

Effects of Long-Term Intake of Mayonnaise Containing Phytosterolester on Blood Cholesterol Concentration in Japanese with Borderline or Mild Cholesterolemia

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Summary In a placebo-controlled double-blind study, we examined the effects on blood lipids during long-term consumption of mayonnaise containing phytosterolester in Japanese with borderline or mild hyperlipidemia. We also examined the safety of this mayonnaise. Fifty-five subjects were divided into 2 groups, one group of which was given for 3 months 15 g/day of a placebo mayonnaise (Placebo), and the other, 15 g/day of mayonnaise containing 884 mg/day of phytosterolester (MSE) for the same period of time. Hematological testing and confirmation of objective and subjective symptoms were conducted every month. Total cholesterol and LDL cholesterol levels did not change in the Placebo group throughout the study, but they did significantly decrease 1, 2, and 3 months after the start of intake in the MSE group. Significant differences were seen between Placebo group and MSE group in the amount of changes in total cholesterol 1, 2, and 3 months after the start of intake, and in LDL cholesterol 1 and 2 months after the start of intake. Furthermore, the results of hematological testing other than for blood lipids and physician interview revealed no adverse events caused by long-term consumption of the test foods. We thus concluded mayonnaise containing phytosterolester to be safe and to lower total serum cholesterol and LDL cholesterol levels.

Key Words: phytosterolester, cholesterol, mayonnaise, safety

Introduction

To prevent hypercholesterolemia it is necessary to decrease the cholesterol in foods, to prevent an increase in blood cholesterol, and to maintain its normal level. Phytosterolester has recently been

highlighted as a food material that lowers blood cholesterol [1]. It is known that the functional mechanism of phytosterolester involves the impediment of cholesterol absorption from the digestive tract, and such efficacy has been validated in a large number of human trials [2]. Its safety has also been confirmed in many human trials. In the US it has been approved as a GRAS (Generally Recognized as Safe)

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substance, while in Japan also the Ministry of Health, Labour and Welfare has recognized margarine containing phytosterolester as a FOSHU (Foods for Specified Health Uses) food. Phytosterol in free bodies is a powder with a high melting point, whereas phytosterolester is a fatty paste with a low melting point. The solubility of the latter in fat, is also high, so it characteristically responds well in foods containing fat.

Thus, phytosterolester not only has a cholesterol-lowering function, but also is a food source that is adaptable in fatty foods. We therefore tested phytosterolester for its cholesterol-lowering ability by putting it in mayonnaise, a very familiar food among Japanese, without age discrimination of subjects. Before this we had ascertained that mayonnaise prepared with at least 870 mg/day of phytosterolester (free body conversion) lowered blood cholesterol when it was given for 1 month to Japanese with borderline or mild hypercholesterolemia [3]. In order to examine the effects on blood cholesterol of mayonnaise containing phytosterolester (intake amount of phytosterolester: 884 mg/day) taken over a longer period of 3 months, the present study was conducted for 3 months as a double-blind, two-group parallel study using a placebo and the test substance. Safety was also examined based on other hematological tests and evaluation of objective and subjective symptoms by physician interview.

Materials and Methods

Table 1 shows the nutritional components and analytical values for phytosterolester amounts of test foods. Placebo chosen was a whole egg type of mayonnaise with a fat component of approx. 75%. Mayonnaise containing phytosterolester (MSE) was

prepared by replacing a portion of the vegetable oil in the Placebo with phytosterolester. Both test foods were exactly the same in terms of other ingredients such as egg, vinegar and seasoning. The major sterols of phytosterolester used for MSE were campesterol, stigmasterol and β -sitosterol and the fatty acids were derived from edible vegetable oils.

The subjects were 57 paid subjects registered with Sogo Ikagaku Kenkyusho Inc. 30 to 60 years of age, and who satisfied the selection criteria of not presently undergoing treatment with any drugs and having a serum total cholesterol of 200 to 280 mg/dl. The subjects were divided into 2 groups such that the serum total cholesterol was nearly the same. Immediately after the start of the study 2 subjects in the MSE group changed their living address, and so the final number of subjects was 55. Of these, 26 were in MSE group and 29, in Placebo group. The backgrounds of subjects in each group are shown in Table 2. There were no significant differences between the 2 groups.

Table 1. Nutritional components and phytosterolester amounts.

Components	Unit	MSE	Placebo
Energy	kcal	106	105
Water	g	2.5	2.6
Protein	g	0.24	0.24
Fat	g	11	11
Ash	g	0.29	0.29
Carbohydrates	g	0.51	0.63
Sodium	mg	100	100
Phytosterolester	mg	884	72

(/15g mayonnaise)

The amount of phytosterolester is converted into that of free sterol. General sterol composition; Campesterol : Stigmasterol : β -sitosterol = 1 : 1 : 2.

Table 2. Background of subjects.

		MSE	Placebo	ALL subjects
		n=26	n=29	n=55
Age	(years old)	48.0 \pm 8.9	45.7 \pm 10.1	46.8 \pm 9.5
BMI	(kg/m ²)	24.6 \pm 3.1	25.9 \pm 3.9	25.3 \pm 3.5
Systolic BP	(mmHg)	133.4 \pm 19.4	132.8 \pm 16.5	133.1 \pm 17.7
Diastolic BP	(mmHg)	80.9 \pm 9.3	83.0 \pm 11.3	82.0 \pm 10.4
Pulse	(beats/min.)	74.5 \pm 8.4	77.0 \pm 8.8	75.8 \pm 8.6
Total cholesterol	(mg/dl)	228.9 \pm 22.7	226.3 \pm 20.2	227.6 \pm 21.3
LDL cholesterol	(mg/dl)	147.3 \pm 20.4	144.4 \pm 22.8	145.7 \pm 21.6
HDL cholesterol	(mg/dl)	53.4 \pm 10.9	48.8 \pm 9.6	51.0 \pm 10.4

Values are means \pm SD. No significant difference between the 2 groups.

The study was conducted in compliance with the spirit of the Helsinki Declaration (adopted in 1964 and amended in 1975, 1983, 1989, 1996, and 2000) and started after the written informed consent of subjects to participate in the study was obtained after a physician fully explained to them the content of the research, the methods, etc.

The study period consisted of a pre-monitoring (non-intake) period of 1 week, an intake period of 3 months, and post-monitoring (non-intake) period of 1 month. During the intake period the subjects were required to consume 1 sachet (15 g) of the test food in addition to their normal diet once a day during the evening meal. During the implementation of the study, subjects were instructed to avoid irresponsible eating and drinking and not alter their normal daily lifestyle. Subjects were instructed to finish their evening meal by 9 pm on the day before blood sampling and to not consume any food or drink thereafter except water.

Survey of food and alcohol intake

It was compulsory for subjects to record in detail in a diary the types of alcoholic beverages consumed and the amounts thereof every day, as well as the foods consumed and the amounts thereof for 3 days prior to testing. Based on the records of consumption, the administrative dietitian calculated the amounts of alcohol consumed and the amounts of nutrients consumed.

Hematological tests

Blood sampling was conducted every month during the intake period. In consideration of the changes in the body due to psychological effects before the start of consumption, the values at the start of consumption adopted were the mean values of 2 blood sample results obtained within 1 week during the pre-monitoring period. The hematological test items were blood cell parameters [red blood cells, white blood cells, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and blood platelet count], total cholesterol, triglycerides, HDL cholesterol, LDL cholesterol, free fatty acids, apolipoprotein determination (AI, B, CIII, and E), β -lipoprotein, blood sugar, HbA1c, albumin, alanine transaminase (ALT), aspartate transaminase (AST), γ -glutamyl transpeptidase (γ -GTP), creatinine, uric acid, blood urea nitrogen (BUN), Na, K, and Cl. Blood sampling was

conducted at the Soiken Clinic (Director: Ken Miyazuka), and Sakai Bio-Chemical Laboratory, Inc. was commissioned for the blood testing.

Blood pressure and physical measurements

At the same time as blood sampling, blood pressure and pulse count were taken, as were physical measurements. Blood pressure and pulse count were measured at least 5 min after rest by using the upper left arm while the subjects were sitting clothed but without shoes.

Examination/interview

A physician examined and interviewed the subjects and thereby investigated the development or not of objective and subjective symptoms including events such as dry cough, skin symptoms, taste abnormalities, headache, and lightheadedness/dizziness, and digestive tract symptoms such as lack of appetite, nausea, vomiting, diarrhea and constipation or other adverse events.

Statistical analysis

The measured values were expressed as the mean \pm SD. Comparison between the groups for blood lipid test values during the intake period was conducted by using two-way analysis of variance, and the coactions between the food groups, and intake period main effects were analyzed. For the changes within the food groups during the intake period, multiple comparison testing was conducted by using the Dunnett test. Corresponding t-test was adopted for the testing at the start of intake and upon the completion of post-monitoring, and non-corresponding t-test was adopted for testing differences between the food groups. The analysis software used was SPSS Ver. 10 (SPSS Co., Ltd.), and in all tests the level of significance on both sides was set at $p < 0.05$.

Results

Food content and volume of alcohol consumed

Table 3 shows the changes in the food content and volume of alcohol consumed during the study period.

A significant decrease was found in the cholesterol intake from the start of the study period to that measured after 2 months in the MSE group, but the difference between the 2 groups was not significant. Aside from this, in the MSE groups significant

Table 3. Changes in food composition and volume of alcohol consumed during the study period.

	Groups	Start	1 month	2 months	3 months	Completion of post-monitoring
Energy (kcal/day)	MSE	2,519.8±563.9	2,488.7±513.3	2,504.4±491.5	2,484.3±344.2	2,320.7±558.2
	Placebo	2,374.9±438.5	2,479.5±377.9	2,357.0±525.2	2,370.4±505.5	2,222.5±486.1
Protein (g/day)	MSE	91.0±21.5	85.2±23.4	80.3±16.6*	84.1±16.8	80.0±19.1*
	Placebo	80.0±19.3	84.8±15.5	73.7±20.1	75.2±19.9	75.2±16.8
Fat (g/day)	MSE	83.1±29.4	95.3±21.9*	92.5±23.3	91.3±22.3	77.7±28.8
	Placebo	78.5±25.9	89.3±24.1	80.1±29.2	86.6±28.1	69.4±22.3
Carbohydrate (g/day)	MSE	296.8±66.4	274.0±50.1	279.7±58.9	279.9±48.8	272.4±61.9
	Placebo	290.1±50.3	284.6±32.3	287.8±63.4	279.9±53.9	277.1±53.8
Cholesterol (mg/day)	MSE	507.5±174.5	469.7±179.9	422.9±154.5*	443.3±134.9	477.9±218.2
	Placebo	428.2±153.8	455.0±117.6	370.9±167.2	397.4±149.4	399.0±110.5
Dietary fiber (g/day)	MSE	13.8±3.5	14.7±4.6	14.0±5.1	14.4±4.8	13.5±4.7
	Placebo	13.6±3.7	15.0±4.4	13.4±4.5	13.5±3.7	13.6±4.1
Alcohol (g/day)	MSE	55.0±82.5	42.1±49.0	49.8±75.8	38.9±45.7	38.5±50.1*
	Placebo	34.9±37.5	42.3±60.4	31.6±37.1	25.9±28.3	25.1±27.0

Values are means±SD. Significantly different from Start: * $p<0.05$, ** $p<0.01$.

Table 4. Changes in test values for blood lipids during the study period.

	Groups	Start	1 month	2 months	3 months	Completion of post-monitoring	Coaction	Main effect
Total cholesterol (mg/dl)	MSE	228.9±22.7	214.5±21.9***	216.1±21.2**	218.3±22.2*	219.6±21.1*	$p<0.05$	n.s
	Placebo	226.3±20.2	223.9±19.2	226.0±22.8	228.3±27.3	224.8±23.1		
LDL cholesterol (mg/dl)	MSE	147.3±20.4	134.3±19.8***	139.6±19.9*	139.0±19.0*	141.8±19.5	$p<0.01$	n.s
	Placebo	144.4±22.8	143.5±20.9	150.9±22.9	148.5±22.3	150.2±20.8		
HDL cholesterol (mg/dl)	MSE	53.4±10.9	54.7±10.8	55.8±8.5	58.3±11.3**	56.2±11.9	n.s	n.s
	Placebo	48.8±9.6	52.1±10.9**	53.0±10.3***	54.0±12.0***	52.3±10.8**		
β-Lipoprotein (mg/dl)	MSE	486.8±95.9	464.0±106.8	451.5±88.8*	439.1±76.0**	478.0±110.7	n.s	n.s
	Placebo	499.1±95.6	498.0±101.5	482.8±78.6	491.0±129.2	501.9±86.3		
NEFA (mEq/liter)	MSE	0.55±0.19	0.56±0.19	0.52±0.19	0.61±0.23	0.53±0.19	n.s	n.s
	Placebo	0.56±0.22	0.52±0.24	0.51±0.23	0.67±0.36	0.48±0.18		
RLP cholesterol (mg/dl)	MSE	10.4±7.5	10.2±11.4	6.8±3.3	7.0±5.3	8.9±6.9	n.s	n.s
	Placebo	11.0±8.2	11.6±12.8	7.7±5.8	11.2±13.1	9.4±6.2		
ApoA I (mg/dl)	MSE	134.1±17.3	143.7±19.0***	143.9±14.9***	146.5±18.5***	142.7±18.8***	n.s	n.s
	Placebo	130.2±17.1	140.7±21.3***	140.5±18.1***	142.6±20.8***	138.9±20.3**		
ApoB (mg/dl)	MSE	110.8±10.6	107.6±11.6	107.1±12.2	108.8±11.8	107.1±11.9	$p<0.05$	n.s
	Placebo	110.6±13.4	112.7±12.8	113.9±14.9	113.0±16.0	112.8±13.3		
Apo III (mg/dl)	MSE	14.7±5.4	14.1±5.6	14.6±6.2	15.0±5.6	14.6±5.1	n.s	n.s
	Placebo	15.0±5.3	14.7±5.4	14.5±4.8	15.1±5.1	14.4±3.4		
Apo E (mg/dl)	MSE	4.66±1.32	4.72±1.26	4.47±1.16	4.47±1.38 _#	4.50±1.20	n.s	n.s
	Placebo	5.14±1.20	5.27±1.39	5.00±1.12	5.32±1.42 _#	4.85±1.03		
Atherosclerosis index (TC-HDLc)/HDLc	MSE	3.45±0.83	3.03±0.71***	2.94±0.63*** _#	2.83±0.59*** _#	3.02±0.66*** _#	n.s	$p<0.05$
	Placebo	3.82±1.02	3.46±0.89**	3.39±0.80**	3.41±1.02**	3.44±0.84**		

Values are means±SD. Significantly different from Start: * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Significant difference between the 2 groups: # $p<0.05$.

decrease was also seen in protein intake after 2 months and at the completion of post-monitoring, as was a decrease in and the volume of alcohol consumed at the completion of post-monitoring, but neither were significantly different between the 2 groups.

Blood lipids

Table 4 shows the changes in values of blood lipids during the study period.

Figure 1 shows the changes in total cholesterol amount in all subjects and in subjects with a total cholesterol of ≥ 220 mg/dl [4] at the start of intake. In the case of all subjects, there were no significant

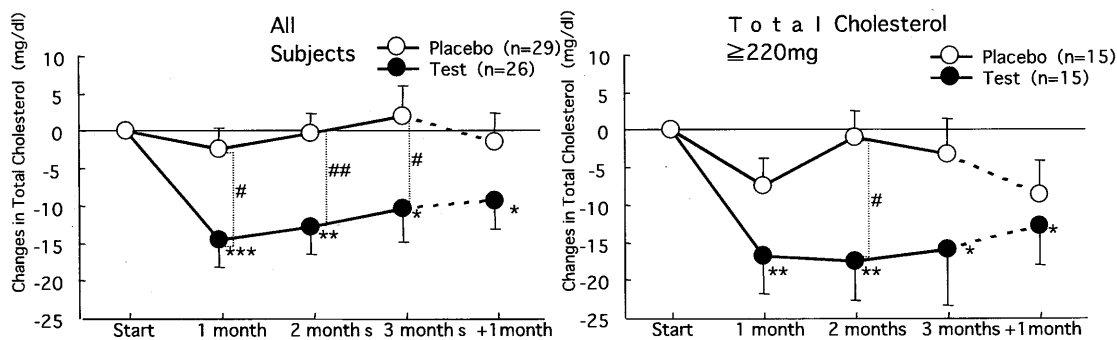


Fig. 1. Changes in total cholesterol amount. Values are means±SE. Significant difference from start. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Significant difference between the 2 groups: # $p < 0.05$, ## $p < 0.01$. +1 month: at the completion of post-monitoring.

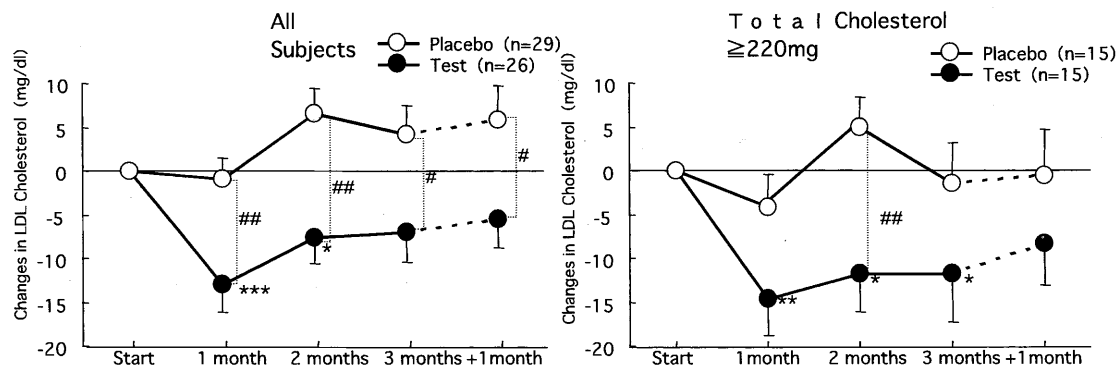


Fig. 2. Changes in LDL cholesterol amount. Values are means±SE. Significant difference from start. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Significant difference between the 2 groups: # $p < 0.05$, ## $p < 0.01$. +1 month: at the completion of post-monitoring.

changes from the start of intake throughout the study period in Placebo group, but in MSE group there was a significant drop from the start of intake throughout the entire intake period. Total cholesterol was significantly lower throughout the entire intake period in the MSE group compared with its level in the Placebo group. In the case of subjects with a total cholesterol of ≥ 220 mg/dl, total cholesterol was slightly lower in the Placebo after 1 month of intake, but the value was not significantly different from the values at the start of intake. Total cholesterol significantly decreased from that at the start of intake in MSE group throughout the entire intake period and also at the completion of post-monitoring. The value was lower in the MSE group than in the Placebo group throughout the entire intake period, and the difference was significant after 2 months of intake.

Figure 2 shows the changes in LDL cholesterol amount in all subjects and in subjects with a total cholesterol of ≥ 220 mg/dl at the start of intake. In the case of all subjects, there were no significant

changes from the start of intake throughout the study period in the Placebo group, but in the MSE group LDL cholesterol significantly decreased from the value at the start of intake at 1 and 2 months of intake. LDL cholesterol was significantly lower consistently during the intake period and at the completion of post-monitoring in the MSE group than in the Placebo group. In the case of subjects with a total cholesterol the ≥ 220 mg/dl, there were no significant changes in LDL cholesterol from the start of intake during the study period in the Placebo group, but the level did significantly decrease from the start of intake throughout the study period in the MSE group. The LDL cholesterol value was lower in the MSE group than in the Placebo group throughout the entire intake period, and the difference was significant after 2 months of intake.

The HDL cholesterol value significantly increased from the start of intake in the Placebo group throughout the entire intake period and at the completion of post-monitoring and significantly increased after 3 months of intake in the MSE group.

Table 5. Changes in blood cells and biochemical examination results during the study period.

	Standard range	Groups	Start	1 month	2 months	3 months	Completion of post-monitoring
White blood cell ($\times 100/\mu\text{l}$)	33-90	MSE	61.3 \pm 16.4	61.0 \pm 13.8	58.7 \pm 12.5	59.7 \pm 13.1	66.5 \pm 17.0*
		Placebo	58.7 \pm 14.0	57.7 \pm 14.1	58.6 \pm 13.3	61.2 \pm 16.1	63.2 \pm 16.1*
Red blood cell ($\times 10,000/\mu\text{l}$)	450-560	MSE	477.6 \pm 31.4 _#	477.0 \pm 34.8 _#	482.4 \pm 38.5 _#	481.0 \pm 37.3 _#	480.2 \pm 37.0
		Placebo	498.9 \pm 40.7 _#	502.1 \pm 41.6 _#	504.3 \pm 39.5 _#	503.5 \pm 38.6 _#	497.2 \pm 40.0
Hb (g/dl)	13.8-17.5	MSE	15.0 \pm 0.7	15.0 \pm 0.8 _#	15.2 \pm 0.9	15.2 \pm 1.0	15.1 \pm 1.0
		Placebo	15.4 \pm 0.9	15.6 \pm 0.9 _#	15.7 \pm 0.9 ^{***}	15.6 \pm 0.9 ^{**}	15.5 \pm 0.9
Hematocrit (%)	37.0-53.0	MSE	44.6 \pm 1.9	44.7 \pm 2.2	45.3 \pm 2.7*	45.5 \pm 2.6*	45.3 \pm 2.5*
		Placebo	45.6 \pm 2.5	45.9 \pm 2.7	46.3 \pm 2.5 ^{**}	46.5 \pm 2.4 ^{***}	45.9 \pm 2.4
MCV (fl)	85-100	MSE	93.2 \pm 3.8	93.4 \pm 4.1	93.8 \pm 3.8 ^{**} _#	94.3 \pm 4.1 ^{***}	94.1 \pm 3.8 ^{***}
		Placebo	91.1 \pm 4.0	91.2 \pm 4.3	91.5 \pm 4.1	92.1 \pm 4.1 ^{***}	92.1 \pm 4.1 ^{***}
MCH (pg)	28-34	MSE	30.9 \pm 1.3	31.1 \pm 1.2	31.1 \pm 1.2	31.0 \pm 1.2	31.0 \pm 1.3
		Placebo	30.5 \pm 1.5	30.6 \pm 1.5	30.8 \pm 1.6 ^{***}	30.8 \pm 1.5 ^{**}	30.7 \pm 1.5*
MCHC (%) (%)	31-35	MSE	33.1 \pm 0.6	33.2 \pm 0.6	33.2 \pm 0.7	33.0 \pm 0.7	32.8 \pm 0.7*
		Placebo	33.3 \pm 0.8	33.5 \pm 0.7	33.3 \pm 0.7	33.2 \pm 0.8	33.1 \pm 0.8
Platelet ($\times 10,000/\mu\text{l}$)	13-35	MSE	25.0 \pm 7.3	25.1 \pm 7.0	25.6 \pm 7.9	25.6 \pm 7.2	24.9 \pm 7.7
		Placebo	22.8 \pm 6.6	23.6 \pm 7.6	23.3 \pm 6.8	23.9 \pm 6.8 ^{**}	23.2 \pm 6.4
Blood glucose (mg/dl)	60-110	MSE	100.2 \pm 23.2	103.6 \pm 31.9	104.8 \pm 32.7	107.0 \pm 34.6*	106.2 \pm 31.8*
		Placebo	102.3 \pm 20.4	107.5 \pm 24.2*	107.2 \pm 25.5	104.9 \pm 26.2	107.3 \pm 24.2
HbA1c (%)	4.3-5.8	MSE	5.0 \pm 0.9	5.1 \pm 1.0	5.3 \pm 1.1 ^{***}	5.2 \pm 1.2 ^{**}	5.4 \pm 1.4 ^{**}
		Placebo	4.9 \pm 0.7	5.0 \pm 0.7	5.2 \pm 0.8 ^{***}	5.0 \pm 0.9*	5.1 \pm 0.8 ^{**}
ALT (IU/liter)	10-40	MSE	30.2 \pm 17.2	28.7 \pm 15.0	29.9 \pm 17.2	33.4 \pm 19.1*	29.2 \pm 20.2
		Placebo	28.5 \pm 13.4	28.7 \pm 14.4	29.4 \pm 16.4	32.1 \pm 13.5	28.8 \pm 15.0
AST (IU/liter)	5-45	MSE	38.0 \pm 32.5	37.7 \pm 27.6	38.6 \pm 30.0	37.9 \pm 30.5	36.8 \pm 33.3
		Placebo	39.6 \pm 34.8	42.1 \pm 35.0	43.4 \pm 38.8	42.3 \pm 34.8	40.6 \pm 35.4
ALP (IU/liter)	100-325	MSE	214.2 \pm 52.5	217.4 \pm 58.0	219.0 \pm 60.5	217.5 \pm 52.0	215.5 \pm 52.9 _#
		Placebo	239.4 \pm 53.2	244.5 \pm 59.3	245.3 \pm 58.6	251.2 \pm 72.0*	253.1 \pm 60.8 _#
γ -GTP (IU/liter)	80 \geq	MSE	90.7 \pm 96.6	81.9 \pm 79.3	84.5 \pm 84.0	85.4 \pm 91.3	77.4 \pm 86.3 ^{**}
		Placebo	79.5 \pm 50.4	81.6 \pm 50.0	87.7 \pm 64.0	91.7 \pm 80.8	80.5 \pm 58.6
LDH (IU/liter)	120-240	MSE	187.1 \pm 27.4	188.5 \pm 36.8	188.5 \pm 28.2	205.4 \pm 37.4 ^{***}	187.7 \pm 35.4
		Placebo	191.1 \pm 24.4	195.3 \pm 34.9	196.6 \pm 29.7	203.5 \pm 25.7 ^{**}	198.3 \pm 41.3
Total protein (g/dl)	6.5-8.5	MSE	7.3 \pm 0.3	7.5 \pm 0.4 ^{**}	7.5 \pm 0.4 ^{**}	7.6 \pm 0.4 ^{***}	7.4 \pm 0.5
		Placebo	7.3 \pm 0.3	7.6 \pm 0.3 ^{***}	7.5 \pm 0.3 ^{***}	7.6 \pm 0.3 ^{***}	7.3 \pm 0.3
Albumin (g/dl)	3.8-5.4	MSE	4.6 \pm 0.2	4.5 \pm 0.2	4.6 \pm 0.2	4.7 \pm 0.2*	4.4 \pm 0.2 ^{***}
		Placebo	4.6 \pm 0.2	4.6 \pm 0.2	4.6 \pm 0.2	4.7 \pm 0.2 ^{**}	4.4 \pm 0.2 ^{***}
A/G ratio	1.1-2.0	MSE	1.7 \pm 0.2	1.6 \pm 0.2 ^{***}	1.6 \pm 0.3 ^{**}	1.6 \pm 0.2 ^{**}	1.5 \pm 0.2 ^{***}
		Placebo	1.7 \pm 0.2	1.5 \pm 0.2 ^{***}	1.6 \pm 0.2 ^{**}	1.6 \pm 0.2	1.5 \pm 0.2 ^{***}
Total bilirubin (mg/dl)	0.2-1.0	MSE	0.72 \pm 0.25	0.66 \pm 0.27	0.67 \pm 0.27	0.64 \pm 0.27	0.61 \pm 0.20*
		Placebo	0.74 \pm 0.23	0.70 \pm 0.23	0.74 \pm 0.26	0.62 \pm 0.25 ^{**}	0.75 \pm 0.34
Na (mEq/liter)	135-147	MSE	138.9 \pm 1.5	139.7 \pm 1.7*	139.2 \pm 1.7	139.4 \pm 1.9	139.7 \pm 1.9*
		Placebo	139.3 \pm 1.3	140.2 \pm 1.1 ^{**}	139.2 \pm 1.5	139.6 \pm 1.6	140.1 \pm 1.1 ^{***}
Cl (mEq/liter)	97-107	MSE	102.4 \pm 2.1	102.6 \pm 1.9	102.1 \pm 2.0	101.6 \pm 1.8	101.4 \pm 2.1*
		Placebo	103.1 \pm 2.2	103.0 \pm 1.8	102.4 \pm 2.1	101.9 \pm 1.7 ^{**}	102.2 \pm 1.7*
K (mEq/liter)	3.5-5.0	MSE	4.5 \pm 0.3	4.6 \pm 0.3 _#	4.6 \pm 0.4	5.5 \pm 0.5 ^{***}	4.7 \pm 0.4
		Placebo	4.5 \pm 0.4	4.4 \pm 0.3 _#	4.5 \pm 0.4	5.4 \pm 0.5 ^{***}	4.7 \pm 0.4*
Ca (mg/dl)	8.5-11.0	MSE	9.4 \pm 0.3	9.4 \pm 0.3	9.2 \pm 0.3*	9.4 \pm 0.3	9.3 \pm 0.3
		Placebo	9.3 \pm 0.3	9.4 \pm 0.4	9.2 \pm 0.4	9.2 \pm 0.3	9.4 \pm 0.3
Mg (mg/dl)	1.5-2.8	MSE	2.4 \pm 0.1	2.4 \pm 0.1	2.4 \pm 0.2	2.5 \pm 0.2 ^{**}	2.4 \pm 0.1
		Placebo	2.5 \pm 0.2	2.4 \pm 0.2	2.4 \pm 0.2	2.5 \pm 0.2	2.4 \pm 0.2
Creatinine (mg/dl)	0.8-1.5	MSE	1.04 \pm 0.14	1.03 \pm 0.12	1.05 \pm 0.14	1.06 \pm 0.15	1.03 \pm 0.15
		Placebo	1.07 \pm 0.12	1.07 \pm 0.13	1.10 \pm 0.14	1.09 \pm 0.13	1.05 \pm 0.12
BUN (mg/dl)	7-23	MSE	15.0 \pm 2.9	14.7 \pm 3.4	14.7 \pm 3.0	14.3 \pm 3.3	14.8 \pm 3.2
		Placebo	15.5 \pm 3.0	14.7 \pm 3.5	14.9 \pm 3.7	15.1 \pm 3.6	15.7 \pm 4.0
Uric acid (mg/dl)	2.7-7.5	MSE	6.4 \pm 1.4	6.4 \pm 1.5	6.2 \pm 1.4	6.2 \pm 1.5	6.1 \pm 1.5
		Placebo	7.0 \pm 1.3	6.9 \pm 1.2	6.8 \pm 1.4	6.5 \pm 1.2 ^{***}	6.4 \pm 1.2 ^{**}

Values are means \pm SD. Significantly different from Start: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Significant difference between the 2 groups: # $p < 0.05$, ## $p < 0.01$.

Table 6. Changes in physical findings during the study period.

	Groups	Start	1 month	2 months	3 months	Completion of post-monitoring
Systolic BP (mmHg)	MSE	133.4±19.4	131.5±14.5	135.8±16.0	135.5±14.9	141.6±16.9**
	Placebo	132.8±16.5	131.0±15.2	133.9±15.0	133.9±13.3	138.9±13.8*
Diastolic BP (mmHg)	MSE	80.9±9.3	80.6±9.5	81.5±8.4	81.2±7.4	80.8±10.1
	Placebo	83.0±11.3	82.3±11.2	83.6±8.9	82.0±10.9	83.0±11.1
Pulse (beats/min.)	MSE	74.5±8.4	74.9±10.0	73.8±7.8	75.0±6.3	75.2±7.0
	Placebo	77.0±8.8	74.7±7.4	75.1±6.9	74.2±6.7	73.6±7.4
BMI	MSE	24.6±3.1	24.7±3.1	24.7±3.0	24.9±2.9***	24.7±2.9
	Placebo	25.9±3.9	26.0±3.9	26.3±3.8***	26.4±3.8***	26.3±3.8***

Values are means±SD. Significantly different from Start: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

However, there was no significant difference between Placebo group and MSE group, and the amount of change was extremely small, 2–5 mg/dl.

The arteriosclerosis index significantly decreased in both groups throughout the entire intake period and at the completion of post-monitoring. The value was also than for the significantly lower for the MSE group compared with Placebo group throughout the entire intake period and at the completion of post-monitoring. There was no coaction between the food groups and intake period, but a main effect was noted.

Although there were no significant changes in the β -lipoprotein level from the start of intake in the MSE group, the value significantly decreased after 2 and 3 months of intake in the MSE group. Apolipoprotein AI significantly increased from the start of intake in both groups during the entire intake period and at the completion of post-monitoring, but there were no differences between the groups. Apolipoprotein B did not significantly change from the start of intake in either group, but a coaction was evident between the food groups and intake period.

Blood cells and biochemical examination of blood

Table 5 shows the changes in blood cells and results for biochemical examination of the blood.

Significant changes were common to both groups in fasting blood glucose, HbA1c, LDH, total protein, albumin, A/G ratio, Na, K, hematocrit, and MCV from the start of intake throughout the intake period. Increases in ALT and Mg and decreases in calcium were also seen in the MSE group.

Physical findings

Table 6 shows the changes in physical findings such as blood pressure, pulse rate, and BMI.

Blood pressure significantly increased in both groups at the completion of post-monitoring com-

pared with that at the start of intake, but the changes were within the normal range. BMI significantly increased 2 and 3 months after the start of intake and at the completion of post-monitoring compared with that at the start of intake in the Placebo group. It also significantly increased 3 months after the start of intake in the MSE group. However, there were no significant differences between the groups throughout the study period.

Examination/Interview

During the study there were no symptoms seen such as dry cough, skin symptoms, taste abnormalities, headache, lightheadedness, or dizziness. The results of physician interviews revealed no abnormal findings in objective and subjective symptoms considered due to the test foods throughout the intake period.

Discussion

Total cholesterol and LDL cholesterol in the MSE group significantly decreased from the start of intake throughout the entire 3-month intake period. The values were also significantly lower in the MSE group than in the Placebo group. The differences between the 2 groups were 8–12 mg/dl for total cholesterol and 11–13 mg/dl for LDL cholesterol. A serum cholesterol lowering effect due to phytosterolester in MSE was thus shown. A comparison of this effect in all subjects and in subjects with a total cholesterol of ≥ 220 mg/dl, who were at risk for arteriosclerosis, revealed that the effect was greater in subjects with a total cholesterol of ≥ 220 mg/dl. This serum cholesterol-lowering effect was also supported by the decreases seen in the arteriosclerosis index and β -lipoprotein in the MSE group.

Comparison of the cholesterol-lowering effect in the present study with the results of studies of intake

of margarine containing phytosterolester in Japan and Europe reveals that the differences in total cholesterol and LDL cholesterol (12.0 and 12.1 mg/dl, respectively) between the MSE group and Placebo group after 1 month of intake in all volunteers in the present study compare favorably with the results of Honma *et al.* [5] (12.4 and 9.1 mg/dl, respectively, after 21 days of intake) and the results of Hendriks *et al.* [6] (10.1 and 7.7 mg/dl, respectively, after 25 days of intake).

Throughout the study period there was no significant difference in the amount of cholesterol consumed between the Placebo group and MSE group, and so it is clear that there was a serum cholesterol-lowering effect of phytosterolester contained in the MSE. Although the value decreased from the start of intake during the intake period in both groups and the difference was significant in the MSE group after 2 months of intake, the serum cholesterol lowering effect in the MSE group was already seen after 1 month of intake. Accordingly, the drop in the amount of cholesterol intake at the second month of intake in the MSE group had no influence on this effect. Also, an examination of the relationship between the amount of change in cholesterol intake and amount of change in total cholesterol at 1, 2, and 3 months of intake in both groups revealed no correlation (data not shown).

Significant changes were sporadically seen in hematological testing other than blood lipids, but in both the MSE group and Placebo group the same changes were seen or, except for K after 3 months of intake, the changes were within the standard range. This increase in K after 3 months of intake was attributed to the greater than normal length of time till serum separation, and was not considered medically problematic. In terms of the evaluation of objective and subjective symptoms by physician interview, there were no adverse events that were likely to become especially problematic as per other studies using phytosterol and phytosterolester [5,7–9], and thus the long-term intake of the present MSE was considered highly safe.

Thus, we showed that mayonnaise containing phytosterolester taken for 3 months lowered serum blood cholesterol more than conventional mayonnaise. The safety of long-term consumption was also demonstrated. Mayonnaise is now a commonly used food of the Japanese, and so the introduction of

mayonnaise containing phytosterolester into daily life would be extremely beneficial from the viewpoint of maintaining and promoting good health.

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