

# The Effects of Probiotic On Serum Lipid Profiles in Patients with type 2 Diabetes Mellitus: A Randomized Clinical Trial

Tahere Tofighiyan<sup>1</sup>, Akram Kooshki<sup>2,\*</sup>, Bibi Leila Hoseini<sup>3</sup>, Maryam Mohammadi<sup>4</sup>

<sup>1</sup>Department of Nursing, School of Nursing and Midwifery, Sabzevar University of Medical Sciences, Sabzevar, Iran

<sup>2</sup>Department of Nutrition & Biochemistry, School of Medicine, Sabzevar University of Medical Sciences, Sabzevar, Iran

<sup>3</sup>School of Nursing and Midwifery, Sabzevar University of Medical Sciences, Sabzevar, Iran

<sup>4</sup>Department of Public Health, School of Health, Shahid Beheshti University of Medical Sciences, Tehran, Iran

\*Corresponding author: kooshki.nutr@gmail.com

**Abstract** Background & Objectives: With regard to probable mechanisms about probiotics effect in cholesterol lowering and since final conclusion about fat-lowering effect of probiotics is not clear; Present study has been conducted to determine effect of probiotics on serum lipid profile level of patients with type II diabetes mellitus. Material and Methods: This double-blind randomized, clinical trial study was done on 44 type-2 diabetic patients, by available sampling. Patients were allocated to either case or control groups by permuted block randomization. Probiotic group received one probiotic tablet and placebo group received one placebo tablet daily for 8 weeks. At baseline and the end of study, 5 ml blood was collected from each patient after a 14-h fasting in order to measure serum lipid profile. Data were analyzed by descriptive and analytic statistics. Results: Demographic parameters, anthropometric and dietary factors were not significantly different between two groups at baseline and the end of week 8. Probiotic intake caused significant decrease in serum total cholesterol ( $p=0.0001$ ), LDL ( $p=0.001$ ) and increase serum HDL ( $p=0.001$ ) in probiotic group. Results showed that observed difference in LDL and HDL between 2 groups was not significant, but serum cholesterol had a significant difference between 2 groups. Conclusions: This study showed that probiotic supplementation is probably effective in improving risk factors for cardiovascular diseases in type 2 diabetes, via serum lipid profile reduction.

**Keywords:** probiotics, serum lipids, diabetes mellitus, type 2

**Cite This Article:** Tahere Tofighiyan, Akram Kooshki, Bibi Leila Hoseini, and Maryam Mohammadi, "The Effects of Probiotic On Serum Lipid Profiles in Patients with type 2 Diabetes Mellitus: A Randomized Clinical Trial." *Journal of Food and Nutrition Research*, vol. 4, no. 12 (2016): 795-798. doi: 10.12691/jfnr-4-12-5.

## 1. Introduction

Real-time use of continuous glucose monitoring (CGM) has recently emerged as a novel tool to improve glycemic control in patients with type 1 diabetes mellitus (T1DM) [1]. While CGM is generally Diabetes is one of common metabolic diseases in the world [1]. WHO has declared that 366 millions of people will be affected to this disease until 2030[2]. Diabetes increases the risk of affecting to atherosclerosis and mortality due to cardiovascular coronary disease [3]. Cardiovascular diseases are the main cause of death in diabetic patients. Relative risk of these diseases is 2-4 times more in diabetic people [4]. Dyslipidemia is one of known risk factors related to diabetes' complications like cardiovascular diseases, which has a high incidence in diabetic patients [5,6]. The risk of heart attack in people with hypercholesterolemia is times more than people with normal level of serum lipid profile [3,7]. Since disorder in fat and glucose metabolism, most of type 2 diabetic patients have high serum triglyceride and cholesterol. High LDL cholesterol and triglyceride,

and low HDL cholesterol are the characteristics of dyslipidemia in type 2 diabetic patients [5,6]. One of the hypercholesterolemia treatments is to apply cholesterol-lowering drugs but these drugs have known side effects [8]. So, recently it appeals to apply other productions such as beneficial compounds like probiotics in reducing blood cholesterol [9]. Probiotics are nonpathogenic live microorganisms which have beneficial effects on host's health. Some of their beneficial effects include: improving indigestion and lactose intolerance, cancer suppress, antibiotic effects and induction of immune system and so on [10,11]. Some reports also have been offered based on probable effect of probiotics on serum cholesterol reduction and hypercholesterolemic effects of probiotics [9].

Although the present studies based on probable effect of probiotics on reducing blood cholesterol and also different results of conducted researches concerning to this issue [12,13,14,15,16]. Final conclusion about probiotics effects on reducing blood cholesterol is unclear yet, so it needs to do more researches. Thus, this study has been aimed to determine the effect of probiotic on serum lipid profile in patients with type 2 diabetes mellitus.

## 2. Material and Methods

### 2.1. Study setting

This study was a double-blind randomized, clinical trial.

### 2.2. Participants

Forty-four type 2 diabetic patients were recruited by available sampling from diabetes clinic of Vaseei Hospital in Sabzevar, Iran 2013.

### 2.3. Sample Size

Sample size was determined according to basic data of Ejtahed's study [17] and using formula " $n = (Z_{1-\alpha/2} + Z_{1-\beta})^2 (\sigma_1^2 + \sigma_2^2) / (\mu_1 - \mu_2)^2$ ". In this formula,  $\alpha=0.05$  and power test was considered 0.8.

### 2.4. Inclusion Criteria

Inclusion criteria were as followed: patients with diabetes mellitus, type 2, body mass index (BMI) <35 kg/m<sup>2</sup> and patients do not have infectious, renal or thyroid disease, hepatitis and cancer.

### 2.5. Exclusion Criteria

Exclusion criteria included: patients who had hyperglycemia due to other diseases except diabetes, past myocardial infarction and brain stroke, using hormone replacement therapy (HRT) in past 6 months and patients received insulin, cholesterol-lowering drugs, probiotic, prebiotic and omega-3 fatty acids and antibiotics from 1 month before and during the study.

### 2.6. Intervention

Patients with overweight and obese that their height was measured at baseline. Patients were allocated to either case or control groups by permuted block randomization ( $n=22$  in each group). Subjects in case group received one probiotic tablet daily and control group received one placebo tablet daily for 8 weeks. Each probiotic tablet is comprised of 100 mg Lactol probiotic. Lactol probiotic include Lactobacillus coagulans and Fructooligosaccharides (FOS). Lactol probiotic is a product made by Bioplus Company of India which is under license of Nature's American Company. Each placebo tablet contained farina. Placebo tablets were produced by Pharmaceutics department of Mashhad School of Pharmacy. In order to making blindness, initially all boxes containing supplementation or placebo were coded by a person other than researcher as A or B, so that the researcher does not diagnose supplementation type. Subjects were advised not to change their diet, physical activity and drug regimen. At baseline and the end of determined period, 5 ml blood was collected from each patient after a 14-h fasting in order to measuring serum lipid profile. Diet recall was obtained by standard questionnaire of "24 hour diet recall" from participants in order to assess probable changes in diet and nutrients intake. This questionnaire was a reliable and valid questionnaire. Patients' follow up was done by

telephone and one-on-one visit in diabetes clinic monthly, in order to monitoring patients with regard to supplementation consumption and preventing from sample reduction. The degree of compliance for each patient was determined according to the number of returned tablets. Participants were weighed at the end of the study again. Laboratory questionnaires and checklists were assessed by 2 faculty members who were expert in this field. Data were analyzed by Nutritionist IV. Laboratory tests and Nutritionists IV software were standard, too.

### 2.7. Research Ethics

The study protocol was approved by Iranian Registry of Clinical Trials (IRCT) and IRCT ID is IRCT2014110519816N1. It also approved by the Ethics committee of Sabzevar University of Medical Sciences (Ethics committee Code: 122.1696). This study was in adherence with the Declaration of Helsinki Written informed consent was obtained from all patients.

### 2.8. Statistical Analysis

Data were analyzed by statistical package for the social sciences (SPSS, Inc., Chicago, III., USA) version 16, using correlation tests, linear regression, t-test and Analyze of Variance (for eliminating interventional variables). Statistical differences were considered significant at  $p < 0.05$ .

## 3. Results

Sample size was 44 persons, but 1 participant from each group excluded due to lack of their cooperation in follow up period. Two groups did not differ significantly in all demographic and clinical characteristics include: gender, physical activity, mean age, anthropometric indices and duration of affecting to diabetes at start of the study (Table 1).

Results showed that probiotic intake causes significant reduction in serum total cholesterol, LDL and increasing serum HDL in probiotic group, while these differences were not significant in placebo group than study's baseline (Table 2). In between groups' comparison, results showed that observed difference in LDL and HDL between 2 groups was not significant, but serum cholesterol had a significant difference between 2 groups (Table 2). Also, analyzing data of food showed that intake of energy, fat, cholesterol and carbohydrate was not significant at baseline and the end of study between 2 groups ( $p > 0.05$ ).

**Table 1. Baseline characteristics if subjects in this study**

Variables	Probiotic group	Placebo	P-Value
	M±SD	M±SD	
Age (y)	53.45± 10.8	54.5 ± 11.10	$p > 0.05$
Duration of disease	7.25± 5.2	7.45± 5.4	$p > 0.05$
Sex			
Men(n)	8(36.36 %)	8(36.36 %)	$p > 0.05$
Women(n)	14(63.63%)	14(63.63%)	
BMI (Kg/m <sup>2</sup> )	22.79±2.7	22.47± 2.3	$p > 0.05$

**Table 2. serum lipid profile of type-2 diabetic subjects at baseline and 8 weeks of probiotic supplementation ( Mean  $\pm$  S.D) in probiotic and placebo groups in this study**

Indices	Groups (n=22 in each group)	Study time		*p-value (within group)	*p-value (betin group)
		Baseline	Week 8		
TG (mg/L)	probiotic	180.67 $\pm$ 112.6	186.00 $\pm$ 163.98	0.851	0.642
	placebo	166.73 $\pm$ 75.83	168.55 $\pm$ 57.39	0.890	
Cholesterol (mg/L)	probiotic	206.33 $\pm$ 47.79	169.67 $\pm$ 42.41	<0.001	0.001
	placebo	184.36 $\pm$ 50.29	196.05 $\pm$ 45.15	0.252	
LDL (mg/dl)	probiotic	123.86 $\pm$ 39.87	96.14 $\pm$ 26.93	0.001	0.301
	placebo	106.26 $\pm$ 44.89	99.40 $\pm$ 36.87	0.302	
HDL (mg/dl)	probiotic	46.34	55.47	0.001	0.742
	placebo	52.21	55.4	0.371	

\* p<0.05 was considered significant.

## 4. Discussion

The present study showed that consuming probiotic supplementation reduces patients' lipid profile concentration in probiotic group. However, there was no significant difference between two groups. Probable mechanisms about the effect of probiotics in cholesterol reduction include: activating bile salt hydrolyses (BSH), de conjugating bowel acids and stopping the circulation of intestinal bowel acids [18,19,20], reducing cholesterol absorption by combining cholesterol with bacterial cell membranes [14,21] and reduction of synthesizing liver cholesterol by producing short-chain fatty acids in digestive system due to fermentation by probiotics[21]. Lay-Gaik declared although suggested mechanisms for lipid profile reduction by probiotics, it is needed more clinical evidences for augmentation this hypothesis.10 These findings are in consistent with Ejtahed et al who had shown that intake of 300 g probiotic yogurt containing *Lactobacillus acidophilus* daily reduces 4.54% total cholesterol and 7.45% LDL cholesterol [22]. Ataie Jafari also showed that probiotic yogurt containing *Lactobacillus acidophilus* and *Bifidobacterium lactis* reduces serum total cholesterol, significantly [15]. In contrast, some studies showed that consuming oral probiotic reduces blood cholesterol 22-33% [15,23]. Greany et al had used probiotic capsules with different strains including: *Lactobacillus acidophilus* and *Bifidobacterium longum* in order to determining their effect on serum lipids. They didn't confirm useful effects of probiotics on serum lipid level. They stated that lack of useful effect can be due to difference in clinical characteristics of samples, amount and duration of consuming probiotics, their type, species and special characteristics [12]. Lewis that used probiotic capsules containing in vitro frozen and dried *Lactobacillus acidophilus*, mentioned however, *Lactobacillus* can decrease cholesterol and is able to being alive in vitro acidic and bile environment, but it had no effect on blood cholesterol in patients affected to hypercholesterolemia. According to him, the reason is due to insufficient time for activation of frozen and dried probiotics, so bacteria get to large intestine and defecate before getting metabolic activation [16]. It seems this issue suggests that dairy products are vector better for prescribing probiotics [22]. Hatakka and Lin also concluded that probiotics consumption have no effect on reduction of serum lipid level [13,24]. These

different results may be due to some differences in species, number and form of probiotic bacteria, duration of intervention, sample size, study design, clinical characteristics and participants' responsibility. Although different findings of researches and with regard to positive effect of probiotics in vitro, so there is need to conduct more researches with a longer duration and different probiotic doses and in a fermented form of dairy products with these bacteria or in a form of tablet or capsule in order to confirming probiotics' ability as an alternative or treatment of anti-fat chemical drugs in clinical condition.

The main problems in the present study included sample loss, placebo preparation and time-consuming of lab kits. In the present study, we had not encountered any side effect or harm in application of probiotics. It is in consistent with some other studies such as Vanderpool and Mosaffa [25,26].

## 5. Conclusion

In summary, this study showed that probiotic supplementation is probably effective in improving risk factors for cardiovascular diseases in type 2 diabetes by serum lipid profile reduction.

## Acknowledgements

This study was supported by Sabzevar University of Medical Sciences, Iran. The authors thank the staff and of diabetic clinic for valuable assistance the authors also grate fully acknowledge the cooperation of the participating patients.

## References

- [1] Marion, J.F. Medical nutrition therapy for diabetes mellitus and hyperglycemia of non-diabetic origin. Food, nutrition and diet therapy. Eds.: Philadelphia: W.B. Saunders Company, 10nd ed., 742-780, 2004.
- [2] Wild, S., Roglic, G., Green, A., Sicree, R., King, H. Global prevalence of diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care*, 27, 1047-1053, 2004.
- [3] Greenland, P., Knoll, M.D., Stamler, J., Neaton, J.D., Dyer, A.R., Garside, D.B. Major risk factors as antecedents of fatal and nonfatal coronary heart disease events. *Journal of the American Medical Association*, 290, 891-897, 2003.

- [4] Jairath. N. Coronary Heart Disease & Risk Factor management. Philadelphia: W.B. Saunders Company, 1999.
- [5] Arauz-Pacheco, C., Parrott, M.A., Raskin, P. American Diabetes Association. Treatment of hypertension in adults with diabetes. *Diabetes Care*, 26, 80-82, 2003.
- [6] Franz, M.J., Bantle, J.P., Beebe, C.A., Brunzell, J.D, Chiasson, J.L., Garg, A. Evidence-based nutrition principles and recommendations for the treatment and prevention of diabetes and related complications. *Diabetes Care*, 25, 148-198, 2002.
- [7] WHO, Diet, nutrition and prevention of chronic diseases, Report of a joint WHO/FAO expert consultation. Geneva: Switzerland, 2003.
- [8] Bliznakov, E.G. Lipid-lowering drugs (statins), cholesterol and coenzyme Q10. *Biomedicine and Pharmacotherapy*, 56, 56-59, 2002.
- [9] Kumar, M., Nagpal, R., Kumar, R., Hemalatha, R., Verma, V., Kumar, A. Cholesterol-lowering probiotics as potential bio-therapeutics for metabolic diseases. Hindawi Publishing Corporation, experimental diabetes research, 2012.
- [10] Ooi, L.G., Liang, M.T. Cholesterol-lowering effects of probiotics and prebiotics: A review of in vivo and in vitro findings. *International Journal of Molecular Sciences*, 17, 2499-2522, 2010.
- [11] Ratna Sudha, M., Chauhan, P., Dixit, K., Babu, S., Jami K. Probiotics as complementary therapy for hypercholesterolemia. *Biology and Medicine*, 1, 4, 2009.
- [12] Greany, K.A., Bonorden, M.J., Hamilton-Reeves, J.M., McMullen, M.H., Wangen, K.E., Phipps, W.R. Probiotic capsules do not lower plasma lipids in young women and men. *European Journal of Clinical Nutrition*, 62, 232-237, 2008.
- [13] Hatakka, K., Mutanen, M., Holma, R., Saxelin, M., Korpela, R. Lactobacillus rhamnosus LC705 together with propionibacterium freudenreichii SSP shermanii JS administered in capsules is ineffective in lowering serum lipids. *The Journal of the American College of Nutrition*, 27, 441-447, 2008.
- [14] Liang, M.T., Shah, N.P. Bile salt deconjugation ability, bile salt hydrolase activity and cholesterol co-precipitation ability of lactobacilli strains. *International Dairy Journal*, 15, 391-398, 2005.
- [15] Ataie-Jafari, A., Larijani, B., Alavi, M., Tahbaz, F. Cholesterol-lowering effect of probiotic yogurt in comparison with ordinary yogurt in mildly to moderately hypercholesterolemia subjects. *Annals of Nutrition and Metabolism*, 54, 22-27, 2009.
- [16] Lewis, S.J., Burmeister S. A double-blind placebo-controlled study of the effects of Lactobacillus acidophilus on plasma lipids. *European Journal of Clinical Nutrition*, 59, 776-780, 2005.
- [17] Ejtahed, H., MohtadiNia, J., HomayouniRad, A., Niafar, M., AsghariJafarabadi, M., Mofid, V. The effects of probiotic and conventional yoghurt on diabetes markers and insulin resistance in type 2 diabetic patients: A randomized controlled clinical trial. *Iranian Journal of Endocrinology and Metabolism*, 13, 1-8, 2011.
- [18] Smet, I.I., Boever, P., Verstraete, W. Cholesterol lowering in pigs through enhanced bacterial bile salt hydrolase activity. *British Journal of Nutrition*, 79, 185-194, 1998.
- [19] Klaver, F.A., vanderMeer, R. The assumed assimilation of cholesterol by lactobacilli and Bifidobacterium bifidum is due to their bile salt-de conjugating activity. *Appl Environ Microbiol*, 59, 1120-1124, 1998.
- [20] Usman, A. Hosono: Bile tolerance, taurocholate de conjugation, and binding of cholesterol by Lactobacillus gasseri strains. *Journal of Dairy Science*, 82, 243-248, 1999.
- [21] Pereira, D.I., Gibson, G.R. Effects of consumption of probiotics and prebiotics on serum lipid levels in humans. *Critical Reviews in Biochemistry and Molecular Biology*, 37, 259-281, 2002.
- [22] Ejtahed, H., MohtadiNia, J., HomayouniRad, A., Niafar, M., Asghari, M., Mofid, V. The effects of probiotic yoghurt consumption on blood pressure and serum lipids in type 2 diabetic patients: Randomized clinical trial. *Iranian Journal of Nutrition Sciences & Food Technology*, 6, 1-12, 2012.
- [23] Tomaro-Duchesneau, C., Jones, M.L., Shah, D., Jain, P., Saha, S, Prakash S: Cholesterol Assimilation by Lactobacillus Probiotic Bacteria: An in vitro investigation. *BioMed Research International* 2014. Article ID 380316.
- [24] Lin, S.Y., Ayres, J.W., Winkler, J.r., Sandine, W.E. Lactobacillus effects on cholesterol: in vitro and in vivo results. *Journal of Dairy Science*, 72, 2885-2899, 1998.
- [25] Vanderpool, C., Yan, F., Polk, B. Mechanisms of probiotic action: Implications for therapeutic applications in inflammatory bowel diseases. *Inflammatory Bowel Diseases*, 14, 1585-1596, 2008.
- [26] Mosaffa, N. Probiotics as a new generation of living drugs. *Journal of Research in Medical Sciences*, 32, 169-174, 2008.