Effects of 2 low-fat stanol ester–containing margarines on serum cholesterol concentrations as part of a low-fat diet in hypercholesterolemic subjects[1–3]

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ABSTRACT

Background: Full-fat sitostanol ester–containing margarine reduces serum total and LDL cholesterol, but the effect of plant stanol ester–containing margarine as part of a low-fat, low-cholesterol diet has not been studied.

Objective: We investigated the cholesterol-lowering effects of 2 novel, low-fat stanol ester–containing margarines as part of a low-fat diet recommended for hypercholesterolemic subjects.

Design: In a parallel, double-blind study, 55 hypercholesterolemic subjects were randomly assigned after a 4-wk high-fat diet (baseline) to 3 low-fat margarine groups: wood stanol ester–containing margarine (WSEM), vegetable oil stanol ester–containing margarine (VOSEM), and control margarine (no stanol esters). The groups consumed the margarines for 8 wk as part of a diet resembling that of the National Cholesterol Education Program's Step II diet. The daily mean total stanol intake was 2.31 and 2.16 g in the WSEM and VOSEM groups, respectively.

Results: During the experimental period, the reduction in serum total cholesterol was 10.6% ($P < 0.001$) and 8.1% ($P < 0.05$) greater and in LDL cholesterol was 13.7% ($P < 0.01$) and 8.6% ($P = 0.072$) greater in the WSEM and VOSEM groups, respectively, than in the control group. Serum campesterol concentrations decreased 34.5% and 41.3% ($P < 0.001$) in the WSEM and VOSEM groups, respectively. Serum HDL cholesterol, sitostanol, campestanol, β-carotene, and fat-soluble vitamin concentrations did not change significantly from baseline.

Conclusions: We conclude that the low-fat, plant stanol ester–containing margarines are effective cholesterol-lowering products in hypercholesterolemic subjects when used as part of a low-fat, low-cholesterol diet. They offer an additional, clinically significant reduction in serum cholesterol concentrations to that obtained with a low-fat diet alone. *Am J Clin Nutr* 1999;69:403–10.

KEY WORDS Cholesterol, low-fat diet, plant stanol esters, sitostanol, campestanol, campesterol, apolipoproteins, hypercholesterolemia, margarine, humans

INTRODUCTION

An increased concentration of LDL cholesterol is the main risk factor for atherosclerotic vascular disease. Considerable efforts have focused on different measures to lower elevated concentrations of LDL cholesterol, such as dietary and pharmacologic measures.

Plant sterols, structurally resembling cholesterol, reduce serum cholesterol concentrations by inhibiting the absorption of both dietary and biliary cholesterol from the small intestine (1, 2). Sitostanol, the saturated form of sitosterol, has been shown to be most effective in this respect (2, 3). Because sitostanol is virtually unabsorbable, it has been considered a safe way to reduce elevated serum cholesterol concentrations. Several studies have shown that 2.0–3.0 g sitostanol from full-fat sitostanol ester–containing margarines or mayonnaises significantly reduces serum total and LDL-cholesterol concentrations without affecting HDL-cholesterol or serum triacylglycerol concentrations (4–9). However, the effect of plant stanols delivered in low-fat margarines on elevated cholesterol concentrations as part of a recommended low-fat, low-cholesterol diet (10) has not been studied.

Therefore, we investigated to what extent the 2 low-fat margarines enriched with wood or vegetable oil–based plant stanols would reduce serum total and LDL-cholesterol concentrations as part of a low-fat, low-cholesterol diet and whether or not these 2 low-fat plant stanol ester–containing margarines would lower serum cholesterol concentrations equally.

SUBJECTS AND METHODS

Subjects

Altogether, 91 subjects were screened for the study from the occupational health care system and former studies carried out at the Department of Clinical Nutrition, University of Kuopio, Kuopio, Finland. To be included in the study, subjects had to have a serum total cholesterol concentration of 5.4–7.5 mmol/L; to have a serum triacylglycerol concentration <3.0 mmol/L; to

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TABLE 1
Baseline characteristics of the subjects in the 3 study groups[1]

Variables	WSEM group (n = 8 M, 10 F)	VOSEM group (n = 6 M, 14 F)	Control group (n = 6 M, 11 F)
Age (y)	43.2 ± 8.2	40.8 ± 9.3	46.0 ± 8.2
Weight (kg)			
Men	83.0 ± 9.5	80.5 ± 12.8	80.4 ± 7.5
Women	64.6 ± 11.0	61.9 ± 7.5	68.1 ± 12.2
Body mass index (kg/m^2)	25.6 ± 4.0	24.2 ± 3.0	25.7 ± 3.5
Waist circumference (cm)			
Men	93.4 ± 8.1	94.4 ± 9.3	94.8 ± 8.4
Women	81.2 ± 12.1	78.1 ± 9.4	83.8 ± 12.8
Lipids (mmol/L)			
Total cholesterol	6.36 ± 0.76	6.15 ± 0.79	5.93 ± 0.64
LDL cholesterol	4.36 ± 0.76	4.21 ± 0.89	4.10 ± 0.60
HDL cholesterol	1.36 ± 0.38	1.37 ± 0.32	1.27 ± 0.27
Triacylglycerols	1.42 ± 0.67	1.25 ± 0.39	1.24 ± 0.66
Blood pressure (mm Hg)			
Systolic	123 ± 8	121 ± 11	127 ± 17
Diastolic	79 ± 6	79 ± 9	81 ± 9

[1] $\bar{x}$ ± SD. There were no significant differences among groups. WSEM, wood stanol ester–containing margarine; VOSEM, vegetable oil stanol ester–containing margarine.

be aged 20–60 y; to have normal liver, kidney, and thyroid function; to not be taking any lipid-lowering drugs or other drugs that might affect lipid concentrations; to be willing to participate; and to not be an abuser of alcohol. On the basis of these criteria, 60 subjects were selected for the study. Five subjects dropped out at the beginning of the run-in period for personal reasons. These subjects did not differ in initial serum lipid concentrations, weight, or lifestyle habits from the 55 subjects who completed the study. Five subjects used low-estrogen oral contraceptives, 6 used postmenopausal estrogen medication, and 3 used calcium channel blockers, diuretics, or both for the treatment of hypertension or ischemic heart disease. Ten of the subjects were smokers. The subjects were requested to maintain their weight, alcohol consumption, smoking habits, and physical activity during the study. Baseline characteristics of the subjects are shown in **Table 1**. The study protocol was approved by the Ethics Committee of the University of Kuopio and all subjects gave their informed consent.

Study design

This double-blind, parallel, randomized study consisted of a 4-wk run-in period (high-fat diet) and an 8-wk experimental period (low-fat, low-cholesterol diet). In 2 subjects, the experimental diet period lasted only 6 wk because of a trip abroad.

Routine laboratory measurements were taken at the screening visit and at the last visit of the study to ensure normal health status. In addition, medical history, drug use, smoking habits, alcohol consumption, and physical activity were reviewed with a questionnaire at the same time points. The subjects started the study by following a high-fat diet for 4 wk. At the end of the run-in period, the subjects were randomly assigned into 3 experimental groups: wood stanol ester–containing margarine (WSEM), vegetable oil stanol ester–containing margarine (VOSEM), and control margarine. Smoking and the phase of menstrual cycle were taken into account in the randomization. After randomization, the subjects followed a low-fat diet for the next 8 wk. Fasting blood samples were taken at the beginning of the run-in (−4 wk) and the experimental diet (0 wk) periods and at weeks 2, 4, and 8. Body weight and side effects were recorded at each visit.

Diets

The composition of the low–erucic acid rapeseed oil–based low-fat margarines (Raisio Group, Raisio, Finland) is presented in **Table 2**. The control margarine contained 35% of energy as fat and no added plant stanols. The 2 test margarines contained 40% of energy as fat and were prepared with use of commercially available plant sterols (wood sterols: Ultra sitosterol, Kaukas Oy, Finland; vegetable sterols: derived principally from soy oil, Archer Daniels Midland Co, Decatur, IL) by recrystallization, hydrogenation to form plant stanols, and esterification to produce fatty acid esters of the obtained plant stanols. The subjects consumed 25 g low-fat margarine/d as part of their low-fat, low-cholesterol diet. The theoretical daily intake of stanols was 2.34 g (2.15 g sitostanol and 0.19 g campestanol) in the WSEM group and 2.20 g (1.50 g sitostanol and 0.70 g campestanol) in the VOSEM group. Vitamin A (5.5 μg/g) and vitamin D (0.07 μg/g) were added to all 3 spreads. The subjects received coded tubs of the test margarines when visiting the laboratory and they were asked to record daily the consumption of the test margarines.

During the run-in period, dietary goals were to consume 36–38% of energy as fat (16–18% as saturated, 14% as monounsaturated, and 6% as polyunsaturated fat), 20% as protein, and 40–44% as carbohydrate. During the experimental period, the diet resembled the Step I diet of the National Cholesterol Education Program (10) and provided 28–30% of energy as fat (8–10% as saturated, 12% as monounsaturated, and 8% as polyunsaturated fatty acids), 20% as protein, and 50–52% as carbohydrate. The goal for cholesterol intake was 35.7 and 23.8 mg/MJ during the run-in and experimental periods, respectively. Except for the 3 test margarines, the diets were composed of normal Finnish food items. The fatty acid compositions were adjusted by changing the quality of spreads, vegetable oils, and liquid milk products during the different study periods. During the run-in

TABLE 2
Composition of low-fat wood stanol ester–containing (WSEM), vegetable oil stanol ester–containing (VOSEM), and control margarines[1]

Nutrients	WSEM margarine	VOSEM margarine	Control margarine
		g	
Fat	10.0	10.0	8.8
Total stanols	2.34	2.20	—
Total unsaturated sterols	0.10	0.15	0.05
Fatty acids			
Polyunsaturated	2.05	2.13	2.10
trans Polyunsaturated	0.03	0.05	0.05
Monounsaturated	4.18	4.08	4.13
trans Monounsaturated	0	0	0.03
Saturated	1.10	1.13	2.03

[1] Values are per 25 g spread.

period, a milk-fat based spread (a blend of 0.6 g milk fat and 0.2 g vegetable oil/g spread), a small amount of rapeseed oil, and 1.5%-fat milk were consumed. During the experimental period, a low-fat test margarine, sunflower oil, and skim or 1.0%-fat milk were used. The compliance of the subjects was improved by providing the spreads, vegetable oils, and liquid milk products free of charge.

The subjects received detailed written and oral instructions about the diets, including the precise amounts of food to be eaten and the quality of food, by main food groups. The diets were calculated for 9 energy intakes: 6.7, 7.6, 8.4, 9.2, 10.1, 10.9, 11.8, 12.6, and 13.4 MJ/d. The energy requirement of each subject was estimated from a 4-d food record that subjects completed before the study and by using the Harris-Benedict formula (11), to which energy needs as a result of physical activity were added.

Adherence to the diets was monitored by examining a 4-d (completed on 3 weekdays and 1 weekend day) food record once during the run-in period and 3 times during the experimental period. The subjects recorded their food consumption after consulting a booklet containing photographs of food portions (12) aimed to help them estimate portion sizes. At every study visit, the subjects met a dietitian who advised them on the practical management of the diets and checked their food records. The diets were planned and the nutrients in the food records were calculated by using the MICRO-NUTRICA dietary analysis program (Finnish Social Insurance Institute, Turku, Finland). The values for the food-composition database were taken from Finnish food analyses and international food-composition tables (13).

Laboratory measurements

Venous blood samples were obtained after a 12-h overnight fast. After ultracentrifugation and precipitation (14), enzymatic colorimetric methods were used to determine cholesterol and triacylglycerols from whole serum and separated lipoproteins by using commercial kits (Monotest Cholesterol and Triacylglycerol GPO-PAP; Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany) with a Kone Specific Clinical Analyzer (Kone Ltd, Espoo, Finland).

Serum samples for β-carotene, fat-soluble vitamins, apolipoprotein (apo) A-I, apo B, and plant sterols were stored at −70°C until analyzed at the end of the study. A Kone Specific Clinical Analyzer and apo A-I and apo B reagents from Kone Corporation were used to analyze apolipoproteins based on immunoprecipitation enhanced by polyethylene glycol at 340 nm. β-Carotene and fat-soluble vitamins were analyzed by HPLC (Perkin-Elmer, Norwalk, CT) on a C_{18} column (Waters, Milford, MA) (15, 16). Serum plant sterols were measured by gas-liquid chromatography (model 5890A; Hewlett-Packard, Palo Alto, CA) equipped with a (0.25 mm internal diameter) 25-m fused silica CP-Sil 5-CB capillary column (Chrompack, Raritan, NJ) (17).

Statistical analyses

Statistical analyses were performed with SPSS for WINDOWS 6.0 statistics program (SPSS Inc, Chicago). Normal distribution of variables was checked with the Shapiro-Wilks test (18). Differences in serum lipid variables were analyzed with repeated-measures multivariate analysis of variance (MANOVA) followed by Student's *t* test in between-group analyses and paired *t* test in within-group analyses. Statistical significance for the continuous response variables (serum lipids, fat-soluble vitamins, apolipoproteins, and mean plant sterols) were tested with a single-measurement, simple-factorial ANOVA followed by Student's *t* test. Logarithmic transformations were used when appropriate. If the initial concentration differed significantly among groups, the concentration was adjusted in the between-groups comparisons by dividing the response variable by the initial concentration. In addition, variables that were not normally distributed, even after logarithmic transformation, and noncontinuous variables were tested with the Kruskal-Wallis test, the chi-square test, or Wilcoxon's matched-pairs signed-rank test. Bonferroni adjustment was used to control the overall α level. The results are expressed as means ±SDs.

RESULTS

Baseline characteristics

There were no significant differences in baseline characteristics among the study groups (Table 1). Body weight decreased marginally during the study in all groups (1.2 ± 1.1, 1.2 ± 1.0, and 1.1 ± 1.3 kg in the WSEM, VOSEM, and control groups, respectively; NS among groups). Physical activity and smoking habits remained stable and no side effects were reported.

Feasibility of the diets

The mean consumption of the test margarines was 98.9%, 98.0%, and 98.0% of the scheduled amount in the WSEM, VOSEM, and control groups, respectively. Thus, the actual daily mean stanol intakes were 2.31 ± 0.03 g (2.13 ± 0.03 g sitostanol and 0.19 ± 0.00 g campestanol) in the WSEM group and 2.16 ± 0.12 g (1.47 ± 0.08 g sitostanol and 0.69 ± 0.04 g campestanol) in the VOSEM group. The small differences in the sitostanol and campestanol intakes were significant between the experimental groups ($P < 0.001$).

There were no significant differences in habitual nutrient intakes before the study among the groups. Nutrient intakes during the experimental diet periods remained stable and were not significantly different among the 3 groups (**Table 3**). Furthermore, the dietary goals were well achieved by all groups. In fact, the mean intake of fat, saturated fatty acids, and dietary cholesterol during the experimental period was even lower than the dietary goals. Energy intake was 44–54 kJ/d lower on average during the experimental than during the run-in period.

TABLE 3
Actual composition of the diets during the study in the 3 study groups[1]

Nutrients	Run-in period			Experimental period[2]		
	WSEM group (n = 18)	VOSEM group (n = 20)	Control group (n = 17)	WSEM group (n = 18)	VOSEM group (n = 20)	Control group (n = 17)
Energy (MJ/d)	8.7 ± 2.0	8.4 ± 1.4	8.0 ± 1.9	7.8 ± 1.4	7.7 ± 1.5	7.1 ± 1.3
Fat (% of energy)	40.6 ± 3.6	39.9 ± 4.6	41.1 ± 3.0	26.4 ± 3.3	25.6 ± 3.9	26.5 ± 3.1
Saturated fatty acids (% of energy)	16.8 ± 1.8	16.9 ± 2.1	16.9 ± 1.5	7.0 ± 1.4	6.8 ± 1.7	7.3 ± 1.6
Monounsaturated fatty acids (% of energy)	14.6 ± 1.6	14.3 ± 2.1	15.0 ± 1.4	8.9 ± 1.5	8.1 ± 1.5	8.6 ± 1.4
Polyunsaturated fatty acid (% of energy)	6.4 ± 1.0	5.9 ± 0.8	6.3 ± 0.7	8.3 ± 0.7	8.3 ± 1.2	8.5 ± 1.2
Protein (% of energy)	16.7 ± 1.9	16.0 ± 1.5	17.0 ± 1.5	18.4 ± 1.6	18.1 ± 2.1	19.2 ± 2.1
Carbohydrate (% of energy)	40.1 ± 3.7	40.7 ± 5.0	39.2 ± 2.6	51.2 ± 4.1	51.8 ± 4.9	50.8 ± 4.8
Alcohol (% of energy)	1.4 ± 1.9	2.2 ± 3.2	1.4 ± 1.8	2.6 ± 3.4	3.1 ± 3.9	2.1 ± 2.3
Cholesterol (mg/MJ)	26 ± 7	34 ± 5	38 ± 7	21 ± 7	18 ± 5	19 ± 5
Fiber (g/MJ)	2.9 ± 0.7	2.9 ± 0.8	2.7 ± 0.5	3.7 ± 0.6	4.0 ± 1.1	4.0 ± 1.0
Vitamin A (μg RE/d)	1258 ± 983	1187 ± 635	1140 ± 548	880 ± 394	928 ± 260	972 ± 323
β-Carotene (μg/d)	3725 ± 3437	4142 ± 3320	3804 ± 1826	3259 ± 2201	3388 ± 1384	3056 ± 920
Vitamin E (mg/d)	11.7 ± 2.2	11.0 ± 2.1	11.2 ± 2.5	16.8 ± 3.0	16.7 ± 3.4	16.8 ± 4.1
Vitamin D (μg/d)	3.1 ± 2.5	3.0 ± 2.4	2.4 ± 1.5	4.9 ± 2.3	4.2 ± 2.0	4.1 ± 1.2

[1] $\bar{x}$ ± SD. There were no significant differences among groups. WSEM, wood stanol ester–containing margarine; VOSEM, vegetable oil stanol ester– containing margarine; RE, retinol equivalents.
[2] Nutrient intakes are presented as the mean from the 3 food records.

Serum lipids and lipoproteins

There were no significant differences between baseline (−4 wk) and 0-wk (at randomization) serum lipids and lipoproteins among the 3 groups. During the run-in period, serum total or lipoprotein lipid concentrations did not change significantly in any of the 3 groups. No significant differences were found between men and women and therefore the results are presented for both sexes combined.

Serum total and LDL-cholesterol concentrations decreased significantly within all study groups during the experimental period. Most of the reduction in serum total and LDL-cholesterol concentrations was achieved after 2 wk. The serum total cholesterol concentration decreased by 18.3%, 15.7%, and 7.7% in the WSEM, VOSEM, and control groups, respectively. The reduction was significantly greater in the WSEM (10.6%, $P < 0.001$) and VOSEM (8.1%, $P < 0.05$) groups than in the control group, but no significant differences were found between the 2 experimental groups (**Table 4**). The serum LDL-cholesterol concentration decreased by 23.6%, 18.4%, and 9.9% in the WSEM, VOSEM, and control groups, respectively. There were significant differences only in the absolute (0.73 mmol/L, $P < 0.01$) and percentage (13.7%, $P < 0.01$) reductions in LDL-cholesterol concentrations between the WSEM and the control groups. The difference in percentage reduction in LDL-cholesterol concentration (8.6%) between the VOSEM and control groups was almost significant after Bonferroni correction ($P = 0.072$). Furthermore, there were no significant differences in absolute or percentage changes between the WSEM and VOSEM groups.

Serum HDL-cholesterol concentrations did not change significantly from baseline in any of the study groups, whereas VLDL cholesterol decreased significantly at 8 wk only in the VOSEM group (Table 4). However, there were no significant differences in VLDL-cholesterol concentrations among the groups at the end of the study. Serum VLDL triacylglycerols decreased significantly from baseline only in the WSEM group (Table 4) and serum HDL-triacylglycerol concentrations did not change significantly in any of the groups (data not shown). LDL triacylglycerols at 8 wk (0.30 ± 0.08 mmol/L) were significantly greater than those at baseline (0.27 ± 0.06 mmol/L) in the control group. There were no significant differences in total, VLDL, or LDL triacylglycerols among the groups at the end of the study.

The decrease from baseline in apo B concentrations at 8 wk in the WSEM (by 0.23 ± 0.16 g/L, 19.2%; $P < 0.001$), VOSEM (by 0.15 ± 0.14 g/L, 13.7%; $P < 0.001$), and control (by 0.06 ± 0.01 g/L, 5.2%; $P < 0.05$) groups was significant and paralleled the decrease in LDL-cholesterol concentrations in all groups. Although HDL cholesterol remained unchanged, apo A-I decreased significantly from baseline in the WSEM (by 0.17 ± 0.17 g/L, 9.0%; $P < 0.01$), VOSEM (by 0.15 ± 0.16 g/L, 8.6%; $P < 0.01$), and control (by 0.10 ± 0.16 g/L, 6.1%; $P < 0.05$) groups at 8 wk. Furthermore, the ratio of apo A-I to apo B increased by 14.3% and 8.3% in the WSEM and VOSEM groups, respectively, but the increase was significant ($P < 0.001$) only in the WSEM group.

Serum β-carotene and fat-soluble vitamins

Serum retinol concentrations did not change significantly in the 3 groups. The absolute concentration of serum β-carotene and α-tocopherol concentrations decreased significantly in the WSEM and VOSEM groups, but in the control group the change in serum β-carotene and α-tocopherol concentrations was not significant (**Table 5**). There was a significant difference in the absolute change in serum β-carotene between the experimental groups and the control group; however, the difference in the absolute change in serum α-tocopherol was significant only between the WSEM and control groups. However, there were no significant changes in serum β-carotene or α-tocopherol concentrations among the groups when the values were related to the serum total cholesterol concentration, ie, when vitamin concentrations were divided by serum total cholesterol concentrations. In fact, the ratio of serum α-tocopherol to total cholesterol increased significantly in all groups.

Serum 25-hydroxyergocalciferol (ercalcidiol) concentrations did not change significantly, whereas the absolute concentration of 25-hydroxycholecalciferol (calcidiol) increased significantly in all groups, but the increase was significantly smaller in the WSEM than in the VOSEM group (Table 5). However, there was

TABLE 4
Serum lipids in the 3 study groups during the experimental period[1]

	WSEM group (n = 18)	VOSEM group (n = 20)	Control (n = 17)
Total cholesterol (mmol/L)[2]			
0 wk	6.55 ± 0.78[3]	6.13 ± 0.81	6.06 ± 0.54
4 wk	5.34 ± 0.74	5.38 ± 0.85	5.69 ± 0.56
8 wk	5.34 ± 0.76[4]	5.15 ± 0.78[4]	5.57 ± 0.49[5]
P (MANOVA)[6]	< 0.001	< 0.001	< 0.01
Change (from 0 to 8 wk)[7]	−1.21 ± 0.61[8]	−0.98 ± 0.59[9]	−0.48 ± 0.49
LDL cholesterol (mmol/L)[10]			
0 wk	4.54 ± 0.72	4.25 ± 0.85	4.27 ± 0.59
4 wk	3.50 ± 0.69	3.54 ± 0.69	3.89 ± 0.62
8 wk	3.48 ± 0.77[4]	3.45 ± 0.76[4]	3.82 ± 0.56[5]
P (MANOVA)[6]	< 0.001	< 0.001	< 0.01
Change (from 0 to 8 wk)[7]	−1.06 ± 0.45[11]	−0.80 ± 0.50	−0.45 ± 0.59
HDL cholesterol (mmol/L)			
0 wk	1.44 ± 0.38	1.41 ± 0.38	1.36 ± 0.26
4 wk	1.38 ± 0.30	1.32 ± 0.37	1.35 ± 0.27
8 wk	1.41 ± 0.33	1.36 ± 0.31	1.37 ± 0.26
P (MANOVA)[6]	NS	NS	NS
Change (from 0 to 8 wk)	−0.03 ± 0.17	−0.05 ± 0.18	0.01 ± 0.15
VLDL cholesterol (mmol/L)			
0 wk	0.57 ± 0.35	0.47 ± 0.24	0.42 ± 0.27
4 wk	0.46 ± 0.22	0.51 ± 0.30	0.46 ± 0.26
8 wk	0.45 ± 0.34	0.34 ± 0.18[12]	0.38 ± 0.28
P (MANOVA)[6]	NS	< 0.01	NS
Change (from 0 to 8 wk)	−0.13 ± 0.40	−0.13 ± 0.21	−0.04 ± 0.28
Total triacylglycerols (mmol/L)[10]			
0 wk	1.45 ± 0.70	1.24 ± 0.50	1.25 ± 0.68
4 wk	1.16 ± 0.54	1.36 ± 0.61	1.19 ± 0.59
8 wk	1.26 ± 0.67	1.13 ± 0.45	1.33 ± 0.80
P (MANOVA)[6]	< 0.05	NS	NS
Change (from 0 to 8 wk)	−0.20 ± 0.55	−0.11 ± 0.41	0.08 ± 0.31
VLDL triacylglycerols (mmol/L)			
0 wk	0.96 ± 0.62	0.76 ± 0.49	0.82 ± 0.63
4 wk	0.73 ± 0.52	0.88 ± 0.56	0.76 ± 0.55
8 wk	0.77 ± 0.64[12]	0.63 ± 0.37	0.85 ± 0.75
P (MANOVA)[6]	< 0.05	NS	NS
Change (from 0 to 8 wk)	−0.19 ± 0.50	−0.13 ± 0.38	0.03 ± 0.28

[1] WSEM, wood stanol ester–containing margarine; VOSEM, vegetable oil stanol ester–containing margarine; MANOVA, repeated-measures multivariate analysis of variance.
[2,10] Significant group-by-time interaction (MANOVA): [2] $P < 0.001$, [10] $P < 0.01$.
[3] $\bar{x}$ ± SD.
[4,5,12] Significantly different from 0 wk (paired t test): [4] $P < 0.001$, [5] $P < 0.01$, [12] $P < 0.05$.
[6] Significant difference in overall within-group changes.
[7] Significant difference among groups, $P < 0.01$ (ANOVA).
[8,9,11] Significantly different from control group (Student's t test and Bonferroni correction): [8] $P < 0.001$, [9] $P < 0.05$, [11] $P < 0.01$.

no significant difference in the percentage increase in calcidiol concentrations among the study groups.

Plant sterols

Baseline concentrations of serum sitostanol and campestanol did not change significantly over the 8-wk study period in the WSEM, VOSEM, and control groups: sitostanol (from 4.6 ± 4.3 to 4.8 ± 7.4 μmol/L, 4.3 ± 5.5 to 3.8 ± 5.3 μmol/L, and 5.5 ± 5.3 to 3.8 ± 4.8 μmol/L, respectively); campestanol (from 3.5 ± 3.0 to 3.2 ± 3.2 μmol/L, 4.5 ± 7.4 to 2.7 ± 5.2 μmol/L, and 4.7 ± 7.9 to 5.7 ± 9.2 μmol/L, respectively). Serum campesterol concentrations did not change significantly in the control group but decreased significantly from baseline ($P < 0.001$) in both experimental groups: from 21.7 ± 6.5 to 14.2 ± 6.0 μmol/L (34.5% change) in the WSEM group and from 27.2 ± 18.7 to 16.0 ± 9.5 μmol/L (41.3% change) in the VOSEM group. Furthermore, serum campesterol concentrations were still significantly decreased in both experimental groups after correction for the reduction in serum cholesterol. In addition, serum sitosterol concentrations tended to decrease in both the experimental groups, but not significantly so.

DISCUSSION

In the present study, the wood- and vegetable oil–based plant stanol ester–containing margarines (WSEM and VOSEM groups, respectively), as part of a low-fat diet, reduced more markedly both serum total and LDL-cholesterol concentrations than did

TABLE 5
Serum β-carotene, retinol, α-tocopherol, calcidiol, and ercalcidiol concentrations and ratios of β-carotene to total cholesterol and of α-tocopherol to total cholesterol in the 3 study groups during the experimental period[1]

	WSEM group (n = 18)	VOSEM group (n = 20)	Control group (n = 17)
β-Carotene (μmol/L)[2]			
0 wk	1.66 ± 1.10	1.47 ± 0.79	1.00 ± 0.37
8 wk	1.22 ± 0.97[3]	1.07 ± 0.54[3]	1.06 ± 0.42
Change (from 0 to 8 wk)[4,5]	−0.44 ± 0.57[6]	−0.40 ± 0.54[7]	0.05 ± 0.26
Retinol (μmol/L)			
0 wk	2.50 ± 0.72	2.21 ± 0.81	2.30 ± 0.66
8 wk	2.36 ± 0.68	2.12 ± 0.82	2.21 ± 0.70
Change (from 0 to 8 wk)	−0.14 ± 0.45	−0.09 ± 0.40	−0.09 ± 0.29
α-Tocopherol (μmol/L)[2]			
0 wk	51.49 ± 8.17	45.10 ± 9.68	44.58 ± 9.86
8 wk	45.27 ± 6.97[8]	41.51 ± 9.30[3]	43.45 ± 9.46
Change (from 0 to 8 wk)[4,9]	−6.22 ± 5.04[7]	−3.59 ± 4.29	−1.13 ± 3.34
Calcidiol (nmol/L)[10]			
0 wk	67.47 ± 23.73	62.48 ± 21.57	73.66 ± 44.26
8 wk	80.62 ± 22.96[11]	96.23 ± 33.68[8]	103.19 ± 43.30[8]
Change (from 0 to 8 wk)[9]	13.15 ± 22.75[12]	33.75 ± 25.72	29.53 ± 24.12
Ercalcidiol (nmol/L)			
0 wk	38.14 ± 42.39	41.19 ± 28.99	63.44 ± 32.76
8 wk	49.06 ± 33.58	42.70 ± 34.65	72.00 ± 41.47
Change (from 0 to 8 wk)	10.92 ± 27.87	1.51 ± 22.42	8.56 ± 32.30
β-Carotene:total cholesterol			
0 wk	0.27 ± 0.21	0.24 ± 0.13	0.17 ± 0.07
8 wk	0.24 ± 0.23	0.21 ± 0.10	0.19 ± 0.08
Change (from 0 to 8 wk)	−0.03 ± 0.12	−0.03 ± 0.08	0.02 ± 0.05
α-Tocopherol:total cholesterol			
0 wk	7.86 ± 0.81	7.40 ± 1.41	7.38 ± 1.55
8 wk	8.50 ± 0.90[8]	8.06 ± 1.35[8]	7.80 ± 1.58[11]
Change (from 0 to 8 wk)	0.65 ± 0.50	0.66 ± 0.66	0.42 ± 0.75

[1] $\bar{x}$ ± SD. WSEM, wood stanol ester–containing margarine; VOSEM, vegetable oil stanol ester–containing margarine; MANOVA, multivariate analysis of variance.

[2,10] Significant group-by-time interaction (MANOVA): [2] $P < 0.01$, [10] $P \leq 0.05$.

[3,8,11] Significantly different from 0 wk (paired t test): [3] $P < 0.01$, [8] $P < 0.001$, [11] $P < 0.05$.

[4] Initial concentrations were nearly significantly different by ANOVA (β-carotene, $P = 0.063$; α-tocopherol, $P = 0.053$) among the study groups; therefore, initial concentrations were taken into account in the between-groups comparisons by dividing the response variable by the initial value.

[5,9] Significant difference among groups (ANOVA): [5] $P < 0.01$, [9] $P < 0.05$.

[6,7] Change significantly different from change in control group (Student's t test with Bonferroni correction): [6] $P < 0.05$, [7] $P < 0.01$.

[12] Significantly different from VOSEM group, $P < 0.05$ (Student's t test with Bonferroni correction).

the low-fat diet alone in subjects with elevated serum total cholesterol concentrations. The cholesterol-lowering effects of the 2 plant stanol ester–containing margarine diets did not differ significantly. These findings indicate that low-fat plant stanol ester–containing margarines, when part of a low-fat diet (10), can reduce serum cholesterol concentrations almost as much as cholesterol-lowering drugs (19, 20).

There have been no studies of the effects on serum cholesterol concentrations of plant stanols as part of a strictly and frequently monitored low-fat, low-cholesterol diet. Moreover, earlier studies used full-fat margarines and mayonnaises (4–9), whereas the present study used low-fat stanol ester–containing margarines (40% of energy as fat, including 9% nonabsorbable stanols). In contrast with Denke's study (21), we found that stanol esters can significantly lower serum cholesterol concentrations even in those with a low cholesterol intake. Note that nonesterified sitostanol suspended in safflower oil and packed into gelatin capsules was used in Denke's study.

The novel finding that plant stanols can reduce serum cholesterol concentrations, even in conjunction with a markedly low dietary cholesterol intake, indicates that plant stanols must inhibit not only the absorption of dietary cholesterol but also that of biliary cholesterol. This is supported by the findings of earlier studies of plant stanol (2, 4, 7, 9), in which the fecal excretion of neutral sterols increased despite a constant dietary cholesterol intake. In addition, in the present study the serum campesterol concentration, which is known to reflect intestinal cholesterol absorption (22, 23), decreased significantly in both stanol ester groups, which agrees with the findings of earlier studies (2, 4, 7, 22, 23). In some studies of plant stanol in diabetic subjects (4, 24), the biliary secretion of cholesterol, which normally ranges from 600 to 1000 mg/d (25), was found to increase significantly (11–16%) (4, 24). An average of 50% of the cholesterol that enters the small intestine is reabsorbed (25). Cholesterol absorption was shown to decrease by 60% in diabetic patients with a daily intake of 3 g sitostanol delivered as fatty acid esters (4, 24).

Sitostanol has been shown to be virtually unabsorbable (26–28), but 12.5% of campestanol was found to be absorbed in a study of intestinal perfusion in humans (29). However, the results from the present study indicate that the absorption of campestanol was also negligible when campestanol was fed as part of a stanol blend containing substantial amounts of sitostanol

(65%). In the present study, the serum campesterol concentration decreased significantly and campestanol decreased nonsignificantly in both stanol ester groups. Therefore, the vegetable oil–based sterol blend can be used after saturation to stanol without an increase in serum campestanol concentration. The absorption of campestanol might be possible when it is not ingested as part of a blend containing competitive components like sitostanol. When campestanol is used as part of the stanol blend that contains substantial amounts of sitostanol, as was used in the present study, campestanol seems not to be absorbed at all (30).

The 2 low-fat test margarines were intended to differ from each other only with respect to the origin of the plant stanols, with the VOSEM margarine containing more campestanol and less sitostanol than the WSEM margarine. However, the actual daily intake of total plant stanol was 6.5% higher in the WSEM than in the VOSEM group. The cholesterol-lowering effect of sitostanol is well documented in the literature, but the effects of campestanol have not been studied, probably because of practical problems in obtaining pure campestanol in reasonable amounts. However, it has been shown in rats (31) that the oleate ester of campesterol can decrease the absorption of dietary cholesterol with the same efficacy as free β-sitosterol, stigmasterol, or the oleate ester of β-sitosterol. Furthermore, recent data from free-living humans indicate that rapeseed oil–derived campesterol could reduce cholesterol absorption and thus reduce serum cholesterol concentration (32). On the basis of these data, campestanol can also be expected to reduce cholesterol absorption. Thus, the difference in stanol compositions is not likely to have an effect on the present results.

On the basis of the food records during both study periods the adherence to the diets was good. Actually, during the low-fat diet the intakes of fat, saturated fatty acids, and dietary cholesterol were even lower than the dietary goals. Note that the intake of dietary cholesterol achieved the goal of the Step II diet of the National Cholesterol Education Program (<200 mg/d) (10), and the intake of saturated fatty acids was close to these goals (<7% of energy) in all study groups. Despite the frequent monitoring, there was a slight decrease in body weight in all study groups during the experimental period. The decrease in weight was primarily due to the lower intake of energy during the experimental than during the run-in period. However, because the weight change was marginal in all groups and because there were no significant differences in weight change among the groups, the decrease in weight cannot explain the findings of the present study.

Low-fat stanol ester–containing margarines appeared to have little effect on serum concentrations of retinol and ercalcidiol. The serum absolute concentration of β-carotene and α-tocopherol decreased significantly in both the stanol ester–containing margarine groups, but this would be expected because β-carotene and α-tocopherol are transported in serum in lipoproteins, whose concentrations decreased during the experimental diet periods. When the serum β-carotene concentrations were related to the serum total cholesterol concentrations, the decrease was not significant in either of the low-fat stanol ester–containing margarine groups. In addition, the decrease in serum α-tocopherol concentration was ascribed to the changes in serum cholesterol concentrations because the ratio of serum α-tocopherol to total cholesterol actually increased significantly in all of the test margarine groups. These findings agree with the findings of Gylling et al (33). The increase in calcidiol concentrations was significantly smaller in the WSEM than in the VOSEM group. However, there were no significant differences among the groups in percentage changes in calcidiol or absolute calcidiol concentrations at the end of the study.

In conclusion, both the low-fat WSEM and VOSEM margarines when used as part of a low-fat, low-cholesterol diet are effective in reducing serum cholesterol concentrations with apparently equal efficacy in subjects with elevated serum cholesterol concentrations. In addition, these margarines offer an additional, clinically significant reduction in serum cholesterol concentrations to that obtained with a low-fat diet alone.

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