Original

A Crossover Study on the Effects of Continuous Soymilk Intake on Lipid Metabolism and Glucose Metabolism in Vietnamese - Lipid Metabolism Improving Effect –

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ABSTRACT *Background and purpose.* Unprepared soymilk is expected to have a physiological effect due to its high protein content, but there are few clinical trials on soymilk intake in Vietnam and other Asian countries targeting patients with dyslipidemia. This study was conducted a crossover study to verify the effect of unprepared soymilk intake on improving lipid metabolism in a joint study with Vietnam National Institute of Nutrition, Nam Dinh University of Nursing, MARUSAN-AI Co., Ltd., and Kyushu Women's University. *Methods.* It was conducted with the approval of the Kyushu Women's University Ethics Review Committee and the Vietnam National Institute of Nutrition Ethics Review Committee. Thirty-seven type 2 diabetic patients with LDL cholesterol borderline or mild dyslipidemia attending hospital in Nam Dinh City, Vietnam, were included in the analysis. The study design was a randomized crossover study of 2 groups, and only the intervention group was given 500 mL/day of unadjusted soymilk (MARUSAN-AI Co., Ltd.) for 8 weeks, and the washout period was 4 weeks. The primary endpoints were LDL-C, TG and the secondary endpoints were TC, HDL-C. *Result.* The amount of change in LDL-C and TG in the intervention group were significantly reduced at both the 4W-intake and 8W-intake. In addition, the amount of change in HDL-C in the intervention group were significantly increased at the 8W-intake. *Conclusion.* For Vietnamese with type 2 diabetes with dyslipidemia, it became clear that the continuously intake of unprepared soymilk could decrease LDL-C and TG and increase HDL-C.

Keywords: Vietnamese, crossover study, unprepared soymilk, LDL cholesterol, lipid metabolism

INTRODUCTION

In the Socialist Republic of Vietnam (hereinafter referred to as Vietnam), lifestyle-related diseases such as diabetes mellitus, obesity and hypertension are increasing as in developed countries (*1-3*). The proportion of people who died of hyper-LDLemia increased by 37.3% in the 10 years from 2009 to 2019 (*4*), which is thought to be due to increased intake of animal foods and fats and lack of exercise (*5-7*). In many meta-analyses, soy protein intake has been reported to reduce serum low-density lipoprotein cholesterol (LDL-C) and serum triglyceride (TG) (*8,9-14*), and soy globulin is expected to have a physiological effect. Furthermore, β-conglycinin, the main component of soy globulin, has been shown to reduce the accumulation of serum triglyceride and visceral fat (*15*). In recent years, Zhang et al. (*16*) have reported a meta-analysis that fasting blood glucose level, fasting insulin level are significantly reduced by soy protein intake. However, according to a report by Le et al. (*17*) in 2014, the energy intake of Vietnamese people is about 2,000 kcal/day, and the protein intake is 61.6 g/day, but the protein intake from soybean products is 2.8 g/day (less than 5%). Soymilk is one of the soybean products that can easily ingest soybean protein. In particular, unprepared soymilk has a high protein content, so soy protein intake is expected to have a physiological effect. Recently, Ta NT et al. have reported that soy protein intake significantly reduced fructosamine (FRA), total cholesterol (TC), and TG in Vietnamese patients type 2 diabetes mellitus (DM) with hyper-LDLemia in a pre- and post-comparison within the soy protein intake group (*18*) . In this way, it has been suggested that the intake of soy protein may improve lifestyle-related diseases in Vietnamese people.

In addition, the soymilk market share in Vietnam is dominated by prepared soymilk with added sucrose, and the protein content is as low as about 2 g/100mL. Therefore, it is considered that the physiological effect of soy protein cannot be sufficiently obtained. Also, there are few clinical trials in Vietnam and other Asian countries targeting patients with hyperlipidemia due to soymilk intake alone.

In this study, a joint research with Vietnam National Institute of Nutrition (NIN), Nam Dinh University of Nursing (NDUN), MARUSAN-AI Co., Ltd., and Kyushu Women's University, we investigated the physiological effects of continuous intaking unprepared soymilk without sucrose (soy protein content 4.5 g/100mL, carbohydrate content 1.5 g/100mL, MARUSAN-AI Co., Ltd. products) in Vietnamese patients with type 2 DM with

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dyslipidemia. In order to examine whether the lipid metabolism is improved by daily intaking 22.5 g of soy protein contained in 500 mL (250 mL x 2 times) of unprepared soymilk for 8 weeks, a crossover study was performed in two groups, the unprepared soymilk intake group (intervention group) and the prepared soymilk non-intake group (control group). A dietary survey confirmed the effects of diet and consumption of the unprepared soymilk on energy and nutrient intake. For evaluating the effect of continuous intake of raw soymilk on improving lipid metabolism and glucose metabolism, changes in LDL-C, TG, and FRA from before to 4 weeks and 8 weeks after intake were used as primary endpoints. Secondary endpoints were changes in TC, high-density lipoprotein cholesterol (HDL-C), and fasting blood glucose (FPG) levels from before to 4 weeks and 8 weeks after intake.

MATERIALS AND METHODS

1. Ethical considerations

This study was conducted in accordance with the Declaration of Helsinki-Ethical Principles of Medical Research Including the Human Body (World Medical Association) and "Ethical Guidelines for Medical and Health Research Including the Human Body" (Ministry of Health, Labor and Welfare, Japan). The ethics review was approved by the Kyushu Women's University Ethics Review Committee (approval number H30-14) and the Vietnam National Institute of Nutrition Ethics Review Committee. After that, informed consent was given to the subjects, and consent was obtained. The research period is from August 2020 to March 2022.

2. Subjects

The sample size of the subjects was set to forty-four. For type 2 DM patients with borderline or mild LDL cholesterolemia who attending hospital in Nam Dinh City, Vietnam, a screening test was conducted to determine eligibility in 45 patients who gave their consent, and those who met all the following inclusion criteria 1) to 4) and did not meet any of the exclusion criteria 1) to 7) were selected. Inclusion criteria: 1) Patients who are type 2 DM diagnosed in past 5 years with FBG > 7 mmol/L, are using oral drug for management DM, had no severe complications and no advisory of dietetics for controlling DM, have hyperlipidemic with high LDL-C (boundary range: 3.10-3.59 mmol/L, mild range: 3.60-4.10 mmol/L) but not yet taking drugs to control hyperlipidemic. 2) Those who can participate in the test during the test period. 3) Person who can drink soymilk continuously for 8 weeks. 4) A person who has given written consent to participate in this study. Exclusion criteria: 1) Person who are DM and have been used insulin for treatment. 2) Person who are allergic to soybeans. 3) Person with serious disease. 4) Person who has a serious medical history or history of gastrectomy. 5) Person taking internal medicine or formula milk for DM. 6) Excessive alcoholic drinkers or excessive smokers. 7) Any other person who are deemed inappropriate to participate in this study.

The sample size was calculated based on the formula [1] proposed by Hassard.

$$N = 2 \times \frac{(Z_\alpha + Z_\beta)^2 . \sigma^2}{(\mu_1 - \mu_2)^2} \quad [1]$$

$Z\alpha = 1.96$, $Z\beta = 0.84$, σ is the standard deviation of LDL-C concentration, μ_1 is the change in LDL-C concentration from baseline to 8 weeks of taking soymilk, μ_2 is change of LDL-C concentration from baseline to 8 weeks of no taking soymilk. We also assume that σ is approximately 0.245 mmol/L (based on results of our previous research in Vietnamese). Therefore, sample size as calculated as 37 subjects. After adding 20% of dropt out, the suggested sample size is 44 (subjects).

3. Study design and schedule

The study design was a crossover study and was randomly assigned to two groups, group A and group B. The study schedule is shown in Figure 1. In the first period, only Group B ingested unprepared soymilk (MARUSAN-AI Co., Ltd., made in Thailand) 500 mL/day (250 mL x 2 times) for 8 weeks, and the washout period was 4 weeks. In the second period, only Group A ingested 500 mL/day (250 mL x 2 times) of unprepared soymilk for 8 weeks.

The nutritional composition of unprepared soymilk is shown in Table 1. The period before intake of unprepared soymilk hereinafter referred to as "before intake", and 4 weeks and 8 weeks after intake of unprepared soymilk hereinafter referred to as "4W-intake" and "8W-intake", respectively. The measurement items were dietary survey, anthropometry, and blood tests, and the test was conducted once for each period for a total of 6 times.

	1st Period			Washout period	2nd Period		
	Before Intake (0W)	4th week	8th week	4 weeks	12th week	16th week	20th week
Group A	Control group				Intervention group		
Group B	Intervention group				Control group		
Soymilk intake status record	Group B →				Group A →		
Measurement items							
1) Dietary survey	●	●	●		●	●	●
2) Anthropometry	●	●	●		●	●	●
3) Blood tests	●	●	●		●	●	●

Figure 1. Study schedule and measurement items

Table 1. Nutritional composition of unprepared soymilk (test beverage)

Nutrients	Unprepared soymilk (per 100mL)
Energy (kcal)	44
Protein (g)	4.5
Lipid (g)	2.2
Carbohydrate (g)	1.5
Sucrose (g)	0.4
Dietary fiber (g)	0.3

4. Anthropometry

Weight and height were measured while participant standing, wearing light clothing and no shoes. Body fat percentage was measured by TANITA scale (model BC- 541N, TANITA Corporation). Waist circumference was measured by the minimum circumference between the umbilical cord and the iliac crest, and hip circumference was measured by the widest circumference of the buttocks.

5. Blood tests

The subjects fasted from 9 pm the day before blood tests, visited the hospital by 9 am on the day of blood tests (water intake was possible), and had blood drawn. The test items are serum total protein (TP), serum albumin (Alb), FPG, TC, LDL-C, HDL-C, TG, alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatinine (Cre), and FRA.

6. Dietary survey

The 24-hour dietary recall method was used for the dietary survey, and a total of 6 surveys were conducted at 0th week, 4th week, 8th week, 12th week, 16th week, and 20th week. Regarding the meals on the previous day, we conducted an interview survey on the meals (type and amount of food) from the time of waking up to the time of bedtime. To answer the intake accurately, we prepared photographs of the actual size of standard vessels (bowl, cup, spoon) used in ordinary households, and conducted an interview survey while showing them. In addition, "Calorie Smile" software (Quest-Computer Co., Ltd.,) which also includes photo data of common dishes, was used during the survey to improve the accuracy of the survey. The calculation of nutrient intake was based on the Vietnamese Food Composition Table published in 2017.

7. Statistical analysis

All values are shown as mean ± standard deviation. Using Microsoft® Excel 2016 for Windows, an unpaired *t*-test were performed on the carry-over effects in Group A and Group B prior to the analysis of a crossover study. The significance level was set to 10%. Statistical analysis software IBM SPSS Statistics 20 (IBM Japan Headquarters, Inc.) was used to analyze the attributes and endpoints of the subjects. Analysis of change between two groups of the same subjects used paired *t*-test and change between two groups of different subjects performed unpaired *t*-test. All tests were two-sided test, and the significance level was set to 5%.

RESULTS

1. Subject selection and attributes

Of the 45 subjects who gave their consent, 42 subjects who met the selection criteria were randomly assigned to each of the two groups, Group A and Group B, by 21. After the start of the study, 5 subjects dropped out due to non-participation in the study (3 subjects) or taking medication for the treatment of dyslipidemia (2 subjects), and finally 19 subjects in group A and 18 subjects in group B, a total of 37 subjects, were included in the analysis (Figure 2). The attributes of the subjects are shown in Table 2. There were no significant differences in age, height, weight, body mass index (BMI), and body fat percentage between the two groups, Group A and Group B.

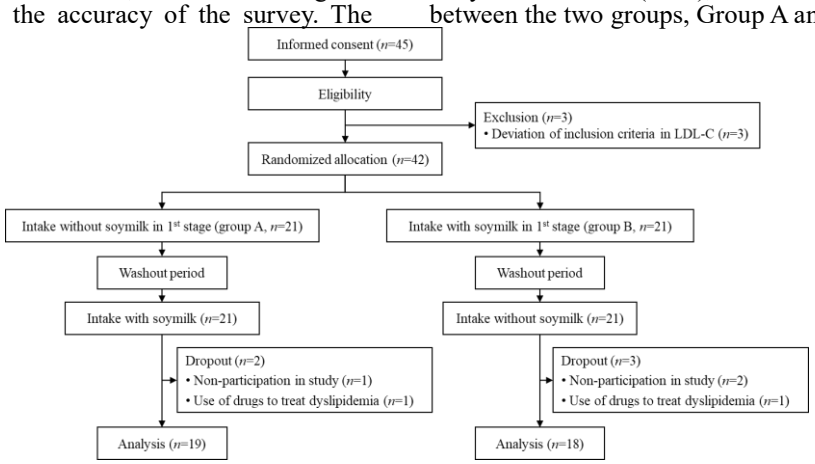

Figure 2. Flow chart from selection of subjects to analysis

Table 2. Subject attributes

	Group A (n=19)	Group B (n=18)	All (n=37)	*p* value†
	Mean ± SD	Mean ± SD	Mean ± SD	
Age (years)	61.8 ± 6.9	64.7 ± 6.3	63.2 ± 6.8	0.21
Height (cm)	155.4 ± 7.7	155.4 ± 6.6	155.4 ± 7.2	1.00
Weight (kg)	58.1 ± 11.6	57.7 ± 7.6	57.9 ± 9.8	0.91
BMI (kg/m^2)	23.9 ± 3.7	23.8 ± 2.5	23.9 ± 3.2	0.93
Body fat percentage (%)	30.9 ± 7.3	30.4 ± 7.2	30.7 ± 7.3	0.83

†: Unpaired t-test, n=37

2. Verification of carry-over effect

As a result of verifying the carry-over effect of the crossover study, LDL-C and TG, which are the primary endpoints of lipid metabolism, had no carry-over effect. However, TC, which is a secondary endpoint, was excluded because it had a carry-over effect. In addition, a carry-over effect was observed in FRA, which is the primary endpoint of glucose metabolism, so it was not possible to analyze glucose metabolism.

3. The amount of change in LDL-C, TG, and HDL-C at the 4W- and 8W-intake

Table 3 shows the amount of change in LDL-C and TG as the primary endpoints and HDL-C as the secondary endpoint at 4W- and 8W-intake. The amount of change from before intake to 4 and 8 weeks of intake period was defined as Δ4W-intake and Δ8W-intake, respectively.

In LDL-C, the control group was 3.43 ± 0.27 mmol/L before intake, 3.66 ± 0.41 mmol/L at 4W-intake, and 3.75 ± 0.53 mmol/L at 8W-intake. The intervention group was 3.46 ± 0.26 mmol/L before intake, 3.09 ± 0.36 mmol/L at 4W-intake, and 2.85 ± 0.32 mmol/L at 8W-intake. The Δ4W-intake in LDL-C was 0.23 ± 0.35 mmol/L in the control group and −0.37 ± 0.30 mmol/L in the intervention group, and the intervention group significantly decreased (p<0.001). The Δ8W-intake was 0.32 ± 0.46 mmol/L in the control group and -0.60 ± 0.28 mmol/L in the intervention group, which was significantly lower in the intervention group (p<0.001).

In TG, the control group was 2.95 ± 1.07 mmol/L before intake, 3.19 ± 1.26 mmol/L at 4W-intake, and 3.01 ± 1.08 mmol/L at 8W-intake. The intervention group was 2.96 ± 1.12 mmol/L before intake, 2.46 ± 0.80 mmol/L at 4W-intake, and 2.38 ± 0.65 mmol/L at 8W-intake. The Δ4W-intake in TG intake was 0.24 ± 0.60 mmol/L in the control group and −0.49 ± 0.64 mmol/L in the intervention group, and the intervention group significantly decreased (p<0.001). The Δ8W-intake was 0.06 ± 0.64 mmol/L in the control group and −0.58 ± 0.79 mmol/L in the intervention group, and the intervention group significantly decreased (p<0.001).

In HDL-C, the control group was 1.14 ± 0.18 mmol/L before intake, 1.14 ± 0.17 mmol/L at 4W-intake, and 1.16 ± 0.21 mmol/L at 8W-intake. The intervention group had 1.17 ± 0.25 mmol/L before intake, 1.21 ± 0.19 mmol/L at 4W-intake, and 1.28 ± 0.19 mmol/L at 8W-intake. There was no significant difference between the two groups in the Δ4W-intake. The Δ8W-intake in HDL-C was 0.02 ± 0.16 mmol/L in the control group and 0.11 ± 0.19 mmol/L in the intervention group, and the intervention group significantly increased (p=0.048).

In addition, the results of the stratified analysis was shown in Table 3. In Δ8W-intake of LDL-C, there was no significant difference between the inner group and outer group in both the control group and the intervention group. In TG, there was no significant difference between the inner group and the outer value group in the control group, but the outer value group showed a significantly lower value in the intervention group (p<0.001). In HDL-C, there was no significant difference between the inner group and outer group in the control group, but the outer value group showed a significantly higher value in the intervention group (p=0.002).

Table. 3. The amount of change in LDL-C, TG, and HDL-C at the 4W- and 8W-intake and stratified analysis of the Δ8W-intake between the inner and outer groups of reference value

Items (unit) (Standard value)	Group		Before intake	4W-intake	8W-intake	Δ4W-intake† Amount of change	Δ4W-intake† *p* value††	Δ8W-intake† Amount of change	Δ8W-intake† *p* value††
LDL-C (mmol/L)	Control	Mean	3.43	3.66	3.75	0.23		0.32	
(< 3.4 mmol/L)	group	SD	0.27	0.41	0.53	0.35	<0.001	0.46	<0.001
	Intervention	Mean	3.46	3.09	2.85	-0.37		-0.60	
	group	SD	0.26	0.36	0.32	0.30		0.28	
Subgroup#								*p* value†††	
Inner group < 3.4 mmol/L (n=17)	Control group	Mean	3.24	3.45	3.52	0.27			
		SD	0.15	0.32	0.43	0.44			
	Intervention group	Mean	3.23	2.91	2.68	-0.56			
		SD	0.11	0.31	0.28	0.29		Control group: 0.57	
Outer group ≥3.4 mmol/L (n=20)	Control group	Mean	3.51	3.75	3.90	0.26		Intervention group: 0.36	
		SD	0.24	0.40	0.53	0.47			
	Intervention group	Mean	3.62	3.26	3.07	-0.62			
		SD	0.20	0.34	0.28	0.26			
TG (mmol/L)	Control	Mean	2.95	3.19	3.01	0.24		0.06	
(1.7-2.25 mmol/L)	group	SD	1.07	1.26	1.08	0.60	<0.001	0.64	<0.001
	Intervention	Mean	2.96	2.46	2.38	-0.49		-0.58	
	group	SD	1.12	0.80	0.65	0.64		0.79	
Subgroup#								*p* value†††	
Inner group < 2.25 mmol/L (n=14)	Control group	Mean	1.98	2.03	2.17	0.20			
		SD	0.34	0.38	0.59	0.64			
	Intervention group	Mean	1.85	1.81	1.86	0.01			
		SD	0.19	0.23	0.16	0.24		Control group: 0.33	
Outer group ≥2.25 mmol/L (n=23)	Control group	Mean	3.55	3.90	3.52	-0.02		Intervention group: <0.001	
		SD	0.91	1.07	0.99	0.63			
	Intervention group	Mean	3.63	2.86	2.69	-0.94			
		SD	0.90	0.76	0.63	0.80			
HDL-C (mmol/L)	Control	Mean	1.14	1.14	1.16	0.01		0.02	
(1.03-1.55 mmol/L)	group	SD	0.18	0.17	0.21	0.14	0.37	0.16	0.048
	Intervention	Mean	1.17	1.21	1.28	0.05		0.11	
	group	SD	0.25	0.19	0.19	0.20		0.19	
Subgroup#								*p* value†††	
Inner group ≥1.03 mmol/L (n=27)	Control group	Mean	1.20	1.20	1.22	0.02			
		SD	0.17	0.16	0.21	0.17			
	Intervention group	Mean	1.28	1.25	1.33	0.05			
		SD	0.13	0.19	0.18	0.14		Control group: 0.89	
Outer group < 1.03mmol/L (n=10)	Control group	Mean	0.98	1.00	1.00	0.02		Intervention group: 0.002	
		SD	0.08	0.10	0.10	0.11			
	Intervention group	Mean	0.87	1.10	1.14	0.27			
		SD	0.26	0.12	0.13	0.23			

†: The amount of change from before intake to 4 and 8 weeks of intake period was defined as Δ4W-intake and Δ8W-intake, respectively. ††: Paired *t*-test, n=37, control group *vs* intervention group. †††: Unpaired t-test, n=37, control group vs intervention group. #: Inner group and outer group mean an inner and outer group the reference value, respectively.

4. The amount of change in Cre, TP, Alb, ALT, and AST at the 4W- and 8W-intake

Table 4 shows the amount of change in Cre, TP, Alb, ALT, and AST, which were conducted as a safety confirmation test. No significant difference was found between the two groups in any of the items.

Table 4. The amount of change in Cre, TP, Alb, ALT, and AST at the 4W- and 8W-intake

Items (unit) (Standard value)	Group		Before intake	4W-intake	8W-intake	Δ4W-intake† Amount of change	Δ4W-intake† *p* value††	Δ8W-intake† Amount of change	Δ8W-intake† *p* value††
Cre (mmol/L)	Control	Mean	83.03	82.94	82.82	-0.13		-0.21	
(62 - 120 mmol/L)	group	SD	16.37	17.14	17.94	7.20	0.94	9.42	0.25
	Intervention	Mean	82.36	82.35	84.29	-0.01		1.94	
	group	SD	16.85	17.89	18.23	4.83		6.33	
TP (mmol/L)	Control	Mean	76.36	76.59	76.54	0.23		0.18	
(3.9 - 6.4 mmol/L)	group	SD	4.24	3.90	4.38	3.00	0.60	3.89	1.0
	Intervention	Mean	76.88	77.52	77.06	0.64		0.18	
	group	SD	4.11	4.64	4.06	3.11		3.43	
Alb (g/L)	Control	Mean	41.69	41.58	41.66	-0.10		-0.03	
(35 - 52 g/L)	group	SD	2.13	1.87	2.10	1.76	0.47	2.03	0.99
	Intervention	Mean	41.70	41.94	41.67	0.24		-0.03	
	group	SD	2.10	2.75	1.87	2.18		1.92	
AST (U/L)	Control	Mean	27.14	28.85	29.42	1.71		2.29	
(< 50 U/L)	group	SD	12.17	19.39	31.21	11.37	0.25	21.85	0.37
	Intervention	Mean	37.81	28.61	33.25	-9.21		-4.56	
	group	SD	78.21	31.17	54.75	47.61		24.47	
ALT (U/L)	Control	Mean	16.29	15.84	16.41	-0.45		0.12	
(< 50 U/L)	group	SD	6.42	5.92	7.37	5.70	0.45	7.72	0.39
	Intervention	Mean	17.87	16.16	16.14	-1.71		-1.73	
	group	SD	13.72	7.02	8.16	8.37		8.07	

†: The amount of change from before intake to 4 and 8 weeks of intake period was defined as Δ4W-intake and Δ8W-intake, respectively. ††: Paired *t*-test, n=37, control group *vs* intervention group.

5. The amount of change in body weight, BMI, waist circumference, and hip circumference at the 4W- and 8W-intake

Table 5 shows the amount of change in body weight, BMI, waist circumference, and hip circumference at the 4W- and 8W-intake. There was a significant difference between the two groups in waist circumference and hip circumference. The Δ8W-intake of waist circumference was 0.83 ± 1.99 cm in the control group and -0.58 ± 2.23 cm in the intervention group, showed significantly lower values in the intervention group (p=0.010). The Δ8W-intake of hip circumference was 0.86 ± 1.49 cm in the control group and -0.05 ± 1.57 cm in the intervention group, showed significantly lower values in the intervention group (p=0.042).

Table 5. The amount of change in body weight, BMI, waist circumference, and hip circumference at the 4W- and 8W-intake

Items (unit)	Group		Before intake	4W-intake	8W-intake	Δ4W-intake† Amount of change	Δ4W-intake† *p* value††	Δ8W-intake† Amount of change	Δ8W-intake† *p* value††
Weight (kg)	Control group	Mean	57.2	57.6	57.4	0.38	0.002	0.17	0.24
		SD	9.6	9.7	9.8	0.68		0.80	
	Intervention group	Mean	57.4	57.2	57.3	-0.18		-0.11	
		SD	9.9	9.7	9.7	0.86		1.15	
BMI (kg / cm^2)	Control group	Mean	23.8	24.0	23.9	0.17	0.002	0.08	0.17
		SD	3.2	3.2	3.3	0.31		0.37	
	Intervention group	Mean	23.9	23.8	23.8	-0.10		-0.07	
		SD	3.3	3.1	3.1	0.46		0.58	
Waist circumference (cm)	Control group	Mean	83.8	84.1	84.6	0.38	0.13	0.83	0.010
		SD	7.8	7.9	8.2	1.80		1.99	
	Intervention group	Mean	83.5	83.2	82.9	-0.30		-0.58	
		SD	7.9	7.8	7.8	1.71		2.23	
Hip circumference (cm)	Control group	Mean	90.4	90.6	91.3	0.21	0.23	0.86	0.042
		SD	5.3	5.3	5.7	1.10		1.49	
	Intervention group	Mean	90.2	90.1	90.2	-0.10		-0.05	
		SD	5.1	5.1	5.1	1.22		1.57	

†: The amount of change from before intake to 4 and 8 weeks of intake period was defined as Δ4W-intake and Δ8W-intake, respectively. ††: Paired *t*-test, n=37, control group *vs* intervention group.

6. The amount of change in nutrient intake at the 4W- and 8W-intake

Table 6 shows the amount of change in energy, protein, lipid, carbohydrate, and sugars at the 4W- and 8W-intake. In Δ4W-intake and Δ8W-intake of energy, lipid, and carbohydrate, there was no significant difference between the intervention group and the control group. In protein intake, Δ4W-intake was -1.4 ± 29.2 g/day in the control group and 21.4 ± 30.1 g/day in the intervention group, and the intervention group significantly increased (p=0.004). The Δ8W-intake was -0.7 ± 29.6 g/day in the control group and 17.8 ± 34.9 g/day in the intervention group, and the intervention group significantly increased (p=0.027). In sugars intake, no significant difference was observed between the two groups in the Δ4W-intake. In Δ8W-intake of sugars was 10.2 ± 18.4 g/day in the control group and 1.4 ± 18.2 g/day in the intervention group, and the control group significantly increased (p=0.040).

Table 6. The amount of change in nutrient intake at the 4W- and 8W-intake

Items (unit)	Group		Before intake	4W-intake	8W-intake	Δ4W-intake† Amount of change	Δ4W-intake† *p* value††	Δ8W-intake† Amount of change	Δ8W-intake† *p* value††
Energy (kcal)	Control group	Mean	1479	1498	1459	19	0.61	-20	0.74
		SD	384	379	375	466		475	
	Intervention group	Mean	1573	1654	1593	80		20	
		SD	428	306	327	475		494	
Protein (g)	Control group	Mean	66.6	65.1	65.9	-1.4	0.004	-0.7	0.027
		SD	20.2	26.5	27.3	29.2		29.6	
	Intervention group	Mean	69.0	90.4	86.8	21.4		17.8	
		SD	24.8	22.7	23.0	30.1		34.9	
Lipid (g)	Control group	Mean	31.5	35.3	30.7	3.9	0.52	-0.8	0.60
		SD	21.7	23.0	18.2	25.8		26.7	
	Intervention group	Mean	37.2	45.5	39.8	8.3		2.6	
		SD	22.2	17.3	18.6	26.9		22.7	
Carbohydrate (g)	Control group	Mean	232.3	230.1	230.1	-2.3	0.40	-2.2	0.39
		SD	68.6	59.0	47.8	78.2		79.0	
	Intervention group	Mean	240.9	220.3	221.6	-20.6		-19.3	
		SD	84.2	60.1	58.4	82.0		84.4	
Sugars (g)	Control group	Mean	11.5	16.7	21.7	5.3	0.21	10.2	0.040
		SD	12.2	19.5	20.7	20.8		18.4	
	Intervention group	Mean	16.7	16.6	18.0	-0.1		1.4	
		SD	16.6	14.6	15.0	18.1		18.2	

†: The amount of change from before intake to 4 and 8 weeks of intake period was defined as Δ4W-intake and Δ8W-intake, respectively. ††: unpaired t-test, n=37, control group vs intervention group.

DISCUSSION

The amount of change in LDL-C and TG in the intervention group significantly decreased at both the 4W- and 8W-intake. In addition, the amount of change in HDL-C in the intervention group significantly decreased at 8W-intake. From the results of these primary and secondary endpoints, the effect of continuous intake of soymilk on improving lipid metabolism was confirmed. Furthermore, as a result of stratified analysis of the Δ8W-intake between the inner and outer groups of reference value in LDL-C, TG, and HDL-C, respectively, in all items, a significant improvement effect was observed in the outlier group of the intervention group.

The American Heart Association (AHA) has reported the mechanism of action of soy protein on serum lipids, such as thyroid hormone and other metabolic hormones, bile acid excretion promoting action, and involvement in LDL receptors (*19*). Also, in 1999, the U.S. Food and Drug Administration (FDA) allowed a soy protein health claim, "Ingesting 25 g of soy protein per day as part of a diet low in saturated fatty acids and cholesterol reduces the risk of heart disease." (*20*) In Japan as well, the cholesterol-reducing effect of soy protein in humans has been recognized, and the Consumer Affairs Agency has approved it as one of the functional substances in Food for Specified Health Uses (FOSHU) (*21*). In addition, it has been reported that triglyceride lowering effect of soy protein is due to the decrease in fatty acid synthesis ability in the liver and the suppression of liver fat accumulation due to the decrease in blood insulin concentration (*22-24*). Although many studies on lipid metabolism of soy protein have been reported so far, few studies have been conducted on the effect of soymilk on the improvement of lipid metabolism disorders, and few studies have been conducted in the Asian region.

In this study, it was confirmed that it can be to reduce LDL-C and TG by continuously unprepared soymilk intake for 8 weeks in Vietnamese patients with type 2 DM who have borderline or mild LDL cholesterolemia. Regarding glucose metabolism, the effect of unprepared soymilk intake could not be clarified because FRA, which is the primary endpoint of glucose metabolism, had a carry-over effect. However, although no significant change in body weight was observed, the amount of change in both waist circumference and hip circumference was significantly reduced at the Δ8W-intake. Waist circumference is adopted as an essential item in the diagnostic criteria for metabolic syndrome in Japan as an indicator of visceral fat accumulation. It is known to increase the risk of which visceral fat accumulation causes hypertension, hyperglycemia, and lipid abnormalities through abnormal adipocytokine secretion, resulting in cardiovascular disease (*25*). In addition, waist circumference has been closely associated with insulin resistance and therefore is recommended as one of the diagnostic criteria for metabolic syndrome by NCEP-ATP III (National Cholesterol Education Program-Adult Treatment Panel III) (*26*). In this study, it found that the continuous intake of unprepared soymilk 500 mL/day for 8 weeks, the waist circumference, which indicates the visceral fat accumulation, was significantly reduced. Therefore, it is suggested that continuous intake of soymilk can be expected to reduce insulin resistance by improving the secretion of adipocytokines, and further research is needed in the future.

Dietary survey revealed that a protein intake was significantly higher in the intervention group than in the control group, both in the Δ4W-intake and in the Δ8W-intake. As 100mL of unprepared soymilk contains 4.5g of protein, it is considered that the intake of 500mL of unprepared soymilk a day significantly

increased protein intake in the intervention group. As mentioned above, it has been reported that soy protein reduces LDL-C and TG and increases HDL-C, and in this study, we were able to obtain similar results from blood tests. Furthermore, despite an increase in energy intake of 80 kcal in the 4th week in the intervention group, the body weight decreased significantly compared to the control group, and waist circumference and hip circumference decreased significantly in the 8th week, respectively. Martinez-Villaluenga C et al. has reported that peptides derived from β-conglycinin, a major component of soy protein, inhibit fatty acid synthase at the molecular level (*27*). It has also been reported that administration of soy protein peptide to rats enhances the thermogenic capacity of mitochondria in brown adipose tissue (*28*). In this way, the results of animal studies have been reported so far, but in this human study, the continuous intake of unprepared soymilk significantly reduced both waist circumference and hip circumference. In the future, we would like to conduct further research to clarify the relationship between the intake of unprepared soymilk and lipid and sugar metabolism.

In conclusion, In this study, it was confirmed that it can be to reduce LDL-C and TG, and to increase HDL-C by continuous intake of unprepared soymilk for 8 weeks in Vietnamese patients with type 2 DM who have borderline or mild LDL cholesterolemia. In addition, it was clarified that the improvement effect was higher in the outer group for TG and HDL-C than in the inner group of reference value. Furthermore, as a new finding, it was revealed that the continuous intake of unprepared soymilk significantly reduced both waist circumference and hip circumference. In the future, we would like to conduct further research to clarify the relationship between the intake of unprepared soymilk and lipid and sugar metabolism.

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CONFLICTS OF INTEREST (COI)

This study will be conducted as a joint research between NIN, NDUN, Kyushu Women's University and MARUSAN-AI Co., Ltd. and will be conducted at a research funding based on a joint research agreement. MARUSAN-AI Co., Ltd. provided research expenses and samples for his COI relationship to be disclosed in this study.

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