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The efficacy of probiotics supplementation on the lipid profiles of obese adolescents : a randomized trial

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ABSTRACT

Background: Obesity is defined as a medical condition characterized by excessive accumulation of body fat and is associated with an increased prevalence of dyslipidemia. Probiotics are living microorganisms, which upon consumption in sufficient numbers, exert health benefits. One of the benefits that has been studied is improving blood lipid profile. The aim of this study was to determine the efficacy of probiotics supplementation on obese adolescents' lipid profiles.

Methods: A randomized, double-blind, controlled clinical trial involving 58 obese adolescents aged 12-15 years in Denpasar City was performed. After the physical activity, nutritional intake, and lipid profile were assessed, subjects were randomized with random block into two groups: the treatment group who received sachet containing probiotics with five strains bacteria (*Streptococcus thermophilus, Lactobacillus rhamnosus, Lactobacillus acidophilus, Bifidobacterium longum, Bifidobacterium bifidum)* and the control group who received placebo for eight weeks. During the study, there were six drop out subjects, and the final analysis was performed on 52 subjects. The data was analyzed using MANCOVA test, with p value of <0,05 considered significant.

Results: In the supplementation group, total cholesterol was decreased by 22,6 mg/dL (95% CI: -33.3 to -12.1, p = 0.0001), LDL was decreased by 16.9 mg/dL (95%CI: - 26.7 to -7.1, p = 0.001), and TG was decreased by 30.8 mg/dL (95%CI: -55.3 to -6.4, p = 0.014). Probiotic supplementation was not proven to increase HDL levels and HDL: LDL ratio with p values of 0.370 and 0.374, respectively.

Conclusion: Probiotics supplementation in obese adolescent could reduce total cholesterol, LDL, TG, but had no effect on improving HDL levels and HDL: LDL ratio.

Keywords: adolescents, dyslipidemia, obesity, probiotic.
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INTRODUCTION

Obesity is a medical condition characterized by excessive accumulation of body fat. Study of obesity in adolescents increases because its prevalence has increased more than four times in the last three decades.[1] The prevalence of overweight and obesity in Indonesia is found mostly in the age group of 5 to 12 years, while in Bali, obesity is most prevalent in the age group of 13 to 15 years.[2,3] Children with obesity are at high risk of becoming obese in adults and potentially suffer from metabolic diseases such as dyslipidemia.[4,5] Increased prevalence of dyslipidemia is in line with the increasing prevalence of obesity, and become the most common risk factor of cardiovascular disease in the future.[6,7]

Consumption of high-calorie, high-fat, and high-cholesterol diet is a cause of obesity.[4] This condition can cause changes in the intestinal microbiota, and altered fat metabolism. In the intestine of obese children, composition of microbiota is altered with Firmicutes as the predominant phyla.[8,9]

Probiotics are supplements consisting of microbiota that is beneficial for digestive health. Probiotics act by balancing the digestive tract microbiota when given in sufficient quantities.[10,11] They are known to have many benefits, one of which is its effect in decreasing cholesterol levels in the blood.[12] The effect of probiotics on cholesterol reduction is characterized by its ability to bind lipids in the small intestine. The important mechanisms of probiotics on cholesterol reduction include: (1) the breakdown of direct cholesterol and deconjugation of bile salts and (2) conversion of cholesterol to coprostanol in the intestine, which is directly excreted through feces.[13,14] Several studies have shown the effect of probiotics on lipid profile. Xiao et al., evaluated the effect of low-fat yogurt containing 10^8

CFU/g *B. longum* BL1 on lipid profiles of 23 adult samples aged 28-60 years. The results showed a significant reduction (P <0.05) in the total serum cholesterol, LDL, and TG, as well as an increase in HDL by 14.5% after probiotics administration for 4 weeks compared with control.[15]

Most of the research on probiotic supplementation in obesity were conducted in adults. This study was conducted to investigate the benefits of probiotic supplementation in improving blood lipid profiles in adolescents with obesity.

METHODS

This study was a randomized double blind clinical trial. Obese adolescents aged 12 to 15 years old with BMI > percentile 95 who met the inclusion criteria were included in the study. The exclusion criteria include obesity caused by genetic disorder, patients with chronic infection, patients with malignancy, immunocompromised patients, administration of medication that affect lipid profile, administration of probiotics more than two weeks, and history of anti-inflammatory medications (NSAID, corticosteroid) consumption.

The study conducted at five junior high schools in Denpasar, chosen by purposive sampling. This study was approved by Research Ethics Committee of Udayana Medical School, Sanglah Hospital.

Sample estimation was calculated by using a hypothesis test for mean of two paired groups, with confidence interval 0.05 and power of 80%. By calculating 10% dropped out, subjects needed for each group were 29, with a total of 58 subjects. The subjects were recruited by consecutive sampling. Subjects and their parents were given explanations about the purpose and procedure of the study, including its potential benefits and risks.

Subjects who were eligible and willing to participate in this study were asked to sign the informed consent. Subsequently, subjects underwent assessment which included food recall, anthropometric status, and lipid profile. After the data was collected, subjects were randomized using a block system. The size of the block selected was block of 4 so that the number of sequences needed was 15 sequences. Each block consisted of 4 subjects, 2 from group A and 2 from group B. Randomization was carried out by researchers using a computer system. The random code was stored by the research assistant in a sealed envelope and opened after the research was completed. The pharmaceutical company is the party that determines code A for probiotic or B for placebo so the researchers and subjects did not know the content of sachets given.

Each subject was given 56 sachets containing probiotic or placebo for 8 weeks, one sachet was taken daily. The probiotics contain 5 strains of microbiota (*Streptococcus thermophilus, Lactobacillus rhamnosus, Lactobacillus acidophilus, Bifidobacterium longum, Bifidobacterium bifidum*) in the amount of $1{,}25 \times 10^9$ CFU. The placebo was made from flour and glucose with the same consistency, taste, and outer packaging as probiotics. The placebo was also produced by the same pharmaceutical company as the probiotics.

Food recall assessment was obtained by questionnaire given to the parents before the intervention. Food recall was obtained for three days to count the total calorie, protein, fat, and carbohydrate. Anthropometric measurement included weight, height, and body mass index (BMI) and plotted using CDC 2000 chart. Total cholesterol and triglyseride were assessed using *enzymatic colorimetriy* method, while LDL and HDL were measured using *Enzymatic homogeneous method.* Blood sampling and laboratory tests were carried out by the Prodia® Clinical Laboratory. Subjects' lipid profile was measured at the beginning and end of the study.

Treatment follow-up was carried out once a week by phone to determine the compliance and adverse effects of probiotics. If there were intoxication or side effects of supplements given, the supplementation would be discontinued. Samples that had discontinued consumption of supplements or did not consume ≥80% of the supplements were recorded as drop outs. The drop-out rate that can be accepted was less than 20%.

Administration of supplementation or placebo was carried out for 8 weeks, divided into 2 periods, each for 4 weeks. To ensure that the subjects consumed the supplements, one of the adult family members, preferably the parents are appointed as supervisors and observers of compliance to take supplements. Evaluation for compliance was carried out by reporting the remaining supplements that were not consumed. Children were asked to bring to school the remaining supplements that are not taken during the first 4 weeks to be submitted to the researcher, and the researchers gave the remaining supplements that must be taken 4 weeks later.

Data analysis was performed by computer programs. Categorical data was presented in percentage. Normally distributed numerical data was presented in mean and standard deviation while the abnormally distributed numerical data was presented in median and interquartile range. The Kolmogorov-Smirnov test was used to determine the normality of data distribution. Data that had an abnormal distribution was transformed. The bivariate test used to analyze the effect of probiotic supplementation on lipid profiles was the Mann-Whitney test. To calculate and analyze the effect of several controlled variables, a multivariate logistic regression test (MANCOVA) was performed. The level of significance used was p <0.05 and 95% confidence interval.

RESULTS

This research was conducted from May to September 2018. There were 127 students with obesity at five junior high schools in Denpasar who met the inclusion criteria. Sixty one subjects were excluded because they refused to participate in this research and consumed NSAIDs, and therefore, 58 subjects were eligible. During this study, there were two subjects from the treatment group and four subjects from the control group who dropped out of this research because of their unwillingness to continue the study and incomplete administration of the supplement. At the end of the study, the total number of analyzed subjects were 52 subjects, 27 subjects from the treatment group and 25 subjects from the control group. Scheme of the recruitment and randomization of subjects was shown in **Figure 1**.

Baseline data of subjects' characteristics were shown in **Table 1.** The subjects consisted of 17 (32.6%) boys and 35 (67.3%) girls who were distributed

comparably between the treatment and control groups. The mean age of the treatment group was 14.0 ± 1.0 years with an age range of 12.2 to 15.9 years and a control group of 14.3 ± 1.2 years with an age range of 11.9 to 15.9 years. At the beginning of the study, anthropometric data included body weight, body height and BMI were obtained; nutritional intake which included calories, protein, fat, and carbohydrates were comparable between the treatment and control group. Comparison of lipid profiles before and after treatment based on research groups is described in Table 2. Based on the table, the overall lipid profile including total cholesterol, LDL, HDL, triglycerides and HDL ratio: LDL at the beginning and end of the study appeared comparable.

The effect of probiotic supplementation was assessed by decreased mean levels of total cholesterol, HDL and TG and increased level of HDL and HDL: LDL ratio at the end of treatment. Changes in levels of lipid profiles reflected the effects of the treatment given. The results of the analysis are presented in Table 3. The difference in the mean levels of total cholesterol, LDL and TG at the beginning and end of the study, in the treatment group compared to the control group was significantly different with p value of <0.05. Non-significant results were obtained from the difference in mean HDL levels and HDL: LDL ratio at the beginning and end of the study, in both study groups with p value of > 0.05.

Multivariate analysis were performed with MANCOVA test by analyzing all dependent variables with control variables including age and BMI. The results of multivariate analysis were presented in Table 4. Probiotic supplementation could significantly reduce the mean cholesterol level by 22.6 mg / dL (95% CI: -33.1 to -12.1) in the treatment group with the p value of 0.0001. LDL levels in the treatment group decreased by 16.9 mg / dL (95%CI: -26.7 - -7.1) with the p value of 0.001. There was a significant decrease in TG levels in the treatment group by 30.8 mg / dL (95% CI: -55.3 - -6.4) with the p value 0.014. There was no significant decrease in the level of HDL in the treatment group with a change of 1.4 mg / dL (95% CI: -4.4 - 1.7) and p value of 0.370. No significant result was also found in the HDL: LDL ratio in the treatment group. There was only a 0.02 increase in the HDL: LDL ratio (95%CI: -0.02 - 0.6) in the treatment group with the p value of 0.374.

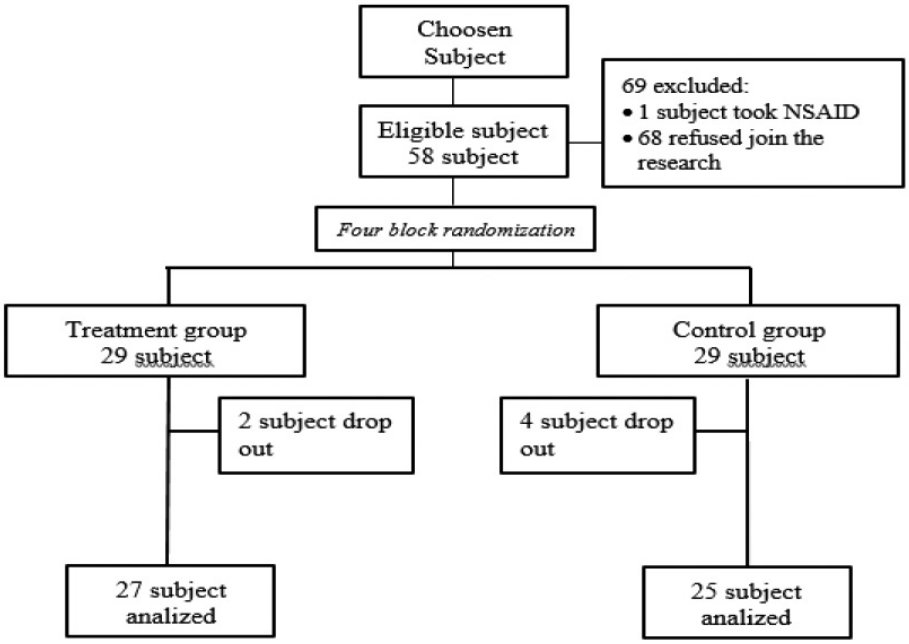

Figure 1. Selection process, randomization, and subject analysis scheme

Table 1. Baseline characteristics of the subject

Characteristic	Group	
	Supplementation	Control
N	27	25
Sex		
Male, n (%)	9 (33,3)	8 (32,0)
Female, n (%)	18 (66,7)	17 (67,3)
Age (year), mean (SD)	14,0 (1,0)	14,3 (1,2)
Body weight, mean (SD) kg	78,5 (11,2)	76,8 (9,3)
Body height, mean (SD) cm	160,5 (5,5)	158,5 (6,8)
BMI (kg/m^2), median (IQR) kg/m^2	30,5 (4,1)	30,7 (3,3)
Calorie intake, median (IQR) kkal	2841,1 (146,3)	2806,1 (325,9)
Protein intake, median (IQR) kkal	88,6 (29,1)	84,6 (30,5)
Fat intake, median (IQR) kkal	82,3 (22,5)	78,6 (16,0)
Carbohydrate intake, median (IQR) kkal	410,4 (61,7)	422,1 (64,6)

SD = standard deviation; IQR = *interquartile range*

DISCUSSION

Obesity is a medical condition in which nutritional disorders occured, characterized by excessive accumulation of body fat. There is a positive relationship between obesity and the appearance of comorbidities including hypertension, dyslipidemia, obstructive sleep apnea, and diabetes.[4] Denpasar is one of the cities with the highest prevalence of obese adolescents in Bali, therefore the study selected research subjects aged 12-15 years at junior high school.[3]

The characteristics of BMI and nutritional intake at the beginning of the study appeared comparable between the treatment and the control group, and therefore, would not influence the results of the study.

Probiotics supplementation in the

Table 2. Comparison Of Lipid Profile Before And After Treatment Based on The Research Groups

Characteristic	Group	
	Supplementation	Control
Total cholesterol		
Initial (mg/dL), mean (SD)	170,7 (31,5)	165,1 (24,8)
End (mg/dL), mean (SD)	153,2 (23,2)	170,7 (24,6)
LDL		
Initial (mg/dL), mean (SD)	114,4 (34,3)	107,8 (22,6)
End (mg/dL), mean (SD)	99,3 (24,0)	109,6 (24,2)
HDL		
Initial (mg/dL), mean (SD)	44,8 (8,1)	43,52 (9,0)
End (mg/dL), mean (SD)	43,4 (7,6)	44,1 (9,1)
Triglyceride		
Initial (mg/dL), mean (SD)	126,2 (82,0)	120,9 (88)
End (mg/dL), mean (SD)	102,2 (54)	129,5 (60)
Rasio HDL : LDL		
Initial (mg/dL), mean (SD)	0,4 (0,3)	0,4 (0,2)
End (mg/dL), mean (SD)	0,5 (0,3)	0,4 (0,2)

Table 3. Comparison of Lipid Profile Before and After Treatment Based on Research Group

Characteristic	Group		p
	Supplementation	Control	
Difference of total cholesterol, mean (SD) mg/dL	-17,3 (21,0)	5,6 (18,5)	0,0001
Difference of LDL, mean (SD) mg/dL	-15,1 (17,0)	1,8 (13,0)	0,0001
Difference of HDL mean (SD) mg/dL	-1,1 (7,0)	0,6 (7,0)	0,248
Difference of TG, mean (SD) mg/dL	-24,0 (50,0)	8,6 (30,5)	0,001
Difference of ratio HDL:LDL, mean (SD)	0,0 (0,1)	0,0 (0,1)	0,695

Table 4. MANCOVA Test Results on the Effect of Probiotic Supplementation on Lipid Profile After Controlling for Age and BMI

Dependent variables	B	Confidence interval (95% CI)	p
Decrease of total cholesterol (mg/dL)	22,6	-33,3 to -12,1	0,0001
Decrease of LDL (mg/dL)	16,9	-26,7 to -7,1	0,001
Increase of HDL (mg/dL)	-1,4	-4,4 to 1,7	0,370
Decrease of Triglyceride (mg/dL)	30,8	-55,3 to -6,4	0,014
Decrease of HDL:LDL ratio	0,02	-0,02 to 0,6	0,374

treatment group decreased the average of total cholesterol, LDL, and triglyceride levels more than the control group with p value of <0.05. Similar results were obtained from studies conducted by Rajkumar et al. (2004) who examined the effects of probiotics # VSL3 and omega-3 fatty acids on insulin sensitivity, blood lipid profile, and inflammation in 60 healthy adult subjects aged 40-60 years. The method of the aforementioned study was a double blind randomized trial comparing probiotics # VSL3 containing 8 bacterial strains (*B. longum, B. infantis, B. breve, L. acidophilus, L. paracasei, L. bulgaricus, L. Plantarum, S. thermophilus*), omega-3 fatty acids, and placebo for 6 weeks. The result showed that probiotics #VSL3 significantly improved lipid profiles (total cholesterol, LDL, HDL, triglycerides and VLDL) with p value of <0.05.[16]

The first mechanism of cholesterol reduction by probiotics is through the production of Bile Salt Hydrolase (BSH), an enzyme which catalyzes the deconjugation of bile acids. The conjugated bile acid will dissolve, decreasing its absorption in the intestine, and thereby increasing its elimination in the feces. Another mechanism is the conversion of cholesterol to coprostanol by the bacteria in the intestine, allowing it to be directly excreted through feces. Both of these mechanisms result in reduced concentration of cholesterol in the blood.[12,13] Prior studies which reported same results as this study used similar strains of bacteria, namely the Lactobacillus, Bifidobacterium, and Streptococcus. The result of this study was in accordance with the theory that cholesterol binding ability is influenced by the growth of certain specific strains of probiotics.[17,18]

HDL levels in this study were not significantly different between the treatment and control group (p = 0.374). Different results were obtained from the research conducted by Kiessling et al. (2002) who examined the increase in HDL cholesterol levels in long-term consumption of fermented dairy products for 6 months. This study was a clinical trial involving 29 healthy adult women aged 19-56 years. The probiotic strain used was *S. thermophilus, L. lactis, B. longum*. The results of this study showed that the administration of fermented milk containing probiotics significantly increased blood HDL levels in the treatment group with a p value of <0.05. Different result from this study was likely due to a shorter duration of treatment. The study also stated that an increase in HDL levels can be caused by the administration of probiotics along with other diets that naturally increase blood HDL levels.[19]

The ratio between HDL and LDL was also not significantly different in both bivariate and multivariate analyzes. Administration of probiotics in a short period of time cannot affect HDL levels and thus, the HDL: LDL ratio will not change significantly.

Probiotics have been widely used in the prevention and treatment of several diseases and considered to be safe for consumption. Side effects can occur especially in immunocompromised individuals and patients with severe health conditions. Mild side effects include abdominal pain, diarrhea or bloating, while severe side effects are very rare, including bacteremia and sepsis in children who are critically ill or

have a low immune system.[20] During the study, there was no report of side effects or toxicity from the treatment given, and therefore, supplementation of probiotics is considered safe for consumption.

This study has several limitations. This study did not analyze the physical activity (exercise) and food recall was only analyzed at the beginning of the study and not involved in data analysis.

CONCLUSION

This study proved that probiotic supplementation in obese adolescents reduced the total cholesterol, LDL levels, and blood triglyceride levels. Probiotic administration in this study did not increase blood HDL levels and did not improve the ratio between HDL and LDL. With the evidence that probiotics improve blood lipid profiles in obese adolescents, it is hoped that probiotic supplementation can be part of the management of obesity in adolescents to prevent dyslipidemia.

CONFLICT OF INTEREST

There is no conflict of interest in this article.

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The researchers declared that there is no funding involved in this research.

AUTHOR CONTRIBUTION

All authors searched for journals included journal analysis, journal selection, and synthesizing the data.

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