[Original Article]

Studies to Confirm the Safety and Serum Low-Density Lipoprotein (LDL) Cholesterol Level-Lowering Effect of a Low-Calorie Mayonnaise Supplemented with Phytosterol Esters

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[ABSTRACT]

To confirm the safety and efficacy of a low-calorie mayonnaise supplemented with phytosterol esters (PEM) at a daily consumption dose of 884 mg given for 12 weeks with the goal of lowering serum low-density lipoprotein (LDL) cholesterol levels, we conducted a double-blind comparative study in healthy adults with marginally high serum levels of LDL cholesterol and total cholesterol using a mayonnaise without phytosterol esters supplementation (CM) as the control food. Serum LDL cholesterol levels decreased significantly in the PEM group as compared with the CM group during the 12-week intervention period (136.3 ± 16.4 vs. 145.0 ± 19.3 mg/dL at Week 8 ($P < 0.05$) and 135.0 ± 17.4 mg/dL vs. 144.9 ± 17.5 mg/dL at Week 12; $P < 0.05$). No observable adverse effects were observed due to the ingestion of PEM in this study. The safety of PEM was again confirmed by an additional clinical study in which healthy adult subjects ingested a 3-fold greater amount of PEM (2,652 mg of phytosterol esters as a daily dose) for 4 weeks. Thus, we concluded that PEM is effective in lowering serum levels of LDL cholesterol and is safe and well tolerated without any clinical problems.

[Key words]

phytosterol ester, mayonnaise, LDL-cholesterol, safety

INTRODUCTION

Recent cause-of-death population statistics in Japan reveal that arteriosclerotic disorders, especially cardiovascular diseases (e.g., myocardial infarction) and cerebrovascular diseases (e.g., cerebral infarction and stroke) have become major causes of death, along with malignant neoplasms¹⁾. According to a report compiled by the Japan Society of Ningen Dock and the All Japan Hospital Association, hypercholesterolemia was the most frequent (26.4%) abnormality detected among persons who underwent medical checkups in 2008²⁾. There has also been a substantial increase in the number of people with abnormal lipid metabolism, which eventually results in hypercholesterolemia or hyperlipidemia. Because persons with hyperlipidemia are at high risk for arteriosclerosis (potentially causing myocardial infarction and/or cerebral infarction³⁾), there is increasing public awareness of the threat of hyperlipidemia as well as related metabolic abnormalities (e.g., obesity and metabolic syndrome).

To prevent the development of hyperlipidemia, serum cholesterol levels should be kept within the normal range by healthy living, although this can be difficult. One potential approach to this goal involves the supplementary use of diet foods containing sufficient amounts of phytosterol esters, which are known to be active in lowering serum cholesterol levels^{4,5)}. The serum level of total cholesterol has long been used as the major criterion for evaluating the anti-

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hypercholesterolemic effect of medical drugs and health foods. However, guidelines published by the Japan Atherosclerosis Society in 2007 indicate that the increase in relative risk for development of coronary heart disease is correlated more closely with the serum level of low-density lipoprotein (LDL) cholesterol than that of total cholesterol. In the present study, therefore, we chose the LDL cholesterol level as the primary criterion for efficacy evaluation; the serum total cholesterol level was retained as a major efficacy evaluation criterion after LDL cholesterol.

Phytosterol esters, in which a phytosterol molecule binds to a fatty acid molecule to form an ester bond, are contained in a wide variety of natural foods: tiny amounts are present in grains and vegetables, whereas greater amounts are found in rapeseeds, soybeans, broccoli, and bran^{6,7)}. After oral intake of phytosterol esters, the ester bond is cleaved to generate free phytosterol in the intestine. There, cholesterol exists in a form of bile acid micelles that permit intestinal absorption. Orally administered phytosterol, whose structure is similar to that of cholesterol, has been shown to be effective in competitively inhibiting the micellization of cholesterol and results in accelerated excretion of unmicellized cholesterol in the feces⁵⁾. Therefore, it is reasonable to consider that oral phytosterol or its ester could lower serum cholesterol levels by reducing the amount of cholesterol absorbed in the intestinal tract^{8,9)}.

Recently, we developed a low-calorie mayonnaise (hereafter referred to as phytosterol ester mayonnaise, abbreviated PEM) supplemented with phytosterol esters (sterol composition of campesterol : stigmasterol : beta-sitosterol in a 1 : 1 : 2, molar ratio; 884 mg per 15 g mayonnaise) using an advanced emulsifying technique. In Japan, many different types of foods that contain emulsified phytosterol supplements or are emulsified phytosterol esters (including those approved as foods for specified health use FOSHU) have been shown to possess an appreciable anti-hypercholesterolemic effect. Although the inhibition of cholesterol micellization has been reported as a potential mechanism of action of this beneficial effect⁵⁾, the relationship between the emulsification state of such foods and their precise anti-hypercholesterolemic effect remains to be investigated.

We thus sought to confirm the efficacy of this low-calorie mayonnaise (PEM), which contains a basal amount of phytosterol esters corresponding to a daily phytosterol ester intake dose of 884 mg, in lowering serum levels of LDL cholesterol and total cholesterol. We conducted a randomized, double-blind, parallel-group clinical study (hereinafter referred

to as the Efficacy Study) using a low-calorie mayonnaise containing only endogenous phytosterol esters, corresponding to a daily phytosterol ester dose of 32 mg, as control mayonnaise (CM). The study subjects were healthy adults with marginally high serum levels of LDL cholesterol and total cholesterol. The safety evaluation of PEM was performed via this Efficacy Study and also by an additional clinical study (hereinafter referred to as the Excessive-intake Safety Study), in which three times as much PEM was ingested.

MATERIALS AND METHODS

Ethics

Both the Efficacy Study and Excessive-intake Safety Study were conducted in accordance with the ethical principles stated in the Declaration of Helsinki¹⁰⁾ and with the Ethical Guidelines for Epidemiological Research¹¹⁾. Approval was received from each study site (or from the research ethics committee commissioned by each study site) and written informed consent was obtained from all subjects. The excessive-intake safety study was approved by the Ethical committee of the Yukokai Medical Organization and the efficacy study approved by this Ethical Committee and the Ethical Committee of the Soujinkai Mareesia Garden Clinic.

Efficacy Study

This study was designed as a randomized, double-blind, controlled parallel-group study.

Subjects: Subjects (men and women aged 20–64 years) who had serum levels of 120–180 mg/dL low-density lipoprotein (LDL) cholesterol and 200–240 mg/dL total cholesterol within the screening period were selected as subjects, based on a notification regarding application for review of FOSHU issued by the regulatory authorities¹²⁾. The major exclusion criterion was the regular use of medical drugs or specially designed health foods that can affect serum lipid concentrations and lipid metabolism. All eligible subjects ($n = 102$) were randomly allocated to the PEM or CM group. The inter-group differences were minimized in terms of gender, age, and baseline serum levels of LDL cholesterol and total cholesterol.

Study foods and intake method: PEM is made by emulsification with vegetable oil, egg, vinegar, plant sterol-ester, viscosity improver, emulsifier, salt, and other seasonings. The nutritional composition of PEM and CM containing daily doses of 884 and 32 mg of phytosterol esters, respectively, in 15 g of study foods are shown in Table 1. One pack (15 g) of the allocated study food was ingested daily as

Table 1 Nutritional composition of the two study foods used in the present two studies (per pack, 15 g)*

Constituents	PEM ^{a)}	CM ^{b)}
Energy (kcal)	45	45
Protein (g)	0.38	0.38
Fat (g)	4.5	4.6
Carbohydrates (g)	0.59	0.54
Sodium (mg)	250	240
Phytosterol esters (mg)	884	32

*Daily intake dose

^{a)} PEM (Phytosterol ester-supplemented mayonnaise)^{b)} CM (Control mayonnaise)

The amount of phytosterol ester is converted into that of free sterol.

part of a meal for 12 weeks. No restrictions were placed on the time of study food intake, but the subjects were asked to record the time and amount of study food ingested in a study diary.

Study schedule and examinations: Body weight, physiological examinations (systolic blood pressure, diastolic blood pressure and pulse rate), and laboratory tests (hematology, blood chemistry and urinalysis) were performed twice during the pre-intervention period (baseline), once every 4 weeks during the intervention period (week 4, 8, and 12), and 4 weeks after the completion of intake (post-week 4). In addition, subjects were instructed to maintain a study diary recording the content of every meal and the extent of exercise measured with a pedometer for the 3 days prior to every examination day and not to change (as much as possible) their daily lifestyle with regard to meals and exercise habits throughout the study period. Moreover, serum levels of beta-sitosterol, campesterol, stigmasterol, beta-carotene, vitamin A, and vitamin E were measured on one day within the pre-intervention period and at week 12.

Methods for evaluation of efficacy and safety: The primary efficacy criteria were serum levels of LDL cholesterol and total cholesterol; secondary efficacy criteria were serum levels of high-density lipoprotein (HDL) cholesterol, remnant-like particle (RLP) cholesterol, triglyceride (TG), apolipoproteins (Apo) B, Apo A-I, Apo C-III, Apo E, LDL/HDL ratio, and Apo B/Apo A-I ratio. Safety evaluations were performed on the basis of data describing subjective symptoms, laboratory tests (hematology, conventional blood chemistry, urinalysis, additional blood chemistry tests), physiological examinations, and serum levels of phytosterols and vitamins. Means values yielded by examinations conducted on two different days during the pre-intervention period were used as baseline values. All statistical tests were two-tailed and level of significance 5%. Numerical values were expressed as mean $\pm$

standard deviation (SD). The unpaired *t*-test was used for an inter-group comparison between the two study groups. Analysis of variance by repetitive determination was performed for “dietary and exercise parameters” and “lipid metabolism parameters” and these data were further analyzed by repeated-measures ANOVA using the Bonferroni correction method.

On the other hand, the unpaired *t*-test was used for an inter-group comparison between the two study groups, and a paired *t*-test was used for an intra-group comparison with the baseline of safety evaluation.

Excessive-intake Safety Study

This study was designed as a randomized, double-blind, controlled parallel-group study. Thirty subjects (16 male/14 female) aged 20–64 years with serum LDL-cholesterol levels ranging from 70 to 180 mg/dL were assigned to either PEM or CM group. Subjects were assigned such that each study group was similar with regard to sex, mean age, and mean serum LDL cholesterol level. All subjects were asked to ingest three packs (45 g in total) of either PEM or CM allotted study food daily with meals for 4 weeks. The daily consumption dose of phytosterol esters for individuals in the PEM group was 2,652 mg, and for individuals in the CM group was 96 mg. Subjective symptoms were self-recorded, and laboratory tests (hematology, blood chemistry, urinalysis), weight, and determination of serum levels of phytosterols and vitamins were performed at the indicated assessment time points.

RESULTS

Efficacy Study

Baseline subject data: Of 102 subjects (PEM group: 51 subjects [30M/21F]; CM group: 51 subjects [34M/17F]) enrolled, one participant in each study group withdrew from the study within 4 weeks after starting of the intervention. Of the remaining 100 subjects, 20 were excluded from the efficacy evaluation due to the presence of an in compliant lifestyle (14 subjects) or a menstrual period overlapping with two or more laboratory examination days during the pre-intervention and/or intervention period (6 subjects); menstruation is known to affect serum cholesterol levels¹³⁾. The study food compliance rate for each of the remaining 80 individual subjects was $\geq 80\%$. Baseline characteristics of the 80 eligible subjects are shown in Table 2. The PEM group comprised 45 subjects (28 men, 17 women), and the CM group 35 subjects (25 men, 10 women). Average ages were 48.8 and 48.9 years, respectively,

and mean weights 66.2 and 65.4 kg. Mean serum levels of LDL cholesterol at baseline for PEM and CM groups were 145.1 and 146.2 mg/dL, respectively; thus, there was no significant difference between the two study groups. Moreover, the serum level of total cholesterol did not differ significantly between the two study groups.

Diet and exercise survey: Mean changes in dietary habitations, as represented by the ingestion of several major nutritional components, and exercise habits, as represented by walking exercise, throughout the pre-intervention and intervention periods are shown in Table 3 for both study groups. There were no significant differences in any of the dietary or exercise parameters between the two study groups.

However, some significant changes from baseline in the intake quantities of energy, lipids, and sodium as well as exercise level were observed at several assessment time points during the intervention period.

Efficacy evaluation: Data describing lipid metabolism profiles in terms of serum levels of various lipid metabolites, including LDL cholesterol and total cholesterol, for the two study groups over the whole study period are shown in Table 4. Changes in serum levels of LDL cholesterol are also illustrated in Fig. 1. The mean serum LDL cholesterol levels for the CM group were 146.2 ± 13.7 mg/dL at baseline; 145.6 ± 18.9 mg/dL at Week 4; 145.0 ± 19.3 mg/dL at Week 8; 144.9 ± 17.5 mg/dL at Week 12. The LDL cholesterol levels of this group were thus

Table 2 Baseline characteristics of subject eligibility for efficacy evaluation in the two study groups

Parameters	Unit	PEM group	CM group	P-value
Sex (male : female)		28 : 17	25 : 10	0.267 ^{a)}
Age	(yr)	48.8 $\pm$ 9.1	48.9 $\pm$ 8.3	0.995 ^{b)}
Height	(cm)	165.6 $\pm$ 9.2	168.1 $\pm$ 10.5	0.268 ^{b)}
Body weight	(kg)	66.2 $\pm$ 11.8	65.4 $\pm$ 12.2	0.767 ^{b)}
BMI	(kg/m ²)	24.0 $\pm$ 2.7	23.0 $\pm$ 2.7	0.112 ^{b)}
Systolic blood pressure	(mmHg)	120.1 $\pm$ 13.6	120.9 $\pm$ 13.1	0.783 ^{b)}
Diastolic blood pressure	(mmHg)	72.6 $\pm$ 11.3	74.5 $\pm$ 9.4	0.427 ^{b)}
Pulse rate	(bpm)	74.5 $\pm$ 8.7	73.9 $\pm$ 6.3	0.753 ^{b)}
LDL-cholesterol	(mg/dL)	145.1 $\pm$ 14.2	146.2 $\pm$ 13.7	0.719 ^{b)}
Total cholesterol	(mg/dL)	228.4 $\pm$ 20.1	229.7 $\pm$ 19.7	0.786 ^{b)}

PEM group: n=45, CM group: n=35

Numbers are mean $\pm$ standard deviation.

^{a)} Fisher's exact probability test.

^{b)} Unpaired *t* test

Table 3 Changes in mean values of dietary and exercise parameters in the two study groups during the study period

Parameters		Group ^{a)}	Baseline	Week 4	Week 8	Week 12	Post-week 4
Energy	(kcal)	PEM	1,782.5 $\pm$ 394.3 ^{b)}	1,707.3 $\pm$ 329.5	1,738.3 $\pm$ 333.3	1,738.1 $\pm$ 291.2	1,811.3 $\pm$ 330.2
		CM	1,788.7 $\pm$ 434.5	1,716.9 $\pm$ 384.8	1,713.0 $\pm$ 417.7	1,653.7 $\pm$ 410.1*	1,678.9 $\pm$ 470.8
Protein	(g)	PEM	63.85 $\pm$ 13.09	65.35 $\pm$ 12.74	65.76 $\pm$ 12.26	64.93 $\pm$ 12.77	67.36 $\pm$ 14.94
		CM	66.39 $\pm$ 16.47	65.81 $\pm$ 12.90	65.85 $\pm$ 15.83	64.52 $\pm$ 14.40	64.09 $\pm$ 17.00
Lipids	(g)	PEM	55.15 $\pm$ 18.88	49.64 $\pm$ 14.69	49.80 $\pm$ 13.49	49.56 $\pm$ 10.83	55.53 $\pm$ 16.36
		CM	56.64 $\pm$ 19.35	54.41 $\pm$ 17.71	52.52 $\pm$ 17.84	47.27 $\pm$ 19.81*	50.19 $\pm$ 18.42
Carbohydrates	(g)	PEM	248.19 $\pm$ 60.20	240.99 $\pm$ 53.95	247.62 $\pm$ 52.03	249.26 $\pm$ 55.37	250.28 $\pm$ 53.65
		CM	243.65 $\pm$ 71.32	232.91 $\pm$ 57.68	235.90 $\pm$ 57.20	234.33 $\pm$ 57.49	234.21 $\pm$ 69.71
Cholesterol	(mg)	PEM	282.8 $\pm$ 112.1	265.6 $\pm$ 107.0	292.0 $\pm$ 103.8	268.2 $\pm$ 114.4	293.5 $\pm$ 123.3
		CM	300.0 $\pm$ 158.0	257.6 $\pm$ 96.7	273.7 $\pm$ 154.8	282.7 $\pm$ 128.7	274.4 $\pm$ 124.7
Na	(mg)	PEM	3,578.5 $\pm$ 1,054.7	3,387.1 $\pm$ 766.5	3,554.7 $\pm$ 918.6	3,456.1 $\pm$ 748.6	3,771.6 $\pm$ 1,131.8
		CM	3,918.8 $\pm$ 1,070.2	3,555.8 $\pm$ 822.9	3,468.9 $\pm$ 822.9	3,440.2 $\pm$ 882.6*	3,568.5 $\pm$ 1,111.6
Walking exercise	(number of steps)	PEM	7,049.3 $\pm$ 3,318.1	7,826.7 $\pm$ 3,968.5	7,616.9 $\pm$ 3,439.2	7,960.7 $\pm$ 3,060.7	8,017.8 $\pm$ 3,289.4
		CM	8,446.1 $\pm$ 3,987.7	8,218.6 $\pm$ 2,853.2	7,781.6 $\pm$ 2,607.9	8,686.2 $\pm$ 2,988.8	7,978.5 $\pm$ 3,419.2

^{a)} PEM group: n=45, CM group: n=35

^{b)} Numbers are mean $\pm$ standard deviation.

*: $P < 0.05$ (repeated measurements ANOVA with the Bonferroni correction method, against baseline)

Table 4 Changes in mean values of lipid metabolism parameters in the two study groups during the study period

Parameters	Group ^{a)}	Baseline	Week 4	Week 8	Week 12	Post-week 4
LDL-cholesterol (mg/dL)	PEM	145.1 ± 14.2 ^{b)}	138.0 ± 17.7**	136.3 ± 16.4**	135.6 ± 17.4**	141.0 ± 22.0
	CM	146.2 ± 13.7	145.6 ± 18.9	145.0 ± 19.3]#	144.9 ± 17.5]#	140.7 ± 14.8
Total cholesterol (mg/dL)	PEM	228.4 ± 20.1	221.3 ± 22.4**	218.5 ± 22.9**	215.3 ± 23.5**	223.3 ± 26.7
	CM	229.7 ± 19.7	228.8 ± 23.8	229.7 ± 23.8]#	225.5 ± 20.2]#	222.0 ± 19.3
HDL-cholesterol (mg/dL)	PEM	59.3 ± 15.0	59.8 ± 15.7	58.4 ± 14.3	58.1 ± 15.3	57.1 ± 15.4
	CM	59.9 ± 12.6	59.5 ± 12.2	59.5 ± 11.9	57.6 ± 10.9	55.1 ± 11.1
RLP-cholesterol (mg/dL)	PEM	5.15 ± 2.16	4.53 ± 2.10	4.89 ± 1.93	4.93 ± 2.01	4.93 ± 2.42
	CM	4.92 ± 1.60	4.76 ± 2.33	5.19 ± 2.49	4.93 ± 1.97	4.69 ± 1.56
TG (mg/dL)	PEM	110.7 ± 56.8	109.0 ± 54.7	115.5 ± 52.8	108.0 ± 56.0	104.6 ± 59.6
	CM	106.0 ± 43.5	108.1 ± 63.0	116.9 ± 57.1	107.4 ± 51.8	105.5 ± 43.8
ApoA-I (mg/dL)	PEM	146.2 ± 23.1	149.7 ± 24.2	148.2 ± 22.7	147.1 ± 24.5	144.8 ± 26.0
	CM	147.7 ± 21.5	151.3 ± 22.4	151.8 ± 23.2	148.6 ± 21.0	142.0 ± 21.0
ApoB (mg/dL)	PEM	111.9 ± 13.0	106.8 ± 15.5**	106.0 ± 14.8**	107.3 ± 16.2**	110.8 ± 16.1
	CM	112.3 ± 12.4	111.2 ± 14.7	111.3 ± 14.8	112.9 ± 13.4	110.7 ± 13.3
ApoC-III (mg/dL)	PEM	9.32 ± 2.75	9.75 ± 2.66	9.91 ± 3.02	9.31 ± 2.78	9.34 ± 3.06
	CM	9.27 ± 2.47	9.82 ± 3.06	9.80 ± 2.77	9.43 ± 2.49	9.20 ± 2.85
ApoE (mg/dL)	PEM	4.18 ± 0.78	4.13 ± 0.85	4.16 ± 0.86	4.06 ± 0.84	4.14 ± 0.91
	CM	4.25 ± 0.85	4.21 ± 1.06	4.23 ± 1.07	4.21 ± 1.00	4.15 ± 0.89
LDL-cholesterol/ HDL-cholesterol	PEM	2.6 ± 0.6	2.5 ± 0.7	2.5 ± 0.6**	2.5 ± 0.6	2.6 ± 0.7
	CM	2.5 ± 0.5	2.5 ± 0.6	2.5 ± 0.6	2.6 ± 0.7	2.7 ± 0.6
ApoB/ApoA-I	PEM	0.79 ± 0.17	0.73 ± 0.17**	0.73 ± 0.16**	0.75 ± 0.17*	0.79 ± 0.18
	CM	0.78 ± 0.15	0.75 ± 0.14	0.75 ± 0.16	0.78 ± 0.17	0.80 ± 0.18

a) PEM group: n=45 (except n=44 at Week 4), CM group: n=35

b) Numbers are mean ± standard deviation.

#: $P < 0.05$ (two sample *t* test, inter-group comparison)

**: $P < 0.01$, *: $P < 0.05$ (repeated measurements ANOVA with the Bonferroni correction method, against baseline)

Fig. 1 Time-course of LDL cholesterol levels over the whole study period as expressed by Mean ± standard error

#: $p < 0.05$ (two sample *t* test, inter-group comparison)

**: $P < 0.01$, *: $P < 0.05$ (repeated measurements ANOVA with the Bonferroni correction method, against baseline)

virtually constant throughout the intervention period. In contrast, the serum levels of LDL cholesterol in the PEM group gradually decreased from 145.1 ± 14.2 mg/dL at baseline to 138.0 ± 17.7 mg/dL at Week 4, 136.3 ± 16.4 mg/dL at Week 8, and 135.6 ± 17.4 mg/dL at Week 12. The values for the PEM

group at Weeks 8 and 12 were significantly lower ($P < 0.05$ each) than the corresponding values for the CM group. The changes in the serum level of LDL cholesterol from baseline at Weeks 8 and 12 for the PEM group were -8.8 and -9.5 mg/dL, respectively; both of these concentrations were significantly greater ($P < 0.05$ each) than the values determined for the CM group (-1.2 and -1.3 mg/dL, respectively).

The serum levels of total cholesterol in the CM group were 229.7 ± 19.7 mg/dL at baseline; 228.8 ± 23.8 mg/dL at Week 4, 229.7 ± 23.8 mg/dL at Week 8, and 225.5 ± 20.2 mg/dL at Week 12. These results imply that the serum cholesterol level was almost constant during the 12-week intervention period. Again in contrast, the serum levels of total cholesterol in the PEM group changed from 228.4 ± 20.1 mg/dL at baseline to 221.3 ± 22.4 mg/dL at Week 4, to 218.5 ± 22.9 mg/dL at Week 8 and 215.3 ± 23.5 mg/dL at Week 12. The levels at Week 8 and Week 12 in the PEM group were significantly ($P < 0.05$ at both time points assessed) lower than the corresponding values for the CM group.

In addition, analysis of variance by repetitive determination

for both serum levels of LDL cholesterol and total cholesterol at baseline, Week 4, Week 8, and Week 12 in the PEM group yielded a significant difference ($P < 0.01$). Repeated measurements ANOVA with Bonferroni correction also revealed a significant difference ($P < 0.01$). In contrast, the analysis of variance by repetitive determination for the CM group did not yield any significant difference.

Pearson's correlation coefficient testing, performed to investigate a possible relationship between the serum levels of LDL cholesterol at baseline and at each of the three assessment points during the intervention period (i.e., Weeks 4, 8, and 12) in both the PEM and CM group, also revealed interesting results. As illustrated in Fig. 2, the correlation coefficient values (r) for the PEM group were 0.620 ($P < 0.001$) at Week 12. Significant correlations also seen at Week 4 (0.811, $P < 0.001$) and at Week 8 (0.676, $P < 0.001$) (data not shown). Those for the CM group were: 0.620 ($P < 0.001$), 0.662 ($P < 0.001$) and 0.558 ($P < 0.01$), respectively.

Serum levels of Apo B in the PEM group at the three assessment time points during the intervention period (106.8 ± 15.5 mg/dL at Week 4, 106.0 ± 14.8 mg/dL at Week 8, and 107.3 ± 16.2 mg/dL at Week 12) were all significantly lower ($P < 0.01$ each) than those at baseline (111.9 ± 13.0 mg/dL). In contrast, virtually constant values for serum Apo B levels were obtained for the CM group (112.3 ± 12.4 mg/dL at baseline, 111.2 ± 14.7 mg/dL at Week 4, 111.3 ± 14.8 mg/dL at Week 8,

and 112.9 ± 13.4 mg/dL at Week 12). No significant changes in the serum levels of HDL cholesterol, RLP cholesterol, triglyceride, ApoA-I, Apo C, and Apo E were detected in the PEM group.

Safety evaluation: Mean values for each parameter of blood chemistry, hematology, and urinalysis for the two study groups measured at baseline and at each assessment time point throughout the study period are shown in Tables 5-1 and 5-2.

Fig. 2 Correlation of LDL cholesterol levels at baseline with those at Week 12 in both the PEM group and CM group. The values for the 80 individual subjects in both study group at baseline and those Week 12 are plotted on the x-axis and the y-axis, respectively, to present linear lines by the least squares method. Note: R values and P values in the figure were determined by Pearson's correlation coefficient test.

— Linear line for PEM group
 - - - Linear line for CM group

Table 5-1 Changes in the mean values of hematological parameters in the two study groups during the study period (1)

Parameters	Unit	Group	Baseline	Week 4	Week 8	Week 12	Post-week 4
WBC	(/ μ L)	PEM	$5,845.6 \pm 1,472.0$	$5,738.6 \pm 1,407.7$	$5,704.4 \pm 1,373.3$	$5,402.2 \pm 1,541.9^{**}$	$5,722.2 \pm 1,528.9$
		CM	$5,907.1 \pm 1,548.1$	$5,931.4 \pm 1,728.4$	$5,617.1 \pm 1,526.7$	$5,591.4 \pm 1,525.7^{**}$	$5,945.7 \pm 1,633.4$
RBC	(10,000/ μ L)	PEM	479.6 ± 40.9	479.9 ± 42.6	482.5 ± 46.0	476.9 ± 44.0	$466.5 \pm 40.0^{**}$
		CM	472.8 ± 37.2	473.5 ± 41.5	474.8 ± 34.2	476.7 ± 38.1	$463.6 \pm 36.4^{**}$
Hb	(g/dL)	PEM	14.32 ± 1.48	14.27 ± 1.55	14.24 ± 1.56	$14.04 \pm 1.55^{**}$	$13.91 \pm 1.56^{**}$
		CM	14.17 ± 1.27	14.18 ± 1.42	14.02 ± 1.26	14.01 ± 1.32	$13.78 \pm 1.35^{**}$
Ht	(%)	PEM	45.36 ± 3.77	$44.90 \pm 4.00^{*}$	45.12 ± 3.96	$44.35 \pm 4.07^{**}$	$44.31 \pm 4.13^{**}$
		CM	45.25 ± 3.50	$44.66 \pm 3.84^{*}$	44.84 ± 3.35	$44.71 \pm 3.47^{*}$	$44.29 \pm 3.75^{**}$
PLT	(10,000/ μ L)	PEM	26.67 ± 5.00	$25.62 \pm 5.08^{**}$	26.91 ± 4.96	26.23 ± 5.20	$25.46 \pm 4.95^{**}$
		CM	26.21 ± 4.31	26.36 ± 4.28	26.66 ± 5.07	$26.99 \pm 5.02^{*}$	26.01 ± 4.46
MCV	(fL)	PEM	94.74 ± 5.10	$93.70 \pm 5.13^{**}$	$93.72 \pm 4.99^{**}$	$93.13 \pm 4.95^{**}$	95.05 ± 5.07
		CM	95.89 ± 5.64	$94.48 \pm 5.34^{**}$	$94.59 \pm 5.66^{**}$	$93.99 \pm 5.96^{**}$	95.68 ± 6.02
MCH	(pg)	PEM	29.87 ± 1.98	$29.74 \pm 2.02^{*}$	$29.53 \pm 1.91^{**}$	$29.44 \pm 1.92^{**}$	29.80 ± 1.98
		CM	30.02 ± 2.15	29.99 ± 2.29	$29.57 \pm 2.26^{**}$	$29.44 \pm 2.33^{**}$	29.76 ± 2.29
MCHC	(%)	PEM	31.52 ± 0.87	$31.72 \pm 0.89^{*}$	31.50 ± 0.92	31.60 ± 0.88	$31.34 \pm 0.86^{*}$
		CM	31.29 ± 0.79	$31.72 \pm 1.04^{**}$	31.23 ± 0.94	31.29 ± 0.97	$31.09 \pm 0.85^{*}$

PEM group: $n = 45$ (except $n = 44$ at Week 4), CM group: $n = 35$

Numbers are mean $\pm$ standard deviation.

** : $P < 0.01$, * : $P < 0.05$ (paired t test, against baseline)

Several parameters exhibited mean changes from baseline that transiently reached a statistically significant level. However, all such changes were modest and remained within the normal range, leading the Medical Investigator to consider them as physiological variations without any clinical significance. A statistically significant inter-group difference was observed in the serum level of Urea nitrogen (BUN) at Week 8, although it was not associated with any clinical conditions. No individual

subjects in either the PEM or CM group demonstrated any laboratory abnormalities of clinical significance.

As shown in Table 6, no clinically significant mean changes in body weight, systolic blood pressure, diastolic blood pressure, or pulse rate were observed in either study group. No individual subjects in either study group were considered by the Medical Investigator to have any clinically significant abnormalities. Of the 102 Intend-To-Treat subjects, 17 (33.3%)

Table 5-2 Changes in the mean values of blood chemistry parameters in the two study groups during the study period (2)

Parameters	Unit	Group	Baseline	Week 4	Week 8	Week 12	Post-week 4
AST	(U/L)	PEM	20.6±4.8	20.3±5.6	19.9±4.7	19.8±5.1*	19.1±5.3**
		CM	20.7±5.0	20.5±5.3	21.3±5.8	19.5±4.5*	19.7±5.2
ALT	(U/L)	PEM	21.1±10.7	20.7±11.0	19.7±9.7	19.4±9.6*	19.9±12.3
		CM	21.1±9.9	21.8±11.9	22.3±13.4	19.5±10.4	20.3±13.1
LDH	(U/L)	PEM	172.0±24.8	169.6±28.5	172.7±27.6	171.7±28.1	168.5±28.0
		CM	170.6±22.3	170.4±22.3	170.2±20.3	168.2±23.2	163.8±24.3*
ALP	(U/L)	PEM	209.5±45.4	209.2±57.2	204.7±52.4	201.7±44.5**	197.2±44.5**
		CM	211.1±55.2	208.9±58.3	206.6±58.1	201.3±55.6**	201.6±53.3*
γ-GTP	(U/L)	PEM	31.4±22.6	34.3±30.9	30.9±23.1	30.1±22.5	32.3±25.2
		CM	29.2±17.8	28.6±19.3	27.6±15.3	25.5±12.0*	28.2±17.5
T-Bil	(mg/dL)	PEM	0.71±0.28	0.69±0.26	0.74±0.31	0.69±0.30	0.78±0.29*
		CM	0.68±0.21	0.73±0.31	0.73±0.23	0.70±0.25	0.73±0.31
TP	(g/dL)	PEM	7.30±0.35	7.22±0.38*	7.25±0.41	7.16±0.38**	7.12±0.40**
		CM	7.19±0.36	7.15±0.31	7.18±0.34	7.10±0.41*	7.05±0.41**
Alb	(g/dL)	PEM	4.49±0.18	4.48±0.21	4.53±0.23	4.45±0.24	4.41±0.24**
		CM	4.48±0.21	4.46±0.21	4.50±0.18	4.46±0.27	4.39±0.26*
A/G ratio		PEM	1.62±0.18	1.66±0.19**	1.68±0.21**	1.66±0.19**	1.64±0.19
		CM	1.66±0.18	1.67±0.20	1.70±0.22	1.71±0.25*	1.67±0.20
Cre	(mg/dL)	PEM	0.723±0.111	0.728±0.111	0.730±0.106	0.714±0.112	0.738±0.123*
		CM	0.729±0.123	0.743±0.136*	0.747±0.130*	0.737±0.134	0.741±0.133
BUN	(mg/dL)	PEM	14.11±3.18	14.15±3.09	14.00±2.88	]#	13.88±3.04
		CM	13.26±2.12	13.28±2.42	12.60±2.07		13.63±2.53
UA	(mg/dL)	PEM	5.43±1.23	5.48±1.23	5.49±1.29	5.47±1.22	5.65±1.40*
		CM	5.37±1.07	5.56±1.12*	5.48±1.20	5.57±1.13*	5.55±1.06**
Glc	(mg/dL)	PEM	90.6±8.3	91.4±9.5	90.2±8.0	90.7±7.8	89.7±8.3
		CM	89.2±9.1	90.8±10.0	90.3±8.3	91.2±8.2*	90.7±7.7
FFA	(μEq/L)	PEM	414.4±154.5	428.0±179.2	401.2±210.1	455.2±203.9	476.9±194.3**
		CM	369.9±99.4	443.6±137.5**	417.2±138.1*	456.4±179.1**	407.5±130.0
Phospholipids	(mg/dL)	PEM	229.5±24.0	225.0±23.7**	223.0±23.2**	222.5±25.9**	225.7±25.6
		CM	231.0±19.2	229.7±26.1	229.3±24.0	228.5±22.6	224.4±20.6*
Na	(mEq/L)	PEM	141.1±1.1	141.2±1.3	141.6±1.6*	140.9±1.1	141.1±1.4
		CM	141.3±1.6	141.4±1.8	141.8±2.0*	141.0±1.7	141.0±1.6
K	(mEq/L)	PEM	4.25±0.23	4.22±0.27	4.26±0.26	4.20±0.23	4.25±0.30
		CM	4.30±0.37	4.25±0.33	4.33±0.32	4.31±0.35	4.28±0.34
Cl	(mEq/L)	PEM	104.1±1.5	104.3±2.1	104.7±2.0**	104.6±1.8*	104.4±1.8
		CM	104.6±1.7	104.0±1.9*	104.6±1.9	105.0±2.0	104.9±2.4

PEM group: n=45 (except n=44 at Week 4), CM group: n=35

Numbers are mean±standard deviation.

#: $P<0.05$ (two sample t test, inter-group comparison)

** $P<0.01$, * $P<0.05$ (paired t test, against baseline)

in the PEM group and 20 (39.2%) in the CM group experienced adverse events during the study period. The most common ($\geq 70\%$) adverse event in both study groups was the common cold. Other adverse events experienced by $\geq 5\%$ of the subjects in both study groups included headache, menorrhagia, diarrhea, and abdominal pain. All of these events were mild and transient, and no relationship was found between any of these adverse events and the study food ingested.

Several lipid metabolism-related laboratory tests were performed before the start and after the end of the 12-week intervention, and the mean values were compared with each other. As shown in Table 7, campesterol levels in the PEM group increased significantly; those in the CM group decreased significantly, resulting in a significant inter-group difference

($P < 0.05$). Although vitamin E levels increased significantly in both study groups, the extent of increase was greater for the CM group than the PEM group.

Excessive-intake Safety Study

Fifteen subjects (8 men, 7 women) were evaluated in both the PEM group and the CM group. The mean ages were 43.3 and 41.3 years, with mean body weights of 60.6 and 61.2 kg and mean BMIs (body-mass indices) of 22.0 and 21.5, respectively, at baseline. There were no statistically significant differences in the mean values of any parameters between the two groups. Study food compliance was $\geq 80\%$ in all subjects.

No statistically significant changes from baseline were detected in any of the physiological parameters (data not shown). Five subjects in the PEM group experienced adverse

Table 6 Changes in the mean values of physiological parameters in the two study groups during the study period

Parameters	Unit	Group	Baseline	Week 4	Week 8	Week 12	Post-week 4
Body weight	(kg)	PEM	66.2 $\pm$ 11.8	66.1 $\pm$ 11.8	66.1 $\pm$ 11.8	66.0 $\pm$ 11.9	66.2 $\pm$ 12.1
		CM	65.4 $\pm$ 12.2	65.5 $\pm$ 12.4	65.8 $\pm$ 12.5	65.6 $\pm$ 12.7	65.7 $\pm$ 12.6
BMI	(kg/m ²)	PEM	24.0 $\pm$ 2.7	23.9 $\pm$ 2.8	23.9 $\pm$ 2.8	23.9 $\pm$ 2.8	23.9 $\pm$ 2.8
		CM	23.0 $\pm$ 2.7	23.0 $\pm$ 2.8	23.1 $\pm$ 2.9	23.1 $\pm$ 2.9	23.1 $\pm$ 2.9
Systolic pressure	(mmHg)	PEM	120.1 $\pm$ 13.6	118.5 $\pm$ 13.9	117.2 $\pm$ 16.1*	119.0 $\pm$ 14.5	119.9 $\pm$ 14.8
		CM	120.9 $\pm$ 13.1	120.4 $\pm$ 11.9	118.8 $\pm$ 15.1	117.3 $\pm$ 14.3**	116.6 $\pm$ 11.7**
Diastolic pressure	(mmHg)	PEM	72.6 $\pm$ 11.3	72.4 $\pm$ 12.7	70.1 $\pm$ 12.7*	71.4 $\pm$ 10.8	72.2 $\pm$ 11.9
		CM	74.5 $\pm$ 9.4	74.5 $\pm$ 9.7	73.9 $\pm$ 10.4	73.8 $\pm$ 10.5	72.7 $\pm$ 8.2
Pulse rate	(bpm)	PEM	74.5 $\pm$ 8.7	73.4 $\pm$ 9.9	74.3 $\pm$ 9.5	72.8 $\pm$ 8.0	71.6 $\pm$ 9.8*
		CM	73.9 $\pm$ 6.3	76.0 $\pm$ 9.3	74.2 $\pm$ 8.4	74.1 $\pm$ 7.1	73.9 $\pm$ 7.5

PEM group: n=45, CM group: n=35

Numbers are mean $\pm$ standard deviation.

**: $P < 0.01$, *: $P < 0.05$ (paired t test, against baseline)

Table 7 Changes in the mean values of additional lipid metabolism-related parameters in the two study groups during the study period

Parameters	Unit	Group	Baseline	Week 12
Beta-sitosterol	($\mu\text{g/mL}$)	PEM	3.40 $\pm$ 1.23	3.03 $\pm$ 1.22**
		CM	3.78 $\pm$ 1.67	2.91 $\pm$ 1.14**
Campesterol	($\mu\text{g/mL}$)	PEM	5.53 $\pm$ 1.93	6.09 $\pm$ 2.47*
		CM	6.30 $\pm$ 2.66	5.01 $\pm$ 1.73**
Stigmasterol	($\mu\text{g/mL}$)	PEM	0.00 $\pm$ 0.00	0.21 $\pm$ 0.46**
		CM	0.00 $\pm$ 0.00	0.13 $\pm$ 0.36*
Beta-carotene	($\mu\text{g/dL}$)	PEM	32.84 $\pm$ 21.75	33.54 $\pm$ 20.38
		CM	32.66 $\pm$ 24.33	36.40 $\pm$ 24.87
Vitamin A	(IU/dL)	PEM	182.5 $\pm$ 48.6	173.0 $\pm$ 50.3*
		CM	192.4 $\pm$ 49.2	185.3 $\pm$ 48.2
Vitamin E	(mg/dL)	PEM	1.263 $\pm$ 0.241	1.657 $\pm$ 0.370**
		CM	1.379 $\pm$ 0.267	1.865 $\pm$ 0.440**

PEM group: n=45, CM group: n=35

Numbers are mean $\pm$ standard deviation.

#: $P < 0.05$ (two sample t test, inter-group comparison)

**: $P < 0.01$, *: $P < 0.05$ (paired t test, against baseline)

events, including common cold, headache, diarrhea, and abdominal pain; seven subjects in the CM group experienced common cold, stomach pain, abdominal pain, malaise, and numbness of the left femoral region. None of the events were considered by the Medical Investigator to be related to study food intake. Although the mean values of several parameters from the laboratory tests, physical measurements, physiological examinations, and serum levels of phytosterols and vitamins for the PEM group changed significantly from baseline, all of these changes were modest and clinically insignificant. No individual subjects in the PEM group exhibited abnormalities in any parameters.

DISCUSSION

In the Efficacy Study, we demonstrated that serum levels of LDL cholesterol and total cholesterol in subjects from the PEM group (who ingested low-calorie mayonnaise containing 886 mg PE daily for 12 weeks) were significantly reduced in comparison to those detected in the control mayonnaise-ingesting group (CM group). The magnitudes of reduced serum LDL cholesterol levels were comparable to those described in previous studies reported by several other research groups using different types of phytosterol-containing foods, including PEM¹⁴⁻¹⁷). These findings suggest that the circulating LDL cholesterol level-lowering effect of phytosterol may be independent of the emulsified state of phytosterols or their esters contained in the food. This hypothesis is supported by a report describing a meta-analysis of phytosterol efficacy¹⁴).

Pearson's correlation coefficient testing performed with the data presented in this paper showed that there is a positive correlation between the serum level of LDL cholesterol during the intervention period and the baseline level in both the PEM and CM groups. Compared to that for the CM group, the regression line for the PEM group is parallel but shifted downward (when constructed on the basis of data obtained at Week 12) (Fig. 2). This finding suggests that the LDL cholesterol-lowering effect of PEM occurs independently of the initial serum level of LDL cholesterol.

Together with the serum level of LDL cholesterol, Apo B levels also displayed a significant decrease ($P < 0.01$) in the PEM group at Weeks 4, 8 and 12 in comparison to the value at baseline. Apo B is known to be an essential proteinaceous component of LDL and a constituent of very low-density lipoprotein (VLDL), intermediate density lipoprotein (IDL),

and chylomicron. Thus, it functions as a potential risk factor for the progression of arteriosclerosis^{18,19}). It has been also reported that Apo B plays a critical role in inducing lipid deposition on the arterial inner wall, a process by which arteriosclerosis is initiated and developed. Furthermore, an antigenic substance which is found in locally accumulating foam cells is composed of oxidized phosphatidylcholine-ApoB complexes^{20,21}). The results of the present study, which show that PEM intake lowered not only the serum level of LDL cholesterol but also that of Apo B, suggest a possible correlation between these two variables.

Both the 12-week intake of phytosterol esters (daily consumption dose of 884 mg/day) and 4-week intake of a dose three times higher (2,652 mg/day) of the same type of food (PEM) were well tolerated without any clinical problems in the present two clinical studies. Because both studies were executed in a "double-blind" manner, it was possible to directly compare the frequency and nature of intake-related adverse events for PEM and the control food (CM). Both studies similarly demonstrated a high safety and tolerability of PEM. This finding is supported by the fact that PEM is designated as Generally Recognized as Safe (GRAS) by the United States Food and Drug Administration.

The effect of phytosterol esters on the intestinal absorption of fat-soluble vitamins is a controversial issue. It is generally accepted that phytosterol esters are active in inhibiting the absorption of these vitamins²²). However, a recent paper reported that a 12-week continuous intake of a dressing containing 800 mg of phytosterol had no such inhibitory effects²³). Consistent with that study, the results of the present studies showed that a 12-week intake of 884 mg/day or a 4-week intake of 2,652 mg/day of phytosterol esters in the form of a low-calorie mayonnaise modestly affected the absorption of beta-carotene, vitamin A and vitamin E. Although this issue remains to be clarified, the PEM used in the present studies does not appear to significantly affect the intestinal absorption of fat-soluble vitamins and may be beneficial to those at risk of hypercholesterolemia.

CONCLUSION

We showed that both a low-calorie mayonnaise containing phytosterol esters (daily dose of 886 mg) for 12 weeks and the same type of food containing a three-fold higher amount of phytosterol esters (daily dose of 2,652 mg) ingested for 4 weeks were safe and well tolerated. The former treatment was

effective in lowering serum levels of LDL cholesterol and total cholesterol to a significant extent in comparison to the control mayonnaise.

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要 旨

植物ステロールエステル含有ローカロリーマヨネーズの LDL コレステロール 低下効果と安全性

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植物ステロールエステルを含有したローカロリーマヨネーズ (PEM) の LDL コレステロール低下効果と安全性を検討することを目的として、血清コレステロール濃度が高めの健常成人を対象に無作為化二重盲検プラセボ対照並行群間比較試験を実施した。一日あたり 884 mg 植物ステロールエステルを含むマヨネーズ (PEM) または対照として植物ステロールエステルを含まないマヨネーズ (CM) を 12 週間毎日摂取することとした。その結果、血清 LDL コレステロール濃度において、PEM 群は CM 群に比べて、8 週および 12 週目で有意に低値を示した ($P < 0.05$)。なお、PEM 摂取に関連する副次作用は認められなかった。また、PEM の安全性は一日摂取目安量の 3 倍量 (植物ステロールエステル含量 2652 mg) 4 週間摂取試験においても確認された。以上の結果より、PEM は血清 LDL コレステロール濃度を低下し、安全な食品であることが示された。

キーワード：植物ステロールエステル，マヨネーズ，LDL-コレステロール，安全性