Original Article

Effects of phytosterol ester-enriched vegetable oil on plasma lipoproteins in healthy men

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It has been reported that phytosterol esters reduce cholesterol absorption and lower serum cholesterol concentration. There have been very few studies published on the effect of dose of phytosterol esters less than 1.0 g/day on plasma cholesterol levels in healthy subjects using commonly consumed foods. In this study, we evaluated the effect of 0.45 g/day (as free sterol) phytosterol ester-enriched dissolved in vegetable oil on plasma lipoproteins in sixty healthy males with slightly elevated total cholesterol concentration. This study was conducted in a randomized, double-blind, placebo-controlled, and arm parallel study. A total of 14 g /day of phytosterol ester-enriched vegetable oil containing 0.45g phytosterol (as the major free sterol) was compared with a control vegetable oil containing 0.04 g phytosterol (as the major free sterol). All subjects did not change their usual dietary habit and consumed foods that included about 360 mg/day cholesterol for 12 weeks. In subjects with higher total cholesterol concentrations (>200mg/dL), the phytosterol enriched-vegetable oil significantly reduced total cholesterol (10.3%, $P<0.05$), very low density (VLDL) lipoprotein cholesterol (22.5%, $P<0.05$), and remnant-like lipoprotein (RLP) cholesterol (24.7%, $P<0.01$) compared with the control vegetable oil. A reduction in low density lipoprotein (LDL) cholesterol concentration was also observed. In particular, the improvement in serum lipoprotein was more pronounced in subjects with higher total cholesterol concentrations. Triglycerides and high density lipoprotein (HDL) cholesterol did not change significantly. Plasma concentration of fat-soluble vitamins (tocopherol and retinol) and β-carotene were not statistically significantly affected by phytosterol ester-enriched vegetable oil. These findings indicate that a daily consumption of phytosterol ester as low as 0.45 g/day (as free sterol) is effective in lowering blood total cholesterol concentration and RLP cholesterol concentration. Lower total cholesterol, VLDL cholesterol and RLP cholesterol due to consumption of the phytosterol ester-enriched vegetable oil may be helpful in reducing the risk of CHD in the population.

Key Words: phytosterols, phytosterol ester, plant sterols, sterols, vegetable oil, cholesterol, lipoprotein, apolipoprotein.

Introduction

Hypercholesterolemia is a well-established risk factor for atherosclerosis.[1] Compelling evidence indicates that lowering total cholesterol or LDL-cholesterol reduces the risk of coronary heart disease (CHD) and mortality from CHD.[2-5] This serum cholesterol-lowering benefit may occur not only in persons with CHD or severely elevated serum cholesterol concentrations, but also in healthy persons with only mild-to-moderate elevations in serum cholesterol.[6-7] There have been a series of studies concerning the serum cholesterol-lowering effect of factors derived from diets. Plant-derived sterols (phytosterols) occurring naturally in nonsaponifiable plant oil material, are structurally related to cholesterol.[8] The most abundant phytosterols in nature are β-sitosterol, campesterol and stigmasterol[9], which inhibit the absorption of dietary cholesterol from the intestine[10-11] due to their higher affinity for mixed micelles compared with cholesterol.[12] More than 40 years of animal and human studies have shown that phytosterols lower serum cholesterol levels.[13-14] The addition of phytosterol to foods with the intended effect of lowering serum cholesterol levels is a recent major development in nutraceutical technology in Europe, North America, and Japan. The FDA has authorised the use, on food labels and in food labelling; of health claims on the association between phytosterol ester (1.3 g/day; 0.75g/day as free phytosterol equivalent) and reduced risk of CHD.[15]

In many clinical studies, the lowest dose of phytosterol which was reported to date is 0.4g/day phytosterol dissolved in diacylglycerol.[16] In the case of dissolving in triacylglycerol, the lowest reported dose is 0.74g/day.[17] Very few studies have investigated the effect of doses of phytosterols less than 1.0 g/day on serum cholesterol levels in normocholesterolaemic subjects.[13-14,16-22]

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Additionally, the administration time frame of clinical trials concerning the serum cholesterol-lowering effect of phytosterol ester are relatively short, such as 3-4 weeks.

Therefore, we investigated whether the lower dose (0.45 g/day as free sterol) of phytosterol ester dissolved in vegetable oil reduces serum cholesterol level in healthy persons with mild elevation in total blood cholesterol.

Methods

Subjects

The volunteers were recruited by advertisement and were screened for eligibility. Eligibility was assessed by review of medical history of hypertension, diabetes, and hyperlipidaemia, by fasting serum lipid profile (total cholesterol, LDL cholesterol, HDL cholesterol and triacylglycerol), and by other measurements of serum biochemical markers. In addition, participants were required to have total cholesterol levels <280 (mg/dL) and TG levels <400 (mg/dL). Subjects were randomly assigned to consume either the bread containing the phytosterol ester enriched vegetable oil or the bread containing the control oil for 12 weeks.

Study products

The oil mixture containing phytosterol ester was prepared by enzymatic transesterification of 10% (w/w) of phytosterol (derived from soybean and rapeseed oil) and 90% (w/w) of refined rapeseed oil (The Nisshin OilliO, Ltd). After the transesterification, the oil was refined by the same method used for common edible vegetable oil. Then, phytosterol ester-enriched vegetable oil (the test oil) was prepared by blending 25g of the oil mixture by enzymatic transesterification, 15g of refined rapeseed oil and 60g of refined rice oil. Consequently, the test oil contained about 0.5g of total phytosterol (as free sterol)/14g oil.

Table 1. Sterols and fatty acids in control oil and sterol enriched vegetable oil

	Control oil 14 g oil/day (N=28)	Phytosterol ester-enriched oil 14 g oil/day (N=32)
Total sterols (g sterols/14g oil)	0.051	0.493
Major sterols (g sterols/14g oil)	0.039	0.445
Sterol ester (sterol equivalents)/ total sterol (%)	47.4	93.1
Composition of major sterol		
β-sitosterol (%)	59.1	45.2
campesterol (%)	13.0	23.5
stigmasterol (%)	3.2	15.9
brassicasterol (%)	1.4	5.7
cholesterol (%)	0.2	0.3
other sterols (%)	23.1	9.4
Total fat as fatty acids (g/100g oil)		
SFA	14.6	15.2
MUFA	50.1	51.0
PUFA	35.3	33.8

SFA = saturated fatty acids; MUFA = monounsaturated fatty acids; PUFA = polyunsaturated fatty acids

The composition of the phytosterol ester-enriched vegetable oil is shown in Table 1. Total content of major sterol (β-sitosterol, campesterol, stigmasterol, brassicasterol) was about 0.45g (as free sterol)/14g oil. Both the test vegetable oil and control oil were very similar in composition of individual fatty acids, and the amount of poly-, mono- unsaturated and saturated fatty acids.

Control or test products, which were given to subjects as bread, were similar in nutritional content, appearance and sensory quality, and delivered in identical containers. The subjects ate each vegetable oil in bread. Both subjects and investigators were blinded for group assignment. Subjects were asked to eat three slices of bread every day. Both products contained a total of 14 g of prepared oil in three slices of bread.

Study design

This study was a randomized, double blind, controlled clinical trial with two parallel treatment arms. The protocol was reviewed and approved by the Ethics Committee of Ochanomizu University prior to the start of the study. The study was conducted according to the Declaration of Helsinki (1996). The procedures were explained in detail to all the volunteer subjects in advance. Signed written informed consent was obtained from all subjects before participating in the study. Total period of this study was 14 weeks consisting of a 2-week washout period and 12-week administration period. All subjects consumed control bread during the washout period to familiarize them with the study product using commonly consumed foods. After 2-week familiarization, subjects were randomly assigned to each treatment group at Week 0 before the start of administration. Compliance with study product consumption was calculated as a percentage of scheduled servings of study product consumed, as evaluated by dietary records, subject interview, and counting of unopened study product packages returned daily.

Diet records

Subjects were asked to complete three dietary records; the first dietary record was conducted for three representative days to register their dietary habits and diets before the start of the study; the second dietary record was performed daily during the administration period; the third dietary record was for three representative days after the administration period. Three representative days indicated three consecutive days (two weekdays [Monday through Friday] and one weekend day [Saturday or Sunday]). Also, before the start of the study, all subjects were provided with thorough instruction regarding completion of dietary records. Dietary record sheets were dispensed to record diets. Dietary records were collected from subjects and analyzed by the dietitian based on the 4th Revision of the Standard Tables of Food Composition in Japan.[23]

Measurements

Serum biochemical markers, haematology, anthropometry, lipid profile and urinalysis testing were completed at Week–2 during the washout period and at Weeks 0,4,8,12 during the administration period. The blood sampling and anthropometric measurements were carried out on the same day. Blood was drawn from an antecubital vein in the

sitting position via tubes at each clinic after an overnight fast. Analyses of serum total cholesterol and triacylglycerol were carried out on a 7450 automated system (Hitachi, Tokyo, Japan). Analyses of serum high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, and very low-density lipoprotein (VLDL) cholesterol were carried out on a rapid electrophoresis scanning (REP) automated system (Helena Laboratories, Saitama, Japan) by agarose-gel electrophoretic methods.[24] Analysis of serum RLP-cholesterol was carried out by immunoabsorption method[25] using a kit of "the RLP-cholesterol [JIMRO2]" (Japan Imuno Research Laboratory Co. Ltd, Japan). Apolipoprotein concentrations were measured nephelometrically using the Behring Laser Nephelometer (Behringwerke AL, Macburg, Germany) with specific antibodies against human apolipoprotein antibodies, respectively. Blood concentrations of fat-soluble vitamins (α-tocopherol, retinol), β-carotenoid, and phytosterols including β-sitosterol, campesterol, and stigmasterol were measured by HPLC. Samples for α-tocopherol, retinol, β-carotenoid and sterol analyses were frozen at –80˚C, and all measures for each subject were completed in the same run.

Statistical analysis:
Statistical analysis was performed with SPSS for WINDOWS (version 10.0J; SPSS Japan Inc., Tokyo, Japan). The results are represented as means ± SE. *P* values less than 0.05 were considered to indicate statistical significance. Differences in baseline characters were analyzed by *t*-test. The difference in raw data was examined by two-way (vegetable oil groups × period) ANOVA. In addition, the significance of differences between the 2 groups for the same period was examined by unpaired Student's *t* test (two-tailed).

Results

Baseline characters of study subjects
After screening the volunteers for eligibility, sixty-two subjects were randomized. Two persons of these randomized subjects dropped out of the study prior to completing the intervention period: one person could not complete the study because of a bone fracture (the lumbar vertebrae) and the other person could not consume the study product whilst on a long-term business trip. Both drop out subjects were in the control group. Sixty persons completed the study (all men, mean age 39.4 ± 1.4 years, mean body weight 71.2 ± 1.4 kg). Anthropometry, lifestyle characteristics, and serum biochemical markers of the subjects are shown in Table 2. There were no significant differences between groups in baseline characteristics. Body weight was similar between the groups in baseline. Throughout this study, mean weight from the start to the end of the intervention period was not significantly reduced in either group. Blood pressure generally did not change over the intervention period, and there were no significant differences between the groups in systolic or diastolic blood pressure changes. Also, all subjects were healthy and there were no significant differences between the groups in serum biochemical markers.

Dietary analyses
Dietary intakes before intervention, during intervention, and after intervention are shown in Table 3. The data during intervention includes the contribution made to dietary intake by the study product. Dietary intakes were similar between the groups before, during, and after intervention. The differences in cholesterol intake were also not significant between the groups and were not significant across the treatment period. The intake of cholesterol was about 360mg/day during intervention.

Table 2. Baseline characteristics of subjects participating in this study

Parameter	Control group 0.05g PS (as free)/ day N=28	Phytosterol ester-enriched group 0.5g PS (as free)/ day N=32
Age (mean ± SE)	39.1 ± 1.9	39.1 ± 2.1
Weight (mean ± SE)	73.9 ± 2.4	68.9 ± 1.5
BMI (mean ± SE)	24.7 ± 0.7	23.8 ± 0.4
Smoking status (n %)	36	30
Alcoholic drinks (times/week)	2.5	1.9
AST (IU/dL)	24.4 ± 1.9	26.0 ± 2.2
ALT (IU/dL)	28.3 ± 3.7	30.9 ± 3.9
γ-GTP (IU/dL)	51.1 ± 7.2	42.5 ± 4.4
Glucose (mg/dL)	96.0 ± 2.5	93.2 ± 1.3
Systolic blood pressure (mmHg) (mean ± SE)	126.4 ± 1.9	125.2 ± 2.0
Dyastolic blood pressure [mmHg] (mean ± SE)	76.6 ± 2.1	76.0 ± 1.7

There were no significant differences between the two groups.
PS = phytosterol

Lipoprotein profile
Serum lipid concentration and responses to exposure are shown in Table 4. Baseline lipid values were similar between the groups. However, total-, VLDL-, and RLP-cholesterol responses were significantly different compared to the control group. There were no significant differences in LDL cholesterol between the groups. When the subjects were stratified according to the analyses of total cholesterol at Week–2, a striking difference was observed (shown in Table 4). In higher total cholesterol subjects (200<total cholesterol) at Week-2, total cholesterol was significantly reduced by 10.3% in the test group at Week 12 against a reduction of 6.0% in the control group. Overall, a greater reduction of serum lipids level was observed in higher total cholesterol subjects.

Triacylglycerol concentrations are shown in Table 5. There were no significant differences between the groups. Apolipoprotein concentrations are shown in Table 6. ApoC was significantly reduced at Week 8 and 12 in subjects with higher total cholesterol level (>200 mg/dL). ApoB and other apolipoprotein concentrations were not significantly reduced compared with the control group.

Table 3. Nutrients analyses from diet records before, during and after the administration period, according to group assignment

Nutrients	Before	During	After
Energy (kcal/day)			
control group	2398.8 ± 104.0	2279.8 ± 16.4	2287.8 ± 101.2
phytosterol enriched group	2246.3 ± 91.3	2288.8 ± 17.3	2246.3 ± 94.5
Protein (g/day)			
control group	92.9 ± 4.1	79.7 ± 0.7	82.9 ± 4.0
phytosterol enriched group	86.7 ± 3.4	80.6 ± 0.7	83.7 ± 4.6
Fat (g/day)			
control group	76.3 ± 4.2	74.0 ± 0.3	75.3 ± 4.0
phytosterol enriched group	70.8 ± 4.4	74.3 ± 0.3	73.8 ± 4.8
Carbohydrate (g/day)			
control group	312.4 ± 14.0	231.2 ± 2.8	301.4 ± 13.6
phytosterol enriched group	302.3 ± 11.1	232.7 ± 3.5	322.0 ± 11.2
Cholesterol (mg/day)			
control group	427.1 ± 39.7	363.2 ± 2.0	417.1 ± 39.4
phytosterol enriched group	373.9 ± 28.2	367.6 ± 1.7	368.9 ± 25.2
Total dietary fibre (g/day)			
control group	16.3 ± 1.2	19.0 ± 0.2	15.3 ± 1.2
phytosterol enriched group	16.3 ± 0.9	19.1± 0.2	16.9 ± 0.9
Soluble fibre (g/day)			
control group	2.2 ± 0.2	1.3 ± 0.0	2.1 ± 0.2
phytosterol enriched group	2.2 ± 0.2	1.3 ± 0.0	2.0 ± 0.2
Retinol (μg/day)			
control group	2599.2 ± 187.5	4714.0 ± 44.7	2699.2 ± 177.5
phytosterol enriched group	2544.4 ± 323.8	4778.1± 67.1	2644.4 ± 383.8
Total vitamin E (mg/day)#			
control group	8.8 ± 0.4	12.7 ± 0.1	8.9 ± 0.6
phytosterol enriched group	9.4 ± 0.6	12.8 ± 0.1	10.4 ± 0.7

There were no significant differences among the three periods and between the two groups. All measurements are represented as mean ± SE
total vtamin E; # total of α -, β -, γ-, and δ-tocopherol

Retinol, α-tocopherol, β-carotene, and sterols

Results of retinol, α-tocopherol and β-carotene analyses are reported in Table 7. Blood concentrations of these fat-soluble vitamins and β-carotene were within normal reference ranges at baseline and following 4, 8, and 12 weeks of intervention. There were no significant differences in vitamin levels at baseline, nor were there any differences in serum vitamin responses between the two groups. Results of the blood sterol analyses are shown in Table 8. The groups receiving phytosterol ester-enriched vegetable oil showed significant differences in campesterol compared with the control group. However, absolute levels of campesterol seemed not clinically important, as the average values of campesterol at Week –2 were almost as much as those at Week 12 (about 3.0 mg/L). Stigmasterol was detectable in plasma and was not shown in Table 8.

Serum biochemical markers

The number of shifts in serum biochemical markers and haematology values from low/normal to high and high/normal to low did not differ between the groups (these data are not shown). Serum biochemical markers responses to intervention were similar between groups, and pairwise comparisons did not indicate a significant difference (these data are not shown).

Discussion

We investigated whether the lower dose (0.45 g/day as the major free sterol) of phytosterol ester dissolved in vegetable oil reduces serum cholesterol level in healthy males with mild elevation in total blood cholesterol level (>200mg/dL). The consumption of a phytosterol ester-enriched vegetable oil (14 g/day) via a commonly consumed food (bread) significantly improved total cholesterol, VLDL cholesterol, and RLP cholesterol and was particularly beneficial in subjects with a higher baseline total cholesterol concentration (>200mg/dL). Table 8 suggests that those with a blood cholesterol concentration >200mg/dL had higher baseline plasma phytosterol levels than those with a blood cholesterol <200 mg/dL. This suggests a difference in cholesterol absorption that may explain the difference in serum cholesterol lowering effect between subjects with higher and the lower blood cholesterol levels.

The reduction (8%) in total cholesterol concentration is within the range of the results of other studies with phytosterols, which found reductions of total cholesterol concentrations from 0.5%[26] to 26%.[11] Although the reduction (7.4%) in LDL cholesterol concentration was not significantly different from the control group, this value was similar in magnitude and direction to those (from 2%[26]

Table 4. Cholesterol concentrations at screening and baseline, and the percentage change (Δ) from baseline to week 4, week 8, and week 12

Cholesterol and week of study	All		Total cholesterol ≤200mg/dL		Total cholesterol >200mg/dL	
	Control N=28	Test N=32	Control N=9	Test N=11	Control N=19	Test N=21
Total cholesterol (mg/dL)						
Screening	212.1 ± 6.1	214.2 ± 5.9	176.9 ± 7.4	177.4 ± 4.6	228.8 ± 4.7	233.4 ± 4.6
Baseline	190.1 ± 5.3	193.8 ± 5.5	167.3 ± 6.5	162.0 ± 4.3	200.9 ± 5.8	210.5 ± 5.0
% Δ from baseline to week 4	-2.5 ± 1.0	-5.0 ± 1.3	-1.9 ± 1.9	-0.3 ± 1.2	-2.8 ± 1.1	-7.5 ± 1.7*
% Δ from baseline to week 8	-2.6 ± 0.9	-4.6 ± 1.3	-2.1 ± 2.2	1.3 ± 1.5	-2.8 ± 1.0	-7.6 ± 1.5*
% Δ from baseline to week 12	-5.0 ± 1.2	-8.0 ± 1.3	-2.9 ± 2.7	-3.6 ± 1.9	-6.0 ± 1.1	-10.3 ± 1.4*
LDL cholesterol (mg/dL)						
Screening	129.4 ± 5.3	132.3 ± 4.8	98.9 ± 5.0	104.0 ± 6.2	143.8 ± 4.5	147.2 ± 3.6
Baseline	114.5 ± 4.5	116.3 ± 4.9	93.1 ± 5.6	91.9 ± 5.5	124.6 ± 4.4	129.1 ± 5.0
% Δ from baseline to week 4	-1.6 ± 1.6	-3.9 ± 1.8	0.2 ± 3.4	0.6 ± 2.0	-2.4 ± 1.8	-6.3 ± 2.5
% Δ from baseline to week 8	-2.5 ± 1.7	-3.7 ± 1.8	-1.2 ± 3.2	1.1 ± 2.1	-3.1 ± 2.0	-6.2 ± 2.5
% Δ from baseline to week 12	-5.3 ± 1.9	-7.4 ± 1.8	-2.1 ± 3.8	-1.6 ± 3.0	-6.9 ± 2.0	-10.5 ± 2.0
HDL cholesterol (mg/dL)						
Screening	60.6 ± 2.7	59.0 ± 2.1	55.4 ± 5.2	56.1 ± 3.4	63.1 ± 3.0	60.5 ± 2.7
Baseline	55.4 ± 2.3	54.8 ± 2.1	51.8 ± 4.1	52.0 ± 3.3	57.1 ± 2.7	56.2 ± 2.8
% Δ from baseline to week 4	-3.8 ± 1.7	-2.2 ± 1.5	-1.2 ± 2.4	1.7 ± 2.9	-5.0 ± 2.2	-4.3 ± 1.5
% Δ from baseline to week 8	0.4 ± 1.9	1.6 ± 1.7	2.7 ± 3.5	4.6 ± 3.7	-0.7 ± 2.2	0.0 ± 1.8
% Δ from baseline to week 12	2.3 ± 1.6	2.7 ±1.9	4.0 ± 2.9	3.3 ± 2.4	1.5 ± 2.0	2.4 ± 2.6
RLP cholesterol (mg/dL)						
Screening	4.7 ± 0.4	4.9 ± 0.4	4.5 ± 0.9	4.1 ± 0.6	4.8 ± 0.3	5.2 ± 0.4
Baseline	3.6 ± 0.4	3.9 ± 0.3	4.0 ± 1.3	3.8 ± 0.6	3.5 ± 0.2	4.0 ± 0.3
% Δ from baseline to week 4	-1.4 ± 4.3	-14.9 ± 4.5*	4.6 ± 4.4	-2.3 ± 9.4	-4.2 ± 5.9	-21.6 ± 4.3*
% Δ from baseline to week 8	3.5 ± 5.4	-15.3 ± 4.5**	6.7 ± 5.9	2.9 ± 8.7	2.0 ± 7.6	-24.8 ± 3.7**
% Δ from baseline to week 12	-12.2 ± 4.2	-21.7 ± 4.1	-3.9 ± 5.7	-15.9 ± 7.8	-16.1 ± 5.4	-24.7 ± 4.7
VLDL cholesterol (mg/dL)						
Screening	10.7 ± 1.2	20.9 ± 8.2	11.3 ± 3.0	20.1 ± 2.4	10.4 ± 1.2	12.3 ± 1.5
Baseline	10.5 ± 1.6	17.7 ± 6.5	12.8 ± 4.6	46.8 ± 9.3	9.4 ± 1.1	11.9 ± 1.5
% Δ from baseline to week 4	20.1 ± 8.6	-1.6 ± 8.1*	17.5 ± 16.3	24.3 ± 26.5	21.3 ± 10.4	-10.9 ± 8.4*
% Δ from baseline to week 8	6.9 ± 9.0	-10.2 ± 10.1	8.5 ± 15.4	40.4 ± 52.5	6.1 ± 11.3	-18.2 ± 6.8
% Δ from baseline to week 12	-13.4 ± 6.9	-22.2 ± 6.2	-6.0 ± 8.4	-22.4 ± 12.2	-16.9 ± 9.4	-22.5 ± 7.0

Screening: average values obtained at week -2, baseline: average values obtained at week 0; all measurements are represented as mean ± SE;
*significantly different from control by t-test (P<0.05), ** significantly different from control by t-test P<0.01.

to 33%[11]) reported previously. After 4 weeks consumption of the phytosterol ester-enriched vegetable oil, total cholesterol concentration was already significantly lower compared with the control oil group (5.0%, 2.5%, respectively) and further slightly decreased until Week 12 (8.0%, 5.0%). Thus, plateau was almost reached within the first 4 weeks after initiation of the treatment. This is in good agreement with published studies in which phytosterols reached the maximal serum cholesterol reduction within a few weeks after initiation of the treatment.[27] Some studies[9,17,19,20,26,28] reported no changes or a decrease in HDL cholesterol concentrations during consumption of phytosterols, in general, only total and LDL cholesterol seem to respond to the consumption of phytosterols. HDL cholesterol levels in the present study did not change - this was in agreement with the previous reports. In the present study, apolipoprotein levels were measured to evaluate lipoprotein profile. Apo B concentration was reduced by 5.7-8.1% in the test group, showing no difference from control. Apo CIII concentration was reduced by 6.1-8.9% in the test group, significantly differing from the control group at Weeks 8,12 in subjects with higher baseline total cholesterol level. The fact that Apo CIII concentration was reduced indicates that the total number of circulating VLDL particles was also reduced.

As a result of a combination of some factors, even at the lowest dose of 0.45 g/day (as the major free sterol) in the studies reported to date,[22] a significant positive response to phytosterols appears to take place. The differences in response to phytosterols between studies seems to be related to the different forms of phytosterols of different types of diets (e.g. low in cholesterol,[26,29-31] low in fat[29]), and the differences in triacylglycerol composition or structure in which the phytosterols are dissolved.[16,22] Free stanols have been shown to inhibit cholesterol absorption more effectively than free sterols.[10] However, esterified sterols and esterified stanols inhibit cholesterol absorption equally[32-33] and lower serum cholesterol levels similarly.[9,33]

Table 5. Triacylglycerol concentrations at screening and baseline, and the percentage change (Δ) from baseline to week 4, week 8, and week 12

Lipid variable and week of study	All		Total cholesterol ≤200mg/dL		Total cholesterol >200mg/dL	
	Control N=28	Test N=32	Control N=9	Test N=11	Control N=19	Test N=21
TG (mg/dL)						
Screening	106.6 ± 10.5	107.6 ± 11.3	113.3 ± 29.7	85.0 ± 12.0	103.4 ± 7.4	119.5 ± 15.6
Baseline	97.4 ± 12.7	101.8 ± 6.7	111.0 ± 39.3	94.2 ± 11.7	90.9 ± 5.0	105.9 ± 8.2
% Δ from baseline to week 4	1.1 ± 3.9	-5.7 ± 5.5	-1.0 ± 6.7	1.7 ± 10.7	2.1 ± 4.8	-9.6 ± 6.3
% Δ from baseline to week 8	-3.7 ± 4.8	-8.2 ± 5.6	-7.0 ± 7.3	0.4 ± 14.2	-2.1 ± 6.2	-12.7 ± 4.2
% Δ from baseline to week 12	-14.1 ± 4.5	-17.0 ± 4.1	-8.8 ± 4.9	-18.8 ± 7.2	-16.6 ± 6.2	-16.1 ± 5.2
LDL-TG (mg/dL)						
Screening	26.7 ± 1.3	27.7 ± 1.8	23.6 ± 3.1	22.1 ± 1.9	28.1 ± 1.2	30.4 ± 2.2
Baseline	23.4 ± 1.0	23.4 ± 1.3	19.9 ± 1.7	19.2 ± 1.1	25.0 ± 1.0	25.6 ± 1.6
% Δ from baseline to week 4	-16.8 ± 2.2	-10.3 ± 2.7	-17.4 ± 3.4	-10.2 ± 4.5	-16.6 ± 2.9	-10.4 ± 3.4
% Δ from baseline to week 8	-13.0 ± 2.5	-12.4 ± 2.5	-15.7 ± 3.6	-10.2 ± 5.5	-11.7 ± 3.3	-13.6 ± 2.5
% Δ from baseline to week 12	-7.5 ± 2.9	-6.3 ± 2.5	-7.5 ± 3.1	-5.3 ± 5.0	-7.5 ± 4.1	-6.8 ± 2.9
HDL-TG (mg/dL)						
Screening	13.6 ± 0.6	13.2 ± 0.8	13.7 ± 1.3	11.5 ± 0.9	13.6 ± 0.6	14.0 ± 1.0
Baseline	13.1 ± 0.7	12.9 ± 0.5	12.8 ± 1.8	12.7 ± 1.1	13.3 ± 0.6	13.1 ± 0.6
% Δ from baseline to week 4	6.7 ± 4.7	3.2 ± 5.7	12.0 ± 7.9	9.2 ± 11.2	4.1 ± 5.8	0.1 ± 6.4
% Δ from baseline to week 8	0.7 ± 5.1	-6.7 ± 5.8	2.4 ± 11.3	3.2 ± 15.1	-0.1 ± 5.5	-11.9 ± 4.1
% Δ from baseline to week 12	-10.6 ± 4.2	-12.1 ± 3.8	-5.9 ± 6.7	-13.2 ± 7.1	-12.8 ± 5.4	-11.6 ± 4.6
VLDL-TG (mg/dL)						
Screening	46.7 ± 7.6	47.8 ± 7.5	56.0 ± 20.7	38.6 ± 11.6	42.3 ± 5.9	52.2 ± 9.6
Baseline	42.9 ± 9.7	47.2 ± 4.9	58.3 ± 29.1	46.8 ± 9.3	35.7 ± 4.4	47.4 ± 5.9
% Δ from baseline to week 4	22.9 ± 8.3	5.2 ± 12.3	17.5 ± 15.9	24.4 ± 26.6	25.4 ± 9.8	-4.9 ± 12.5
% Δ from baseline to week 8	10.0 ± 10.1	10.4 ± 18.9	4.8 ± 15.6	40.6 ± 52.5	12.5 ± 13.1	-5.4 ± 9.4
% Δ from baseline to week 12	-12.6 ± 8.6	-18.2 ± 8.4	3.4 ± 14.5	-22.3 ± 12.2	-20.2 ± 10.4	-16.0 ± 11.4

Screening: average values obtained at week -2, baseline: average values obtained at week 0; All measurements are represented as mean ± SE; there were no significant differences between the 2 groups.

Therefore, esterification of sterols result in a greater reduction of cholesterol absorption efficiency. Some investigators[9,18,34] have also indicated that there is a non-linear relationship between phytosterol intake and serum cholesterol level reduction. As phytosterols need to be in a free state to inhibit cholesterol absorption,[35-37] intestinal hydrolysis of phytosterol esters by cholesterol esterase may be a rate-limiting factor at the high level of phytosterol ester intake, in determining the efficacy of phytosterols to lower serum cholesterol levels. But, the hydrolysis of phytosterol ester does not seem to be a rate-limiting factor at this low level of phytosterol ester as well as in the results reported by Sjerksma *et al.*[18] Differences in serum cholesterol reduction observed in published studies may also be related to the simultaneous consumption of different types of diets (e.g. low in cholesterol).[26,29-31] Mussner *et al.*,[29] indicated that in dividing the subjects in tertiles according to their dietary intakes of cholesterol, energy, fat and saturated fatty acids, subjects with a high dietary intake of these components particularly benefit from consuming phytosterol ester-enriched margarine. In those subjects with high dietary intake of cholesterol (273 to 843 mg/day, average 426±166mg/day), LDL cholesterol concentration decreased by 11.6% compared with a 5.4% reduction of LDL cholesterol concentration in the whole group.

Denke *et al.*,[25] also suggested that a low level of dietary cholesterol attenuates the effectiveness of a low-dose phytosterol. The same may apply to the intake of phytosterols. The Japanese consume more phytosterols from their habitual foods than Caucasians. The intake of phytosterols by Caucasians is estimated to be about 250mg/day.[13,39] However, it is reported that the intake of phytosterols by the Japanese is 400 mg/day.[40] In addition, Japanese people generally ingest less calories and fat than Caucasians. Thus, since the ratio of phytosterol to fat in the Japanese diet is considered to be relatively higher than the the Caucasian diet, it is possible that the effect of serum cholesterol lowering by phytosterols is strengthened. These two points may explain the differences in serum cholesterol lowering effects. Other potential explanations for the significant effect of the lowest phytosterol dose in studies reported to date may be related to the differences in coexisting triacylglycerol composition or structure in which the phytosterol ester is dissolved. Saito *et al.,*[22] recently showed that the lowest effective dose of phytosterols was 0.8 g/day in humans. On the other hand, Goto *et al.,*[16] reported that serum total cholesterol concentration decreased by ingestion of 0.4 g/ day phytosterols dissolved in diacylglycerol. This dose is lower than the dose authorized by the FDA.[15] Also, Sugano *et al.,* reported that the effect of sitosterol on serum cholesterol changed with the kind of dietary fat source.[38] These results suggest

that the level of serum cholesterol lowering-effect by phytosterols may change depending on the kind of oil in which the phytosterols are dissolved in. Plasma concentration of fat-soluble vitamins, β-carotene, phytosterol, serum biochemical markers and haematology parameters were not statistically significantly affected by phytosterol ester-enriched vegetable oil. Opinions vary as to the effect of phytosterol on serum fat-soluble vitamins and β-carotene levels.[41-42] Some recent investigations have reported that ingested phytosterols may reduce serum β-carotene levels. This was not observed in the current study, possibly because the subjects (Japanese) in this study consumed more β-carotene than subjects in other studies. Thus, this phytosterol ester-enriched vegetable oil is considered to be safe and to have no known side effects.

The current study has shown that the consumption of a low dose (0.45 g/day) of phytosterol esters (as the major free sterol) improved the blood lipid status of healthy subjects with slightly elevated total blood cholesterol concentration. This was achieved by ingesting a common food (bread) fortified with phytosterols. This suggests that the daily consumption of a phytosterol ester-enriched vegetable oil may lower the risk of atherosclerosis in healthy subjects with slightly elevated total cholesterol concentrations. It is anticipated that many individuals will find phytosterol enriched foods easier to incorporate into their daily diet than other available blood cholesterol-lowering foods, such as soy proteins.

Table 6. Apolipoprotein concentrations at screening and baseline, and the percentage change (Δ) from baseline to week 4, week 8, and week 12

Apolipoprotein variable and week of study	All		Total cholesterol ≤200mg/dL		Total cholesterol >200mg/dL	
	Control N=28	Test N=32	Control N=9	Test N=11	Control N=19	Test N=21
Apolipoprotein A1 (mg/dL)						
Screening	149.3 ± 4.4	143.8 ± 3.7	138.1 ± 8.9	135.9 ± 6.4	154.6 ± 4.7	147.6 ± 4.3
Baseline	135.2 ± 3.7	134.1 ± 3.9	128.4 ± 5.8	127.0 ± 5.7	138.4 ± 4.6	137.8 ± 5.0
% Δ from baseline to week 4	-3.9 ± 1.2	-3.1 ± 1.4	-2.9 ± 1.4	0.7 ± 1.7	-4.3 ± 1.7	-5.1 ± 1.7
% Δ from baseline to wee k8	0.1 ± 1.2	-0.2 ± 1.5	1.1 ± 2.5	1.6 ± 2.8	-0.4 ± 1.3	-1.1 ± 1.7
% Δ from baseline to week 12	1.7 ± 1.3	1.0 ± 1.7	2.8 ± 2.5	0.5 ± 1.7	1.2 ± 1.6	1.2 ± 2.5
Apolipoprotein A2 (mg/dL)						
Screening	32.8 ± 1.0	32.6 ± 0.7	30.5 ± 1.5	31.2 ± 1.6	33.8 ± 1.2	33.3 ± 0.8
Baseline	29.5 ± 0.6	30.0 ± 0.6	28.5 ± 1.0	28.4 ± 0.9	30.0 ± 0.7	30.8 ± 0.7
% Δ from baseline to week 4	-5.6 ± 1.2	-5.4 ± 1.0	-3.9 ± 1.4	-2.2 ± 1.6	-6.3 ±1.6	-7.1 ± 1.1
% Δ from baseline to wee k8	-3.6 ± 1.4	-3.5 ± 1.4	0.2 ± 2.4	-2.9 ± 2.2	-5.4 ± 1.5	-3.8 ± 1.9
% Δ from baseline to week 12	-3.9 ± 1.5	-4.3 ± 1.6	-0.3 ± 2.8	-6.4 ± 1.9	-5.6 ± 1.7	-3.2 ± 2.3
Apolipoprotein B (mg/dL)						
Screening	99.4 ± 4.0	104.2 ± 4.4	80.4 ± 5.2	79.7 ± 5.1	108.4 ± 3.9	115.9 ± 4.1
Baseline	87.4 ± 3.5	90.0 ± 3.9	74.9 ± 5.8	70.8 ± 3.7	93.4 ± 3.6	100.0 ± 4.3
% Δ from baseline to week 4	-2.4 ± 2.0	-5.1 ± 2.1	-1.0 ± 2.0	0.1 ± 1.7	-3.1 ± 2.0	-7.9 ± 2.3
% Δ from baseline to wee k8	-2.5 ± 2.1	-3.8 ± 2.1	-1.5 ± 2.3	-1.7 ± 2.0	-2.9 ± 1.9	-5.7 ± 2.2
% Δ from baseline to week 12	-4.0 ± 2.2	-6.2 ± 2.2	-1.2 ± 2.7	-2.6 ± 2.6	-5.3 ± 1.7	-8.1 ± 2.0
Apolipoprotein C2 (mg/dL)						
Screening	4.6 ± 0.5	4.2 ± 0.3	4.5 ± 1.1	2.9 ± 0.6	4.7 ± 0.5	4.9 ± 0.3
Baseline	4.2 ± 0.5	3.9 ± 0.3	4.3 ± 1.4	3.0 ± 0.5	4.1 ± 0.4	4.4 ± 0.3
% Δ from baseline to week 4	-0.3 ± 7.5	-7.7 ± 5.5	10.9 ± 22.4	6.1 ± 13.2	-5.6 ± 3.7	-14.9 ± 4.4
% Δ from baseline to wee k8	-10.8 ± 6.6	-9.1 ± 5.8	-2.4 ± 18.9	3.1 ± 15.4	-14.7 ± 4.4	-15.5 ± 3.5
% Δ from baseline to week 12	-14.3 ± 5.9	-14.8 ± 4.8	5.3 ± 14.3	-7.9 ± 10.4	-23.6 ± 4.2	-18.4 ± 4.9
Apolipoprotein C3 (mg/dL)						
Screening	9.9 ± 0.4	9.8 ± 0.5	9.2 ± 1.0	8.5 ± 0.6	10.2 ± 0.5	10.4 ± 0.7
Baseline	8.6 ± 0.4	9.0 ± 0.3	8.3 ± 1.1	8.2 ± 0.5	8.7 ± 0.3	9.4 ± 0.4
% Δ from baseline to week 4	-0.2 ± 1.9	-5.0 ± 2.8	-1.3 ± 2.4	2.3 ± 5.1	0.3 ± 2.5	-8.9 ± 3.1
% Δ from baseline to wee k8	0.1 ± 2.0	-4.1 ± 2.3	-0.9 ± 4.1	-0.1 ± 5.0	0.5 ± 2.4	-6.1 ± 2.2*
% Δ from baseline to week 12	-1.7 ± 1.9	-5.9 ± 2.4	1.6 ± 3.3	-4.3 ± 3.9	-3.3 ± 2.3	-6.8 ± 3.1*
Apolipoprotein E (mg/dL)						
Screening	4.1 ± 0.2	4.4 ± 0.2	3.9 ± 0.5	3.7 ± 0.4	4.1 ± 0.2	4.7 ± 0.3
Baseline	3.6 ± 0.2	3.9 ± 0.2	3.6 ± 0.5	3.7 ± 0.5	3.6 ± 0.2	4.0 ± 0.2
% Δ from baseline to week 4	-2.7 ± 1.6	-3.0 ± 1.8	-1.7 ± 3.4	1.9 ± 3.1	-3.2 ± 1.8	-5.6 ± 2.0
% Δ from baseline to week 8	-4.8 ± 1.6	-4.6 ± 1.8	-5.4 ± 3.2	-0.4 ± 3.5	-4.5 ± 2.0	-6.8 ± 2.0
% Δ from baseline to week 12	-4.6 ± 1.9	-4.5 ± 1.8	-2.4 ± 4.1	-2.0 ± 3.1	-5.6 ± 2.1	-5.7 ± 2.3

Screening: average values obtained at week -2, baseline; average values obtained at week 0; all measurements are represented as mean±S.E
#significantly different from control by *t*-test, $P<0.05$

Table 7. Fat-soluble vitamins and β-carotene concentrations at screening, baseline and each week

Lipid variable and week of study	All		Total cholesterol ≤200mg/dL		Total cholesterol >200mg/dL	
	Control N=28	Test N=32	Control N=9	Test N=11	Control N=19	Test N=21
Retinol (μg/dL)						
Screening	66.3 ± 2.9	66.6 ± 2.2	64.0 ± 5.5	63.1 ± 2.6	67.3 ± 3.4	68.3 ± 3.0
Baseline	58.1 ± 2.1	59.6 ± 1.9	58.9 ± 3.7	53.9 ± 3.0	57.7 ± 2.6	62.6 ± 2.3
at week 4	49.1 ± 1.7	51.0 ± 1.7	50.1 ± 3.4	48.6 ± 2.8	48.6 ± 1.9	52.3 ± 2.1
at week 8	48.3 ± 2.0	50.6 ± 1.7	49.6 ± 5.0	46.7 ± 3.4	47.7 ± 1.9	52.6 ± 1.9
at week 12	47.0 ± 1.5	46.6 ± 1.6	49.0 ± 3.2	45.1 ± 2.6	46.0 ± 1.7	47.4 ± 2.0
β -Carotene (μg/dL)						
Screening	25.6 ± 4.3	24.4 ± 2.9	21.2 ± 4.0	23.2 ± 4.4	27.7 ± 6.1	25.0 ± 3.8
Baseline	34.4 ± 4.3	38.2 ± 4.0	26.3 ± 4.9	34.5 ± 5.2	38.2 ± 5.7	40.1 ± 5.4
at week 4	38.8 ± 4.0	41.1 ± 4.3	29.7 ± 3.4	35.9 ± 6.0	43.1 ± 5.5	43.9 ± 5.8
at week 8	40.9 ± 3.7	43.2 ± 4.7	31.4 ± 4.2	35.6 ± 5.1	45.4 ± 4.9	47.2 ± 6.5
at week 12	36.0 ± 4.1	38.1 ± 4.1	25.1 ± 3.5	32.7 ± 5.2	41.1± 5.5	40.9 ± 5.6
α -Tocopherol (mg/dL)						
Screening	1.1 ± 0.1	1.2 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.2 ± 0.0	1.3 ± 0.1
Baseline	1.0 ± 0.0	1.0 ± 0.0	0.9 ± 0.1	0.8 ± 0.0	1.0 ± 0.0	1.1 ± 0.1
at week 4	1.0 ± 0.0	1.0 ± 0.0	0.9 ± 0.1	0.8 ± 0.0	1.0 ± 0.0	1.0 ± 0.1
at week 8	1.0 ± 0.0	1.0 ± 0.0	0.9 ± 0.1	0.9 ± 0.0	1.0 ± 0.0	1.1± 0.1
at week 12	1.0 ± 0.0	1.0 ± 0.0	0.9 ± 0.1	0.8 ± 0.0*	1.0 ± 0.0	1.1± 0.0

Screening : average values obtained at week -2, baseline : average values obtained at week 0; all measurements are represented as mean ± SE.
* significantly different from control by t-test, $P<0.05$

Table 8. Phytosterol concentrations at screening, baseline and each week

Phytosterol variable and week of study	All		Total cholesterol ≤200mg/dL		Total cholesterol >200mg/dL	
	Control N=28	Test N=32	Control N=9	Test N=11	Control N=19	Test N=21
β -sitosterol [mg/L]						
Screening	2.4 ± 0.2	2.4 ± 0.2	1.9 ± 0.2	2.0 ± 0.2	2.6 ± 0.2	2.6 ± 0.2
Baseline	1.8 ± 0.1	1.9 ± 0.2	1.5 ± 0.1	1.5 ± 0.2	1.9 ± 0.2	2.1 ± 0.2
at week 4	1.7 ± 0.1	2.1 ± 0.2*	1.5 ± 0.1	1.9 ± 0.2	1.8 ± 0.2	2.3 ± 0.2
at week 8	1.4 ± 0.1	1.8 ± 0.1*	1.2 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.9 ± 0.2
at week 12	1.6 ± 0.1	2.0 ± 0.2	1.5 ± 0.2	1.7 ± 0.2	1.6 ± 0.1	2.1± 0.2
Campesterol [mg/L]						
Screening	2.9 ± 0.2	3.0 ± 0.2	2.3 ± 0.2	2.4 ± 0.3	3.2 ± 0.2	3.3 ± 0.3
Baseline	2.2 ± 0.1	2.3 ± 0.2	1.9 ± 0.2	1.9 ± 0.2	2.4 ± 0.2	2.6 ± 0.3
at week 4	2.0 ± 0.1	3.0 ± 0.2*	1.8 ± 0.1	2.6 ± 0.3*	2.1 ± 0.2	3.2 ± 0.3**
at week 8	1.8 ± 0.1	2.7 ± 0.2*	1.7 ± 0.1	2.3 ± 0.2*	1.9 ± 0.2	3.0 ± 0.3**
at week 12	2.0 ± 0.1	3.0 ± 0.2*	1.9 ± 0.2	2.6 ± 0.3	2.0 ± 0.1	3.2 ± 0.3**

Screening : average values obtained at week -2, baseline : average values obtained at week 0; All measurements are represented as mean ± SE
*significantly different from control by *t*-test, $P<0.05$, ** significantly different from control by *t*-test, $P<0.01$

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