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Inulin-Enriched Low Fat Milk Improved Lipid Profile in Indonesian Hypercholesterolemic Adults

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Abstract. Hypercholesterolemia is known as the major risk factor contributing to cardiovascular disease and the prevalence is relatively high in Indonesia. Inulin is one of the soluble fiber with promising effect to improve lipid profile. However, the effects of inulin-enriched products have not yet been confirmed in Indonesian hypercholesterolemic population. OBJECTIVE: To study the effect of inulin-enriched low fat milk to the lipid profile of Indonesian hypercholesterolemic adults. MATERIAL AND METHOD: Thirty-three subjects were given instruction to drink low fat milk with total inulin of 6g/day for 6 weeks. Body composition and lipid profiles were measured before and after intervention. RESULTS: 6 weeks of inulin-enriched low fat milk administration resulted in a significant decrease of total cholesterol (TC, -11 mg/dl, $p<0.05$), low-density lipoprotein (LDL, -10.5 mg/dl, $p<0.05$), high-density lipoprotein (HDL, -2.21 mg/dl, $p<0.05$), and significant increase of skeletal muscle mass (SMM, 200g, $p=0.008$). Reduction in TC and LDL were found to be moderately positively correlated with its baseline ($r= 0.582$, $r=0.491$, $p<0.05$, consecutively). CONCLUSION: Inulin-enriched low fat milk effectively improved lipid profile and increased SMM in Indonesian hypercholesterolemic subjects. The degree of reduction was found to be moderately positively correlated with the baseline of TC and LDL.

Keywords: inulin, low fat milk, hypercholesterolemia, lipid profile.

1. Introduction

Hypercholesterolemia, a condition characterized by high levels of cholesterol in the blood, is known as the major risk factor contributing to cardiovascular disease. According to World Health Organization statistics, a third of ischaemic heart disease is attributable to high cholesterol and is estimated to cause 2.6 million deaths and 29.7 million disability adjusted life years [1]. In Indonesia, the prevalence of hypercholesterolemia is relatively high. As published by Ministry of Health Republic Indonesia (2013), more than 35% of Indonesian adults had high total cholesterol and more than 75% had high LDL cholesterol [2].

Nutritional theraphy for the treatment of hypercholesterolemia has been increasingly studied. Soluble fiber has long been used for the treatment of hypercholesterolemia as positive results in improving lipid profile are observed in studies. Inulin is one of the natural soluble fiber which has been used since the late 1980s in a large range of food, including milk and dairy products [3]–[6]. As a prebiotic, inulin does not only have beneficial effect on gut microbiota, but also on cholesterol level [5].

Several studies of inulin intake and its benefits on blood lipid profile have been conducted. Previously, inulin supplementation (7-18 g/day, 4-6 weeks length) seemed to decrease blood cholesterol effectively in hypercholesterolemic subjects [3]–[5], [7]. Similar results were also seen in a meta-analysis conducted by Wu et al. and a study in Thai population [8], [9]. Though the effects of inulin-enriched products on the lipid profile have been confirmed in many ethnicities, little of them is known in Indonesian population. Therefore, this research aims to study the effect of inulin-enriched low fat milk to the lipid profile of Indonesian hypercholesteremic adults.

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2. Subjects and Methods

2.1. Subjects

Subjects were recruited through a health program called "cholesterol challenge". All participants included in this study were Indonesian, aged ≥20 years old, classified as hypercholesterolemia (total cholesterol level >200 mg/dl or LDL levels >100 mg/dl), not taking any medication for the treatment of hyperlipidemia or any drug treatment which would affect their plasma lipid levels. Subjects who were pregnant and/or breastfeeding, taking medication, had any type of diabetes and/or cardiovascular disease, abnormal gastrointestional digestion/ absorption, intolerance/allergy to milk products were excluded from the study. Subjects who consumed products less than 80% were excluded.

All subjects underwent a screening including measurement of body composition and diabetes and cardiovascular family history through questionnaires. This study was approved by Institute of Research Ethics Committee Atma Jaya Catholic University of Indonesia. Informed written consent was obtained from all participants.

Among 54 interested individuals, 52 were eligible to participate in the study as they met the inclusion criteria. Nineteen subjects (36.54 %) withdrew from the study; 9 subjects had adverse events (lactose intolerance, diarrhea, nausea, and bloating), 8 subjects were noncompliant and 3 subjects requested withdrawal from the study because of illness (unrelated to products/ any gastrointestinal disease). Thirty-three subjects (63.46 %) completed the study and their results were included.

2.2. Materials

The inulin-enriched low fat milk (Tropicana Slim® Low Fat Milk Vanilla, BPOM RI MD: 861328001350) were supplied by PT. Nutrifood Indonesia. A serving unit of the sample inulin-enriched low fat milk contained 3 g of inulin. The samples were prepared by diluting 45 g of inulin-enriched low fat milk into 200 ml of warm water. The composition of the product are shown in Table 1.

Table 1: Macronutrient composition of inulin-enriched low fat milk (per 45 g)

Composition	Amount
Fats (g)	0.5
Proteins (g)	7
Carbohydrates (g)	34
Dietary fibers (g)	3
Natrium (mg)	110
Potassium (mg)	340
Total energy (kkal)	160

2.3. Study Protocol

A quasi-experimental study was performed. All subjects were given the instruction to consume sample as complementary drink twice daily, once in the morning and once in the evening for 6 weeks. Subjects were suggested to reach physical activity level at least 10 metabolic equivalent hour/ week (MET.h/week). Nutrition education class about fat and cholesterol were also provided for the subjects in order to provide more information about the importance of healthy eating pattern to reduce cholesterol level.

2.3.1. Measurements

Body composition including body mass index (BMI), skeletal muscle mass (SMM), and fat mass (FM) of subjects were measured by InBody 720 (InBody Co Ltd, Korea). BMI categories for public health actions in Asians is used for subgroup analyses [10]. All subjects were requested to record their exercise routine through physical activity questionnaires which were converted to MET according to the 2011 Compendium of Physical Activities [11].

Blood samples were collected before and after intervention from fasted subjects for determination of biochemical parameter including cholesterol total (TC), high-density lipoprotein cholesterol (HDL), and triglyceride (TG) in corporation with Prodia Clinical Laboratorium. Low-density lipoprotein (LDL) cholesterol was calculated using the Friedewald equation (1972) with following formula : LDL-indirect = TC - HDL - TG/5.0 (mg/dl). All measurements were performed before and after the intervention.

2.3.2. Statistical analysis

Statistical analysis was performed using SPSS (version 21.0, IBM, USA). Shapiro Wilk tests for normality were performed for all data. Paired sample T-Test and Wilcoxon signed-rank test (nonparametric dependent t-test) were used to measure the significance of mean before and after treatment in all data and subgroup analyses. The mean difference in each subgroup were independently measured by Kruskal-Wallis test and Mann-Whitney U test. Pearson's correlation was used to evaluate relationships between baseline and Δ reduction of lipid parameters. Significance was assigned at p-value of <0.05.

3. Results

3.1. Subject Characteristics

Demographic and baseline characteristics of all subjects are shown in Table 3. Mean age was 31.2±7.0 years old, the mean BMI was 25.32±4.53 kg/m^2 and most of the subjects (69%) did not reach the required physical activity. Comparison of body composition before and after intervention is presented in Table 2. The increase of SMM after 6-week supplementation of product was identified to be significant (p=0.008), but BMI and fat mass remained unchanged.

Table 2: Characteristics of subjects (n=33)

Baseline characteristics		Number (%) or mean±SD
Age (years)		31.2±7.0
BMI (kg/m^2) group		25.32±4.53
	Normal	13 (39)
	Overweight	6 (18)
	Obese	14 (43)
Sex		
	Male	15 (45)
	Female	18 (55)
Physical activity (MET.h/week)		
	<10	20 (61)
	≥10	13 (39)

Table 3: Comparison of body composition parameters of all subjects before and after (n = 33)

	Before		After		Mean difference	p-value
	Mean	SD	Mean	SD		
BMI (kg/m^2)	25.32	4.53	24.37	4.50	0.95	0.476
SMM (kg)	25.81	6.74	26.05	6.73	0.2	**0.008**[a]
FM (kg)	20.80	8.25	20.56	8.08	0.24	0.123

All values are x̄ ± SD; BMI, body mass index (kg/m^2); SMM, skeletal muscle mass (kg); FM, fat mass (kg)
[a]significantly different after intervention (p<0.05)

3.2. Blood Lipid Analyses

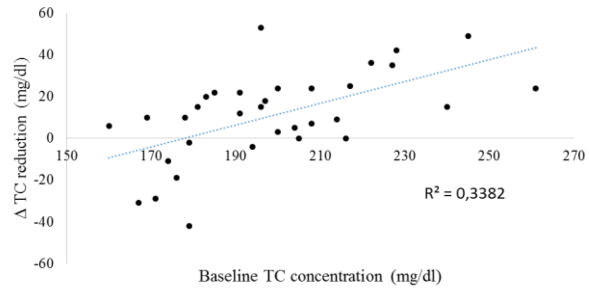
TC, LDL, serum HDL, and triglycerides comparisons before and after study are shown in Table 4. TC and LDL were significantly lower after intervention, about 11 mg/dl and 10.5 mg/dl (p= 0.006, p= 0.003) lower compared to the initial level. HDL also had a significant decrease, which was about 2.21 mg/dl (p= 0.048).

Table 4: Comparison of lipid profiles of all subjects before and after (n = 33)

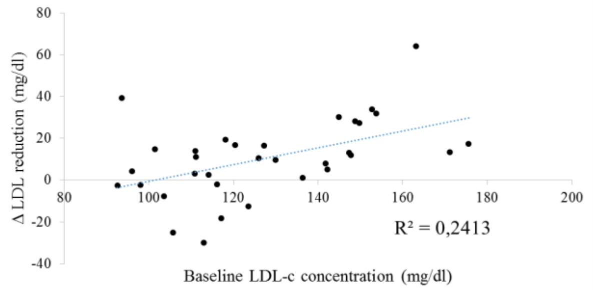
	Before		After		Mean difference	p-value
	Mean	SD	Mean	SD		
Total cholesterol (mg/dl)	198.85	24.00	187.85	20.94	11	**0.006** [a]
LDL indirect (mg/dl)	127.45	23.01	116.95	21.4	10.5	**0.003** [a]
HDL (mg/dl)	51.88	10.68	49.67	8.52	2.21	**0.048** [a]
Triglyceride (mg/dl)	100.09	55.81	106.18	53.69	6.72	0.282

All values are x̄ ± SD.
[a]significantly different after intervention (p<0.05)

Pearson's correlation were performed in purpose to see the correlation of lipid profile reduction compared to the baseline. Mainly, moderate significant correlations were found for TC and LDL (Fig. 1). The subjects' initial TC (mean baseline) were moderately correlated with the TC reduction (r=0.582, n=33, p< 0.05) (Fig. 1a). There was also a moderate significant correlation between initial LDL concentration and LDL reduction (r= 0.491, n=33, p<0.05) (Fig. 1b).



A.



B.

Fig. 1: Correlation between baseline and Δ reduction (n=33); a) TC, b) LDL.

3.3. Subgroup Analyses

Subgroup analyses were performed on both body composition and blood lipid parameters as shown in Table 5. SMM, TC and LDL showed significance among parameters examined. Based on BMI classification, obese subjects appeared to have the most significant increment of SMM and decrease of TC and LDL compared to normal and overweight subjects. Based on gender, more significant improvement were experienced by female subjects rather than male. Based on physical activity level, all subjects experienced significant decrease in LDL but the SMM increased significantly only in <10 MET group.

Table 5: Subgroup analyses before and after supplementation of inulin-enriched low fat milk.

	SMM		p	TC		p	LDL indirect		p
	before	after		before	After		before	After	
BMI group									
Normal	22.16±6.0	22.28±6	NS	197.92±26	187.92±15.3	NS	127.21±23.7	116.04±17.88	NS
Overweight	26.97±7.5	27.38±7.5	NS	185.67±21.8	181.83±22.0	NS	113.10±19.7	113.00±19.15	NS
Obese	28.71±5.8	28.97±5.7	0.038*	205.35±22.1	190.35±25.6	0.034*	133.81±22.3	119.47±26.01	0.019*
p	0.022**	0.022**		NS	NS		NS	NS	
Sex									
Male	31.55±4.7	31.76±4.7	NS	202.13±27.4	193.33±23.8	NS	135.65±23.4	126.01±20.35	0.017*
Female	21.02±3.8	21.28±3.7	0.004*	196.11±21.2	183.28±17.7	0.037*	120.11±20.9	109.39±19.71	0.043*
p	<0.001**	<0.001**		NS	NS		NS	0.027**	
Physical activity									
<10 MET	24.86±7.2	25.09±7.1	0.007*	198.25±22	188.05±17.5	NS	125.30±21.6	115.45±17.78	0.033*
≥10 MET	27.28±5.9	27.51±6.0	NS	199.77±27.8	187.54±26.1	NS	130.75±25.6	119.24±26.69	0.025*
p	NS	NS		NS	NS		NS	NS	

All values are x̄ ± SD; SMM, skeletal muscle mass (kg); TC, total cholesterol (mg/dl); LDL, low density lipoprotein cholesterol (indirect, mg/dl); MET, metabolic equivalent (MET.h/week)
*significantly different after intervention (p <0.05)
**significantly different between group (p< 0.05)

4. Discussions

This study showed that inulin supplementation for 6 weeks in hypercholesterolemic subjects decreased TC, LDL, HDL and increased SMM significantly (p< 0.05). The result of this study was in line with other researches. Study conducted in Spain showed that 12.5 g/day administration of inulin for 5 weeks resulted in 8% decrease in LDL-c with no changes in TC, HDL-c, and TG observed [12]. Comparably, our study showed that the samples could decrease 8.34% LDL-c, contributed to 10.5 mg/dl mean decrease in Indonesian subjects. Another study in Lithuania showed a significant decrease of 12% LDL-c and tendency towards lower TC but no effect on TG concentration after a 28-days period of consumption of yoghurt with 5 g/day inulin in patients with metabolic syndrome [13]. Our study indicated that 6 g inulin supplementation in low fat milk could give relatively same beneficial effect in improving TC and LDL compared to similar studies [12]–[14].

The decrease of both TC and LDL-c were in line with our hypothesis and other researches. In contrast, we also found that the good cholesterol, HDL-c, also significantly decreased. However, this effect can be categorized as minor and did not pass the recommended threshold values that could negatively affect cardiovascular morbidity (not less than 40 mg/dL) [13]. In several studies, the effect of inulin consumption on HDL-c were found to be either increased modestly or nonsignificant so the evidence of inulin on HDL-c were noted to remain inconclusive [15], [16].

In this present study, inulin-enriched low fat milk administration for 6 weeks significantly increased SMM. This result might be caused by higher protein intake of subjects from milk sample. The total protein intake of subjects was not measured but at least additional 14 g/day of protein were consumed from supplemented low-fat milk, contributing ≈25% of recommended daily intake of protein for adults [17]. Supplementation of higher protein intake with low fat diet tend to increase lean body mass and increase fat loss. Increasing protein intake can reduce the risk of muscle mass loss, and might even increase muscle mass [18].

The correlation of TC and LDL shown in our study, proved by Pearson’s correlation (Fig 1), indicated that subjects with the highest baseline were correlated with greater reduction of TC and LDL level. Similarly, in subgroup analyses, obese subjects tend to have higher TC, LDL and SMM and thus experienced the most significant changes to improve all of the parameters after the intervention. Previously, subjects with metabolic syndrome experienced a significant decrease of LDL with 5g/day of inulin supplementation [13]. On the other hand, a study with 10 g of inulin applied to healthy subjects had no effect on lipid parameters [19]. Most likely, subjects with dyslipidemia-one of the major risk factors in metabolic syndrome- will get more benefits from the intervention.

Subgroup analyses results showed that mean baseline of SMM in female was lower than male. By this difference, the intervention in female subjects increased their SMM significantly compared to male subjects. This gender-specific findings might be caused by several factors such as the difference in daily protein requirement and the significant baseline difference between male and female. The recommended protein intake for the average male is 63 g/day, whereas female needs about 50 g/day [20]. Therefore, 14 g protein served in samples contributed higher RDI in female subjects rather than male.

As a soluble dietary fiber, inulin has been extensively studied as a prebiotics. Inulin can modulate gut flora composition, mainly *Bifidobacteria,* which has been shown to be positively correlated with lower TC and LDL-c [14], [21]–[23]. The beneficial effects of inulin on lipid profile are mainly mediated by at least two known mechanisms. First, inulin decreases cholesterol absorption accompanied by enhanced cholesterol excretion via feces. Prebiotics may contribute to cholesterol reduction by increasing fecal bile acid excretion, reducing intestinal cholesterol absorption, and effects in several expressions regarding the cholesterol biosynthesis. Second, inulin administration produces short-chain fatty acids (SCFAs) such as butyrate, propionate, and acetate by intestinal bacterial microflora. Butyrate, the major fermentation product from inulin, is known to inhibit liver cholesterol synthesis and has been found to prevent diet-induced obesity, improve insulin sensitivity, and also increase fatty acid oxidation. Acetate may act as precursor for cholesterol synthesis, while propionate could inhibit hepatic cholesterol synthesis by decreasing the use of acetate as a precursor of cholesterol [5], [14], [24].

Some gastrointestinal symptoms experienced by subjects during the study were rumbling, flatulence, bloating, looser stools but no severe side effects were reported. It is well known that consumption of dietary fibers in large quantities may cause digestive problems because of the osmotic effect, which lead to an increased presence of water in the large intestine. Furthermore, study in 84 healthy subjects reported that even at the highest dose of 20 g/day, inulin were very well tolerated [25].

This study did not include food record diet pattern of each subjects which may influence cholesterol level. Moreover, longer duration of intervention, larger population, measurement of serum SCFA, and the exploration of gut microbiota are recommended for future studies. Despite those limitations, this study might be the first to report the effect inulin-enriched low fat milk on improving cholesterol and also increasing SMM significantly.

5. Conclusion

The present study did support the hypothesis that 6-week administration of 6 g/day inulin-enriched low fat milk was effective to improve the lipid profile of Indonesian hypercholesterolemic subjects. Inulin-enriched low fat milk mainly reduced TC, LDL, HDL and increased SMM significantly. Further investigations and better study design are required to assure the effect and mechanism of inulin on lipid metabolism.

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