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SHORT-TERM CONSUMPTION OF PROBIOTIC YOGURT IMPROVED HDL-C OF TYPE 2 DIABETES MELLITUS PATIENTS: A DOUBLE-BLIND RANDOMIZED CONTROLLED TRIAL

Lily Arsanti Lestari [1,4,✉], ***Dian Ratnasari*** [1], ***Elsa Fairuz Azizah*** [1], ***Ivana Noor Farida*** [1], ***Farah Nuriannisa*** [2], ***Kartika Yuliani*** [2], ***Rio Jati Kusuma*** [1], ***Emy Huriyati*** [1], ***Nyoman Kertia*** [3]

[1] Department of Nutrition and Health, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Jalan Farmako, Sekip Utara, Yogyakarta, Indonesia 55281
[2] Department of Public Health, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Jalan Farmako, Sekip Utara, Yogyakarta, Indonesia 55281
[3] Department of Internal Medicine, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Jalan Farmako, Sekip Utara, Yogyakarta, Indonesia 55281
[4] Institute for Halal Industry & System, Universitas Gadjah Mada, Jalan Kaliurang, Yogyakarta, Indonesia 55281

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Abstract

Background and aims*: Cardiovascular disease is the main complication and cause of morbidity and mortality in type 2 diabetes mellitus (T2DM) patients. The main cause of complication in T2DM is oxidative stress caused by insulin resistance, hence it can increase lipid profiles (cholesterol, LDL, and triglycerides) which exacerbates endothelial dysfunction. Among various functional foods with antioxidant effects, probiotic foods have been reported to suppress oxidative stress, and also improve the fasting blood glucose (FBG) and lipid profile in patients with T2DM. The aim of this clinical trial is to study the effects of probiotics and conventional yogurt on FBG and lipid profile in patients with T2DM.* ***Material and method****: Thirty-eight patients with T2DM, aged 30 to 60 years old, were assigned to two groups in this randomized, double-blind, controlled clinical trial. The subjects in the intervention group consumed 100 ml/day probiotic yogurt containing Lactobacillus acidophilus La-5 and Bifidobacterium lactis BB-12, whereas subjects in the control group consumed 100 ml/day conventional yogurt for four weeks. Anthropometric indices, dietary intake, physical activity, serum FBG, and lipid profile were evaluated at the beginning and end of the intervention.* ***Results****: Consumption of 100 mL/day conventional yogurt could significantly reduce the fasting blood glucose (FBG) level, whereas probiotic yogurt could not reduce FBG significantly. Although the total cholesterol and triglyceride were not improved after yogurt consumption, both type of yogurt could improve HDL-C level.* ***Conclusion****: Both conventional yogurt or probiotic yogurt could be used as functional food since it improved the HDL-C in type 2 DM patients.*

key words: *conventional yogurt, probiotic yogurt, Lactobacillus acidophilus La-5, Bifidobacterium lactis BB-12*

✉ Department of Nutrition and Health, Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Jalan Farmako, Sekip Utara, Yogyakarta, Indonesia 55281.
corresponding author e-mail: santi_wap@yahoo.com / lily_al@ugm.ac.id

Background and aims

Diabetes mellitus (DM) is a group of metabolic diseases characterized by hyperglycemia that occur due to abnormal insulin secretion, insulin action or both. In Indonesia, type 2 DM is one of the top ten causes of death due to non-communicable diseases. In 2020, type 2 DM is also estimated to be a disease with the highest increase in economic burden in Indonesia [1]. Various complications can occur in type 2 DM patients, one of them is caused by the conditions of poor blood glucose control (hyperglycemia) [2].

Insulin resistance in type 2 DM patients causes changes in lipid profiles characterized by elevated total cholesterol, triglycerides, LDL-C levels, and decreased HDL level. This can cause endothelial dysfunction since LDL is atherogenic, easily adheres to blood vessel walls and binds cholesterol, whereas HDL have anti-atherogenic, anti-oxidative, anti-inflammatory and anti-apoptotic effects. Decreased HDL level is caused by impaired HDL metabolism due to insulin resistance [3,4]. Endothelial dysfunction is also influenced by TRL (triglycerides-rich lipoprotein) which passes through endothelial cells and is taken by macrophages, which can form foam cells causing a continuous formation of fat [5].

Cardiovascular disease in type 2 DM patients is caused by conditions of hyperglycemia. Continuous hyperglycemia is closely related to abnormalities of lipid metabolism (dyslipidemia) so that it can cause endothelial damage [6-8]. In addition, changes in lipid absorption in the intestinal tract cause high cholesterol synthesis which increases the risk of cardiovascular disease [9].

Food containing probiotics can be used as secondary prevention of complications of type 2 DM which is being investigated by Ejtahed et al. [6] and Rezaei et al. [10]. A study conducted by Ejtahed et al., [6], showed that administration of probiotics in the form of probiotic yogurt products can reduce oxidative stress. Consequently, fasting blood glucose (FBG) and fasting insulin level of type 2 DM patient can decrease. Some strains of lactic acid bacteria can act as antioxidants and reduce oxidative stress. Probiotic yogurt can increase the concentration of HDL-C as a result of the content of sphingolipid in the milk as the basic ingredient of yogurt and the sphingolipid in cell membranes of probiotic bacteria. Sphingolipid has an influence on cholesterol metabolism and transportation which later also affects HDL metabolism [11,12]. Other bacteria such as the combination of *Lactobacillus acidophilus* and *Bifidobacterium lactis* have also been studied and it can reduce cardiovascular biomarkers in people with type 2 DM. However, in several studies, this combination has not been proven effective in reducing cardiovascular biomarkers (LDL, triglycerides, and total cholesterol) and requires further research [6,13].

Most of probiotic yogurt studies in type 2 DM gave probiotic yogurt at a dose of 300 mL/day to the subjects. In the other hand, consumption of milk and its products are low among Indonesian society. Hence, in this study the subjects only given 100 mL/day probiotic yogurt. According to a study conducted by Yerlikaya [14], probiotic yogurt has a health effect at a dose of 100 mL with the content of bacterial amount of 10^6-10^8 CFU/mL. Therefore, taking into account the characteristic of Indonesian people who have low levels of yogurt consumption, we were interested in examining the effectiveness of giving probiotic yogurt (containing *Lactobacillus acidophilus* La-5 and *Bifidobacterium lactis* BB-12) and conventional yogurt at a dose of 100 mL/day (bacterial count $> 10^6$ CFU/mL) on the FBG, total cholesterol,

LDL, HDL, and triglycerides level of type 2 DM patients.

Material and methods

Study Design

The design of this study was a randomized controlled trial (RCT) with a pre-post test group design. This study was began by the production of probiotic yogurt which contain *Lactobacillus acidophilus* La-5 as much as 10^8 CFU/gram and *Bifidobacterium lactis* BB-12 as much as 10^6 CFU/gram. The conventional yogurt contained yogurt starter cultures namely *Streptococcus thermophilus* and *Lactobacillus bulgaricus* [10].

Subjects

This clinical trial was carried out on diabetic patients who visited *Puskesmas* or primary health care in Yogyakarta, Indonesia during March 2018 until June 2018. The participants were chosen through reviewing medical records filed in the primary health care. The eligible patients were contacted and invited to participate in the study.

The standard sample size was calculated using the formula hypothesis testing for two populations means from Lemeshow et al. [15], which considering the previous research by Rezaei *et al.* [10]. The number of subjects needed based on these calculations was 16 patients each group or 32 patients in total for this study. Considering there might be drop out during the study, we added a 20% of subjects hence 38 peoples were enrolled for this study.

The inclusion criteria were 1) diagnosed with type II diabetes in the aged between 30-65 years old; 2) fasting blood glucose more than 126 mg/dL; 3) consumption of glucose lowering drugs such as Glibenclamide or Metformin; 4) Not taking statin or cholesterol-lowering drugs; 5) not taking antibiotic during the study and a month before this study began; 6) have a refrigerator; 7) ability to follow the treatment regimen and collaborate with the researchers. The exclusion criteria were 1) diagnosed with any complications (except hypertension and dyslipidemia); 2) lactose intolerance; 3) pregnancy and breastfeeding; 4) consuming any probiotic products for 2 weeks before this study began. The drop-out criteria were diagnosed any complications during this study, diarrhea, and consumption of antibiotic during the study. Before beginning of the study, patient's demographics and general information, including gender, age, diabetes duration were collected.

Intervention

The intervention was carried out for four weeks. Subjects were given yogurt for four weeks at a dose of 100 mL/day. Subjects consumed yogurt every day in the morning or after the subject has breakfast. Researchers will give yogurt to the subject's house once a week and dig up data on food intake (24-hour recall questionnaire) and physical activity (International Physical Activity Questionnaire/IPAQ). To monitor whether the yogurt is consumed or not, the researcher also provides a checklist sheet for yogurt consumption to the subject to be filled every day. Food intake information was analysed with Nutrisurvey software to determine the intake of energy and macronutrients.

The blood sample were collected twice, pre-intervention and post-intervention. Measurement of fasting blood glucose and lipid profiles is carried out by the HiLab Diagnostic Center, Yogyakarta, Indonesia using the subject's blood serum. 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

minutes. Fasting blood glucose (FBG) was measured by the Hexokinase method using Abbott reagent, analyse with Achitect C8000. Lipid profiles was measured using Sekisui reagent and analyzed with Achitect C8000. Total cholesterol (TC) was measured by the CHOD-PAP method, HDL and LDL was measured by the Homogeneous Enzymatic Direct method. Triglyceride (TG) was measured by Glycerol Blanking method.

Double blinding is done by the yogurt producer by sticking a blue leaf sticker and red strawberry sticker on the cup as product coding. After the intervention period was completed, the producers informed the researchers about the coding, blue leaf label stickers for conventional yogurt and red strawberry label stickers for probiotic yogurt.

During this study, all the research ethics principles were respected. The study commenced after gaining approval from the Ethics Committee of Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Indonesia. 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.

Statistical Methods

All data were processed with the IBM SPSS version 22. Normal distribution of quantitative data was checked with Shapiro-Wilk. In the first step of analysis, demographic variables were evaluated using descriptive statistics. To analyze the significant differences of the FBG and lipid profiles level before and after the intervention, we used paired t-test (if the data is normally distributed) or the Wilcoxon test (if the data is not normally distributed) in each group.

Results

As many as 38 patients with type 2 diabetes mellitus were enrolled and 32 patients completed the intervention (Figure 1). Six patients were drop out from this study because of diarrhea (n=1), stopped taking both metformin and sulfonylurea (n=3), took simvastatin (n=1) and took antibiotic drug (n=1). All of the baseline characteristics were similar between the control and the intervention group (p>0,05), the data were presented in Table 1.

In this study, we also examined the physical activity of subjects in both groups. The result showed that there was no difference between the control and the intervention groups. There were 3 categories of physical activity namely heavy, medium, and mild. Table 2 showed that most of the subjects had heavy physical activity.

Based on the analysis of dietary characteristic during intervention, weight, and BMI after intervention in control group, we can conclude that there was significant difference between pre and post intervention for carbohydrate intake, from 151.48±42.54 to 170.99±52.16 g/d. In intervention group, there were significant increase of energy, protein, and fat intake, with p=0.023; p=0.003; p=0.034 respectively. There were significant reductions in weight and BMI. Both control and intervention group had a decreased weight and BMI with p=0.003; p=0.002; p=0.007; p=0.005 respectively. The data were shown in Table 3.

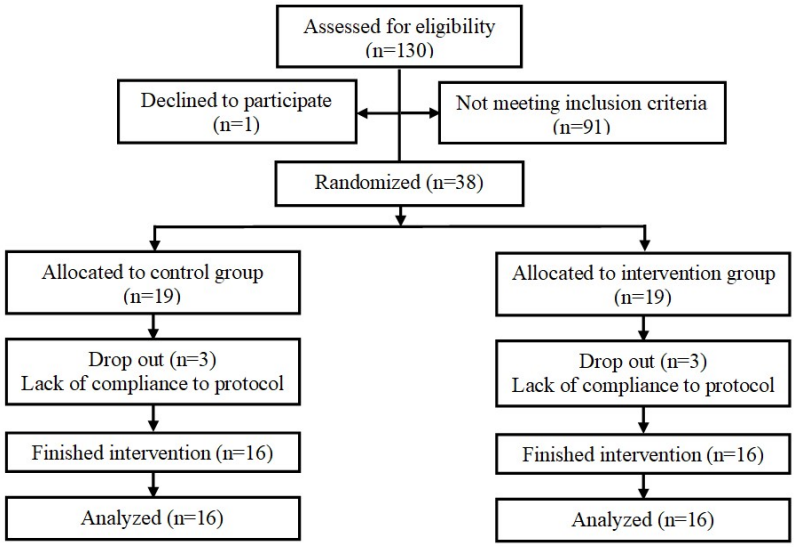

Figure 1. *Schematic diagram of the subjects screening, recruitment, and intervention.*

Table 1. Subjects' characteristics at baseline.

		Group		
Variable		Control	Intervention	p
Gender	Male	5 (31.3%)	5 (31.3%)	1.000[a]
	Female	11 (68.8%)	11 (68.8%)	
Smoking Habits	Yes	0 (0%)	3 (18.8%)	0.113[b]
	No	16 (100%)	13 (81.3%)	
Metformin Consumption	Yes	11 (68.8%)	12 (75%)	0.500[b]
	No	5 (31.3%)	4 (25%)	
Sulfonylurea Consumption	Yes	9 (56.3%)	8 (50%)	0.723[a]
	No	7 (43.8%)	8 (50%)	
Drug compliance	Yes	13 (81.3%)	10 (62.5%)	0.217[b]
	No	3 (18.8%)	6 (37.5%)	
Hypertension Status	Yes	2 (12.5%)	5 (31.3%)	0.197[b]
	No	14 (87.5%)	11 (68.8%)	
Age (years)		53 (10)	56 (7)	0.290[d]
BMI		27.74 ± 3.16	27.62 ± 4.58	0.929[c]
Duration of Diabetes (months)		48 (36)	30 (60)	0.985[d]
Weight (kg)		64.65 (14.6)	67.20 (10.8)	0.910[d]
Total Energy Intake (kcal)		1066.71 ± 288.88	1114.78 ± 296.98	0.646[c]
Protein Intake (gram)		30.68 (12.55)	30.58 (24.15)	0.895[d]
Carbohydrate Intake (gram)		151.48 ± 42.54	158.91 ± 45.94	0.638[c]
Fat Intake (gram)		34.98 (17.75)	33.1 (29.9)	0.651[d]
Dietary Fiber Intake (gram)		6.93 (3.01)	7.90 (7.54)	0.706[d]
Cholesterol Intake (mg)		82.78 (136.78)	51.48 (171.98)	0.624[d]

The data were presented as mean ± standard deviation or median (IQR) [a]chi square, [b]fisher's exact test, [c]Independent t-test, [d]mann-whitney

Table 2. Physical activity (METs) and Category of Physical activity (CP)

		Control	Intervention	p	
Physical activity (METs)		3885.4 (2109.2)	4280.6 (2553.9)	0.706[a]	
CP	Heavy	13 (81.3%)	13 (81.3%)	0.934[b]	
	Medium	2 (12.5%)	3 (18.8%)		
	Mild	1 (6.3%)	0 (0%)		

a= Mann-Whitney test b= Kruskal Wallis test

Table 3. Food intake characteristic of the subjects in conventional and probiotic groups before and after intervention

Variable	Control		Intervention		p''
	Pre	Post	Pre	Post	
Energy (kcal)	1066.71 ± 288.88	1190.74 ± 331.56	1114.78 ± 296.98	1349.28 ± 502.02	0.300[d]
p'	0.078[a]		0.023[a]*		
Protein (g)	30.68 (12.55)	35.54 (14.42)	30.58 (24.15)	45.04 (19.71)	0.040[c*]
p'	0.326[b]		0.003[b]*		
Carbohydrate (g)	151.48 ± 42.54	170.99 ± 52.16	158.91 ± 45.94	187.81 ± 63.65	0.420[d]
p'	0.037[a]*		0.079[a]		
Fat (g)	34.98 (17.75)	42.76 (18)	33.1 (29.9)	41.28 (19.46)	0.970[c]
p'	0.326[b]		0.034[b]*		
Dietary fiber (g)	6.93 (3.01)	7.63 (4.99)	7.9 (7.54)	8.46 (4.02)	0.651[c]
p'	0.469[b]		0.959[b]		
Cholesterol	82.78 (136.78)	121.67 (124.82)	51.48 (171.98)	158.21 (199.71)	0.624[c]
p'	0.148[b]		0.070[b]		
SFA (g)	17.25 (7.04)	19.06 (10.27)	16.15 (18.54)	16.53 (11.01)	0.678[c]
	0.737		0.717		
PUFA (g)	4.2 (2.21)	4.59 (3.68)	3.98 (3.64)	5.3 (5.01)	0.534[c]
	0.816[b]		0.023[b*]		
Weight	64.65 (14.58)	63.65 (13.88)	67.2 (10.8)	66.2 (10.53)	0.806[c]
p'	0.003		0.007		
BMI	27.74±3.16	27.33 ±2.99	27.62 ±4.58	27.3 ±4.46	0.981[d]
p'	0.002		0.005		

[a]*Paired-Sample T test* [b]*Wilcoxon test* [c]*Mann-Whitney test* [c]*Independent Sample T test*
SFA: saturated fatty acid
PUFA: poly unsaturated fatty acid
The data were presented as mean ± standard deviation or median (IQR)

Fasting blood glucose data was analyzed using Wilcoxon test and showed that there was a significantly decrease of fasting blood glucose in the conventional group from 153 (79) mg/dL to 114 (57) mg/dL (p=0.028). Meanwhile, the fasting blood glucose in the probiotic group is not significantly decreased, from 139 (144) to 126 (100) mg/dL (p=0.173). In the intervention group, the mean cholesterol level increase from 165.38 ± 27.35 to 213,81 ± 30.8 mg/dL which was significant according to paired t-test (p<0.05). In the control group, there was a significant increase in mean cholesterol from 155.25±35.52 to 193.19±42.03 mg/dL with p<0.001. There was no significant difference in LDL level between pre and post-test for control and intervention groups. The p-value for the pre-test and post-test of the control and intervention group are p=0.098, p=0.570 respectively. Further analysis was conducted, the Wilcoxon test described that there was a significant difference between the HDL level before and after intervention in control and intervention group (p=0.011, p=0.001 respectively). In this study, there was no significant difference (p> 0.05) in the triglyceride (TG) level before and after intervention for control and intervention group.

Table 4. Fasting Blood Glucose and Lipids Profile Levels.

Variable	Group					
	Control			Intervention		
	Pre Test	Post Test	P	Pre Test	Post Test	p
FBG (mg/dL)	153 (79.25)	114.5 (56.5)	0.028[b]*	139.5 (144)	126 (99.75)	0.173[b]
TC (mg/dL)	165.38±27.35	213.81±30.81	<0.001[a]*	155.25±35.52	193.19±42.03	<0.001[a]*
HDL (mg/dL)	37.75 (11.95)	41.80 (6.98)	0.011[b]*	36.60 (7.73)	41.30 (11.35)	0.001[b]*
LDL (mg/dL)	128 (40)	148.5 (39)	0.098[b]	130 (62)	120.5 (55)	0.570[b]
TG (mg/dL)	133.5 (87)	137 (82)	0.737[b]	144 (87)	159.5 (102)	0.737[b]

[a]*Paired-Sample T test* [b]*Wilcoxon test*
The data were presented as mean ± standard deviation or median (IQR)

Discussion

We investigated the effect of probiotic yogurt and conventional yogurt on fasting blood glucose (FBG) and lipid profiles in type 2 diabetes mellitus patients (T2DM). The results showed that the FBG level was decreased in each group, but the control group had a higher reduction in FBG compared with the intervention group. Many previous studies concluded that consuming of yogurt could improve of the gut microbiota balance, by increasing gram-positive bacteria. This might be the main possible mechanisms on the lowering blood glucose in T2DM patients. The increased of gram-positive bacteria could decrease of gram negative bacteria, so it could reduce lipopolysaccharide (LPS) levels, and then could decreased of Toll- like receptor-4 (TLR4) as a cause of inflammation and damage to cells β pancreass [16].

Short chain fatty acid (SCFA) production by probiotic bacteria also may take a role as reducing proinflammatory marker and increasing glucagon-like peptide-1 (GLP-1) secretion, which is linked to improve insulin secretion and glucose uptake [17]. Probiotic bacteria could also increase antioxidant activity through increased erythrocyte superoxide dismutase (ESD) activity, glutathione peroxidase, and total antioxidants, hence it could decrease of oxidative stress in people with T2DM. Probiotic bacteria such as *Lactobacillus acidophilus* also capable to prevent oxidative damage induced by streptozotocin (STZ) in the pancreas by inhibiting lipid peroxidation and nitric-oxid formation [18].

Streptococcus thermophilus and *Lactobacillus bulgaricus* have biosynthesis ability, like gamma-aminobutyric acid (GABA), that is a neurotransmitter produced by the pancreas. GABA can regenerate and prevent apoptosis in β-cell pancreas, leading to the improvement of insulin resistance condition in T2DM patients [19].

In the present study, the control group had a higher reduction in fasting blood glucose compared with the intervention group. This might be due to the viability of *Bifidobacterium,* an anaerob bacteria, in probiotic yogurt may depend on oxygen concentration [20]. The type of packaging material is also considered in the loss of viability of these bacteria. The material should be oxygen impermeable, such as glass bottle or high-density polyethylene (HDPE) packaging which can protect *Bifidobacterium* from oxygen toxicity [21]. Unfortunately, the packaging that was used in this study was a plastic cup, which is permeable to oxygen. On the other hand, there might be a negative interaction between *S. thermophilus* and *Bifidobacterium*. Post-fermentation acidification from *S. thermophilus* can adversely affect the viability of *Bifidobacterium* during and after

fermentation, resulting in lower viability of *Bifidobacterium* during storage [22]. Decreasing *Bifidobacterium* in probiotic yogurt may reduce its health benefit.

We found that there was a significant increase of total cholesterol (TC) in both groups ($p<0.05$). In the probiotic yogurt group, there was an increase of TC as much as 37.94 mg/dL, while in the conventional yogurt group, the TC was increased by 48.43 mg/dL. Probiotic yogurt could not improve the TC levels in patients with type II diabetes. However, probiotic yogurt could control the TC levels within the normal range (<200 mg/dL). Conventional yogurt consumption increased the LDL-C levels of subjects in the conventional group although it was not statistically significant and the LDL-C levels still within the normal range (<150 mg/dL). Meanwhile, probiotic yogurt consumption did not significantly decrease LDL-C levels. This result is inconsistent with the results from previous studies reporting such effects in patients with type 2 diabetes and hypercholesterolemic patients. This discrepancy may be due to differences in doses of probiotics, interventions period, sample size, clinical characteristic of participants, or different study design [10]. According to previous study by Rezaei *et al*. [10], daily intake of 100 gram of probiotic yogurt containing *Lactobacillus acidophilus* La-5 and *Bifidobacterium lactis* BB-12 for 4 weeks may decreased FBG and lowering total cholesterol. Ataei-Jafari *et al*. [23] reported that consumption 300 gram of probiotic yogurt everyday had a significant reduction in serum cholesterol level of hypercholesterolemic patients. Ejtahed *et al*. [24] also reported that consumption of 300 g of probiotic yogurt containing *Lactobacillus acidophilus* La-5 and *Bifidobacterium lactis* BB-12 for 6 weeks in type II diabetes patients caused a 4.54% decrease in total cholesterol and a 7.45% decrease in LDL compared with the control group. Zhang *et al*. [25] conducted meta-analysis study and summarized that probiotic yogurt consumption could significantly improve glucose metabolism which could contribute to LDL level improvement in patients with type 2 diabetes after more than 7 weeks intervention. Hence the inconsistent results from this study was might be due to inappropriate duration of intervention.

Meta-analysis of 18 clinical trials by Berger et al. [26], reporting that high fat, especially saturated fat, and high cholesterol intake from dietary showed a significant increase in serum total cholesterol. There is a plausible mechanism for the effect of dietary cholesterol on serum lipid concentrations. Higher total cholesterol, LDL, and triglycerides, as well as lower HDL, are known risk factors for CVD. Given that cholesterol is synthesized in the body, there is compensation for absorption of additional dietary cholesterol by reducing cholesterol synthesis [27]. Major dietary sources of cholesterol include egg yolks, butter, fish, shrimp, cheese, beef, pork, and poultry. The relation between dietary cholesterol and serum cholesterol has been estimated to be linear with cholesterol intake up to 600 mg/day. Studies have reported a nonlinear relation for intakes of cholesterol >600 mg/day, with little effect on serum lipid concentration in most people. In healthy adults, about 1 gram of cholesterol is synthesized and 0.3 gram is consumed per day. The body maintains a relatively constant amount of cholesterol (150–200 mg/dL). This is done mainly through controlling the level of *de novo* synthesis. Dietary intake of cholesterol in part regulates the level of cholesterol synthesis. Both of these cholesterols are then used in the formation of membranes and in the synthesis of the steroid hormones and bile acids [9]. Bile acid synthesis uses most of this cholesterol.

Consumption of conventional yogurt or probiotic yogurt could increase the HDL level in a patient with type 2 diabetes mellitus significantly. The increasing HDL level was higher in intervention group. In the study conducted by Sadrzadeh et al. [28] showed that there was no significant difference in HDL level between conventional yogurt group and probiotic yogurt group. A significant difference of HDL level was only observed in the control group which consumed milk. The beneficial effect observed in his study was caused by the consumption of milk fat containing sphingolipid. Therefore, we assumed that the increase of HDL levels observed in both groups could be caused by sphingolipids in yogurts and in cell membranes of bacteria. Sphingolipids can be found in lipid-rich structures and have effects on cholesterol metabolism and transport [29]. Yoghurt intake could increase the sphingolipid intake by 11%. There are some studies revealed the effect of increasing HDL levels by consuming yogurt disregarded to the content of probiotic bacteria. Mohammadshahi et al. [30] found that probiotic yogurt consumption could increase the HDL level in T2DM patient significantly (43.66 ± 6.80 - 50.42 ± 6.64; p = 0.023) and may be used as an alternative intervention to improve dyslipidemia. The result from several studies can be varied because of the differences in the type of microorganism, and some limitation in the study, such as the number of subject and the period of intervention.

The LDL level was decreased in the intervention group, whereas it increased in control group. The decreased of LDL level in the intervention group could be affect by some mechanisms such as bile salt deconjugation, cholesterol assimilation, and cholesterol synthesis blockade in hepar. Bile salt deconjugation was done by *L. acidophilus* La-5 and *B. animalis subsp. lactis* BB-12 through bile salt hydrolase (BSH) activity. Cholesterol assimilation could be processed in gut by living cell of probiotics. The result of cholesterol assimilation was cholesterol absorption blockade which could increase LDL absorption from plasma to hepatic cells or the other cells [31]. Cholesterol synthesis blockade in hepar would happen if probiotics produced acetate, propionate, and butyrate from dietary fiber fermentation process in gut. Those SCFAs could induce both cholesterol synthesis blockade and/or deport LDL to hepar which were initiating reduction of plasma LDL [9].

In control group, the LDL level was increased. This could be affected by saturated fatty acids (SFA) intake [32,33]. High SFA diet could reduce the catabolism of LDL apoB-100 fractional, hence it will increase plasma LDL levels, however this effect is very dependent on types of saturated fatty acids consumed [34]. Intake of SFA could induce the increase of activity and concentration of CETP. Increase of plasma CETP concentrations caused an elevation in plasma LDL-C concentration [35].

Abnormality of TG level on T2DM patients, can be affected by insulin resistance. Insulin resistance associated with lipoprotein lipase (LPL) dysfunction. LPL is an important enzyme for removal of TG from plasma, so impaired LPL is the main cause of hypertriglyceridemia in T2DM patient [36,37]. Insulin resistance also induce the lipolysis and release of plasma non-esterified fatty acid (NEFA) from adipose tissue [38], and approximately 60% of hepatic triglycerides are derived from plasma NEFA [39].

On the other hand, butyric acid, one of short chain fatty acids (SCFA) produced by probiotic bacteria [40], is capable to suppress cyclic adenosine activitymonophosphate (cAMP), and protein kinase A (PKA) in adipocyte tissue, which could affect the decrease of lipase activity

such as adipose triglycerides lipase (ATGL) and hormone-sensitive lipase (HSL), which could reduce the lipolysis in adipocyte tissue [41]. Decreased of lipolysis could reduce the levels of NEFA, furthermore could reduce TG synthesis in the liver, reduce Apo B secretion, and reduce VLDL secretion which is rich in TG, hence probiotic yogurt could repair the TG level [42].

In control group, there was a significant increase in carbohydrate intake ($p = 0.039$). The increase level of TG on control group could influenced by diet [43]. Carbohydrates intake could contribute to the development insulin resistance and diabetes mellitus [44]. High carbohydrate induces the hepatocytes triglyceride (HPTG) from overproduction of VLDL-TG, and cause reduced clearance of VLDL-TG [45]. The sources of fatty acids that used in HPTG synthesis could come from fatty acids derived from the *de novo* lipogenesis pathway because the carbohydrates can be converted to fatty acids through the process of *de novo* lipogenesis [46]. Jung and Choi [47] also reported that consumption of high carbohydrates (HC) meal that contains 79.1% of carbohydrates was associated with an increased of TG level after consumption HC meal (39.9 mg/dL increased after 2 hours and 109 mg/dL after 4 hours).

Previous study showed that hypertriglyceridemia was not associated with dietary fiber intake on diabetic subjects, and high protein-low fat (HP–LF) diet with a protein to fat ratio of 1.5 could improve TG concentrations compared with a low protein-high fat (LP–HF) diet on obese subjects with newly diagnosed type 2 diabetes mellitus [48,49].

Conclusion

Consumption of 100 mL/day conventional yogurt could significantly reduce the fasting blood glucose (FBG) level, whereas probiotic yogurt could not reduce FBG significantly. Both type of yogurt could improve HDL-C level. Hence, they can be recommended as a functional food for patients with type 2 diabetes mellitus.

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Conflict of interests. The authors declare that there was no conflict of interest in preparing this manuscript.

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