 MET-min/week was considered as low physical activity and the amounts of physical activity between 600 to 3000 MET-min/week were considered as moderated physical activity. The study protocol has been registered at Iranian Registry of Clinical Trials (Register code: IRCT201601027479N2 available at: http://www.irct.ir) as supporting information to this publication. The study was approved by ethics committee of Isfahan University of Medical Sciences and written informed consent was obtained from all subjects.

Intervention

The probiotic soy milk contained 2×10^7 cfu/ml of *lactobacillus plantarum A7*. Lactobacillus plantarum is one of the most active and common probiotics that makes it a very suitable candidate for probiotic supplementation in patients with nephropathy. Conventional and probiotic soy milk were made every three day and dispensed to the participants. The probiotic soy milk were sampled in the day of delivery and microbiologically analyzed every two weeks. MRS Broth (MRS agar: Merck, Darmstadt, Germany and bile: Sigma–Aldrich, Inc., Reyde, USA) and pour plate method were used to evaluate the total colony count of *lactobacillus plantarum A7* in probiotic soy milk. As a result of the colony counting test, we concluded that the survival rate and concentration of *lactobacillus plantarum A7* in probiotic soy milk remains steady between the first day of production and the third day at 2-4°C. Our study had a 2-week run-in phase prior to the beginning of the intervention in which participant should avoid from consumption of any fermented or probiotic food. They were also asked to avoid from consumption of any dietary supplement and report any change of their medications to the researchers.

Biochemical analysis

To analyze biochemical factors of participants, 10 mL of venous blood were collected at baseline and after the 8th week of intervention in the early morning after an overnight fast and within 1-2 h, the blood samples were centrifuged with 3500 rpm for 10 minute and the sera were put in a −80°C freezer until they were used for subsequent biochemical analysis. Appropriate biochemical analyses were used to assay concentration of total cholesterol (cholesterol oxidase/peroxidase), triglycerides (glycerol phosphate oxidase/peroxidase), HDL-C (detergent), LDL-C (detergent), serum creatinine (alkaline picrate), phosphorus (phosphomolybdate/UV) in sera (Biosystems analyzer A-15, Spain). All the tests were performed in a blinded fashion, in pairs (before and after the intervention) at the same time, in the same analytic run, and in random order to reduce systematic error and inter-assay variability. We also assayed the adherence to the intervention by measuring the serum concentration of genistein by TR-FIA method (Labmaster, Finland).Volunteers did not know that adherence was determined by serum concentration of genistein.

Statistical analysis

Kolmogrov-Smirnov test was used to examine the normality of variables before any statistical comparison was made. If any of variables did not follow the normal distribution, log transformation was conducted. Quantitative variables are reported as mean±SD. To detect any significant difference between the control and the intervention group independent samples t-test was used. We also used paired t-test to report the difference in each group before and after the intervention. To adjust for the confounding factors and compare the effects of consumption of probiotic soy milk and conventional soy milk on lipid profiles between the two groups, Analysis of covariance (ANCOVA) was used. Statistical analyses were conducted using the Statistical Package for Social Science version 23 (SPSS Inc., USA), and $p<0.05$ was reported significant.

Results

From 44 patients participating in this study two men in the probiotic group were excluded due to hospitalization (n=1) and loss to follow up (n=1). Two persons (a man and a woman) in the soy milk group were also excluded due to poor compliance and loss to follow up. At the end, a total of 40 patients [intervention (n=20) and control (n=20)] completed the study. The mean±SDs for age, weight, height and BMI of study participants were 55.2±7.7 year, 71.2±10.9 kg, 163.2±6.2 cm, and 26.63±3.1 kg/m^2 respectively. There was no statistically significant difference between the intervention and control groups in the baseline anthropometric variables (Table 1). On the basis of the 3-day 24-hour recall, no significant difference were detected between the two groups in baseline dietary variables, except for dietary carbohydrate intake ($p<0.001$) (Table 1). Baseline physical activity levels were not markedly different between soymilk and probiotic soy milk groups (p=0.29). There were 11 and 10 women in intervention and control groups respectively and no statistically significant difference was seen between these two groups (p=0.75). Possible difference of lipid panel, serum creatinine, serum phosphorus and serum genistein were tested at baseline and they are presented in Table 2. None of the aforementioned variables showed significant difference at the beginning of the study between the two groups.

When the analyses were adjusted for gender and dietary carbohydrate intake which the latter was significantly different at baseline between control and intervention groups, consumption of probiotic soy milk resulted in a statistically significant increase in serum genistein (17.6±15.3 vs. 4.5±2.3, p=0.002) and eGFR (15.9±10.8 vs. 3.2±8.4, $p<0.001$) compared with control group (Table 2). Probiotic soy milk also led to decreased LDL-C (-9.2±10.4 vs. -2.2±5.2, p=0.01), total cholesterol (T-Chol) (-12.4±4.8 vs. -4.87±14.7, p=0.04), non-HDL cholesterol (non-HDL-C) (-15.3±4.5 vs. -5.9±14.7, p=0.01) and serum TG (-14.6±12.5 vs. -3.9±9.3, p=0.007) compared with control group in diabetic patients with nephropathy. We did not detect any significant effect of probiotic soy milk on serum HDL-C (1.11±3.38 vs. 0.90±2.7, p=0.8) and serum phosphorus (-0.14±0.10vs. 0.05±0.5, p=0.1).

Adherence was good in both groups, with only minor complaints being reported by some patients (4 in probiotic soy milk and 5 in conventional soy milk) of flatulence and beany flavor of soy milk at the beginning of the trial.

Table 1. General characteristics and nutrient intake of study participants who received either conventional soy milk or probiotic soy milk[1]

	Intervention group[2] (n=20)	Control group[3] (n=20)	p.value[4]
Age (yr.)	56.9±8.1	53.6±7.19	0.18
Duration of disease (yr.)	8.7±2.1	6.9±4.9	0.46
Weight (kg)	70.8±10.7	71.6±11.4	0.82
Height (cm)	162.9±6.6	163.6±6.0	0.74
BMI (kg/m2)	26.68±3.19	26.58±3.27	0.92
PA low/moderate (%)[6]	16/4 (80/20)	13/7 (65/35)	0.29[5]
Calorie intake (Kcal/day)	2105.8±149.7	2173.4±143.6	0.15
Protein intake (g/day)	61.6±8.0	62.2±5.3	0.77
Fat intake (g/day)	90.5±8.6	92.9±11.6	0.47
Carbohydrate intake (g/day)	275±24.7	309.7±29.2	p<0.001*
Dietary cholesterol intake (mg/day)	271.2±19.8	279.2±25.2	0.62
Dietary fiber intake (g/day)	17.6±2.7	18.7±3.7	0.30 [1]

*All values except for PA are means±SDs. [2] Received 200ml/day of probiotic soy milk during the study [3] Received 200ml/day of conventional soy milk during the study [4] Obtained from independent samples t-test [5] Obtained from χ^2 test [6] Reported in frequency (percent). * Statistically significant. BMI, Body mass index. PA, physical activity.*

Table 2. lipid panel and eGFR at baseline and after the intervention in diabetic patients with nephropathy[1]

	Intervention group (n=20)[2]			Control group (n=20)[3]				
	Before intervention	After intervention	Change	Before intervention	After intervention	Change	P1[4]	P2[5]
HDL-C (mg/dl)	48.7±8.9	49.8±9.3	1.11±2.8	46.2±6.1[6]	47.1±6.4	0.90±2.7	0.31	0.82
LDL-C (mg/dl)	112.1±17.3	102.9±18.4	-9.2±10.4*	117.8±19.8[7]	115.6±17.9	-2.2±5.2	0.34	0.012*
T-Chol (mg/dl)	191.2±20.3	178.8±19.7	-12.4±4.8*	189.7±25.5[8]	184.8±22.7	-4.87±14.7	0.83	0.045*
Non HDL-C (mg/dl)	142.5±20.0	127.2±21.3	-15.3±4.5*	143.5±25.5[9]	137.6±21.9	-5.9±14.7	0.89	0.011*
TG (mg/dl)	170.4±75.5	155.7±77.4	-14.6±12.5*	180.5±53.2[10]	176.6±49.01	-3.9±9.3	0.62	0.007*
Serum creatinine (mg/dl)	1.01±0.11	0.83±0.16	-0.17±0.11*	1.03±0.16	1.00±0.14	-0.03±0.08	0.51	<0.001*
Serum phosphorus (mg/dl)	4.48±0.47	4.33±0.44	-0.14±0.10	4.38±0.67[12]	4.44±0.59	0.05±0.5	0.61	0.106
Serum genistein (nmol/l)	24.31±13.9	41.9±16.0	17.6±15.3*	22.33±13.4[13]	26.8±12.8	4.5±10.6	0.65	0.002*
eGFR (ml/min/1.73m^2) [6]	71.5±9.5	87.5±14.2	15.9±10.8*	72.1±9.1[14]	75.4±11.13	3.2±8.4	0.84	<0.001*

*[1] All values are means±SDs. [2] Received 200ml/day of probiotic soy milk during the study [3] Received 200ml/day of conventional soy milk during the study. [4] P-values for baseline differences, obtained from independent samples t-test [5] P-values for differences between intervention and control group, Obtained from ANCOVA test adjusted for gender and dietary carbohydrate intake. [6] Obtained from CKD-EPI equation (2009) not adjusted for body surface * Statistically significant. HDL-C, HDL cholesterol. LDL-C, LDL cholesterol. T-Chol, Total cholesterol. Non HDL-C, non-HDL cholesterol. TG, Triglycerides. eGFR, Estimated glomerular filtration rate.*

Discussion

To the best of our knowledge, this is the first time that the effect of probiotic soy milk is examined on lipid profile in type 2 diabetic patients with diabetic nephropathy. In the current study, consumption of probiotic soy milk led to improved lipid panel but it had no beneficial effect on HDL-C, while renal function were also improved as a result of probiotic soy milk consumption.

In the present study, no statistically significant change were observed in weight, BMI, dietary intakes, energy intake and physical activity within groups throughout the study, hence none of the observed effects could be associated with the change of the above-mentioned factors. It must be taken into consideration that there was a statistically significant difference in baseline dietary intake of carbohydrate between intervention and control groups which its probable influence was adjusted using ANCOVA. To adjust for possible confounding effect of gender, it was also entered as a probable confounder in ANCOVA model. However the observed changes caused by the effect of gender were negligible.

We measured serum genistein concentration to evaluate the effect of probiotic soy milk on serum genistein, compared with conventional soy milk and also to confirm the adherence of participants to the intervention. Serum genistein concentrations were elevated in all of the volunteers in both groups demonstrating that adherence in both groups was good. In addition, 90 percent of the participants used at least 90% (50 bottles out of 56 bottles) of their probiotic or conventional soy milk. Consumption of conventional soy milk led to a non-significant rise (4.5±10.6 nmol/l, p=0.07) in serum genistein, while probiotic soy milk resulted in a significant increment in serum genistein compared with soy milk group (17.6±15.3 nmol/l, p=0.002). Consistent with our results, Rekha et al., Tsangalis et al. and Kano et al. reported that β-glucosidase activity of probiotic bacteria such as lactic bacteria, markedly converts the glucosidic form of soy isoflavones into the aglyconic form which is biologically more active (22-24). The aglyconic form of genistein are absorbed more rapidly and in higher amounts than the glucoseides and may also be more active in reducing cardiovascular risk factors (25).

In the present study, administration of probiotic soy milk significantly lowered serum LDL-C, T-Chol, and non-HDL-C compared with conventional soy milk. Similar findings have also been indicated in type 2 diabetic patients who were taking 300 g of probiotic yogurt for 6 weeks (15). While Mazloom (26), Firouzi (27) reported no significant change in serum LDL-C and total cholesterol in diabetic patients as a result of probiotic supplementation. Kawase (28) also observed no significant improvement in total cholesterol concentration. The results from the recent trials which administered soy products to the patients with DN indicated beneficial effects on kidney function and a significant reduction in T-Chol and LDL-C (17, 29), our findings also indicated that a non-significant but a favorable within-group trend in conventional soy milk group for LDL-C (-2.2±5.2 mg/dl, p=0.08), T-Chol (-4.87±14.7, p=0.1) and non-HDL-C (-5.9±14.7, p=0.09). Current findings and those of previous studies, support our primary hypothesis regarding an additive or even synergistic beneficial effects of administration of a probiotic along with an appropriate medium such as soy milk. Lactic bacteria might affect the serum cholesterol levels by different mechanisms. One mechanism is through metabolites of lactic bacteria -mostly short chain fatty acids (especially propionate) – which can inhibit the enzymatic synthesis of cholesterol; another one is through easing removal of cholesterol through the feces, as it also can bind with cholesterol and bile salts and block cholesterol reabsorption, and finally, lactic bacteria are able to assimilate the cholesterol (30). However, these effects are dependent on viability and activity of lactobacillus spp. and need a suitable medium to deliver the probiotics to appropriate location where they can colonize and cholesterol is absorbed. Soy milk has been proposed for such an action, by several studies as an alternative for dairy products (19, 31).

The current study also showed that the consumption of probiotic soy milk resulted in a significant reduction in serum TG compared with conventional soy milk, although the control group also showed a promising but non-significant reduction in serum triglyceride levels (-3.9±9.3 mg/dl, p=0.08). In contrast with our findings, several previous studies reported that probiotic supplementation do not significantly improve TG levels in healthy subjects or diabetic patients (15, 32, 33). Whereas, number of previous studies reported soy-based products have resulted in reduction of TG. Azadbakht et al. reported that consumption of soy protein in type 2 diabetic patients with nephropathy resulted in a significant (p=0.002) decrease in serum triglyceride level after 7 weeks (29). Administration of a soy-based beverage also led to a significant decrement in serum triglyceride among children with familial hypercholesterolemia (34). Grundy et al. also reported that soy protein could result in a significant reduction in plasma TG when levels were elevated (35). Considering the findings of previous studies and the significant difference in TG between intervention and control groups in the current study; it seems that the observed effect of probiotic soy milk on serum TG in our study is due to the effect of soy milk (29) which is probably enhanced by lactobacillus plantarum A7. The underlying mechanism of soy effects on triglyceride levels is unresolved; however it is suggested that some bioactive compounds such as saponin, PUFAs or soy protein, individually or in a synergistic way may be responsible for this effect (36). Additionally, beta-glucosidase activity of lactic acid bacteria leads to an improvement of bioavailability of soy bioactive compounds and better digestion of soy proteins which enhances the potential beneficial effects of soy milk (24, 37).

Findings from the current study showed that consumption of probiotic soy milk did not result in a significant change on HDL-C in type 2 diabetic patients with nephropathy. Similar findings were reported in previous studies regarding the effect of probiotic supplementation on HDL-C concentration in type 2 diabetic patients (15, 26, 27). On the other hand, Moroti and colleagues (16) examined the effect of a daily dose of 200 ml of a symbiotic shake on lipid profile of 20 diabetic patients and showed a significant increase in HDL-C. Kawase et al. also reported that administration of probiotics (from lactobacillus family) could significantly increase HDL-C in healthy men (28). Mix administration of probiotics and fructo-oligosaccharides and restricting the participants to only health men may be a probable reason for conflicting results.

Restriction of phosphorus is favorable in decelerating the progression of nephropathy (38). In the current study, we omitted foods rich in phosphorus and restricted dietary intake of phosphorus to 1500 mg/day. The question was that, might the phytase activity of lactobacillus plantarum A7 convert phytate -which is the major storage form of phosphorus in food- to phosphate and result in an increased serum phosphate or not? In the present study, consumption of probiotic soy milk did not affect serum phosphorus in diabetic patients with nephropathy. The reduction of serum phosphorus in probiotic soy milk, conventional soy milk and the between group difference was not statistically significant (Table 2). Consistent with our study Zamudio et al. evaluated the phytase activity of 6 lactic acid bacteria, and showed that this phytate degradation activity was very low (39).

Findings from our study indicated that administration of probiotic soy milk in T2D patients with nephropathy improved glomerular function and bring about a significant increment in eGFR ($p<0.001$) compared with conventional soy milk. This result was supporting this idea that, probiotic soy milk compared with conventional soy milk not only improved lipid panel in patients with diabetic nephropathy, but also might be able to improve renal function in these patients. It seems that the synergistic effect of soy and probiotics might have a promising effect on diabetic nephropathy through its impact on lipid profile (29). In line with our findings, Jin et al. showed that hypercholesterolemia is associated with progressive glomerular injury (40). Hyperlipidemia induces renal injury due to stimulation of TGF-β, and leads to increased production of reactive oxygen species and a damage to the glomeruli and glomerular glycocalyx. A strong body of evidence suggests that hyperlipidemia worsens diabetes-associated micro-vascular diseases, increases glomerular injury, increases tubulointerstitial fibrosis, and stimulates the progression of DN (10).

The current study was the first to examine the effect of probiotic soy milk on lipid profile in type 2 diabetic patients with nephropathy. However, some limitations of our clinical trial must be noted. Although the duration of this study was appropriate to detect a probable improvement in lipid profile and no serious side-effect was reported, long term effects of consumption of soy milk containing lactobacillus plantarum A7 must be investigated in future trials. As the present study did not aim to compare the effect of probiotic soy milk in different genders, although the results were adjusted for gender; however, possible gender-based differences could not be completely ruled out, and it is suggested forthcoming studies investigate the effect of probiotic soy milk in a gender-based fashion. Furthermore, the effect of this trial on the composition of fecal residue and population of gut flora needs to be evaluated in the future studies. Additional studies are needed to confirm our results.

In conclusion, consumption of soy milk containing lactobacillus plantarum A7 in type two diabetic patients with nephropathy was safe and well tolerated and had favorable effects on LDL-C, T-Chol, non-HDL-C and serum TG and improved kidney function. Finding from our study suggests that soy milk containing lactobacillus plantarum may be able to correct lipid profile and decelerate the progression of nephropathy in diabetic patients.

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