European Journal of Clinical Nutrition (2000) 54, 715–725
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www.nature.com/ejcn

Comparison of the effects of plant sterol ester and plant stanol ester-enriched margarines in lowering serum cholesterol concentrations in hypercholesterolaemic subjects on a low-fat diet

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Objective: To investigate cholesterol-lowering effects of stanol ester (STAEST) and sterol ester (STEEST)-enriched margarines as part of a low-fat diet.
Design: According to a Latin square model randomized double-blind repeated measures design with three test margarines and three periods.
Setting: Outpatient clinical trial with free-living subjects.
Subjects: Thirty-four hypercholesterolaemic subjects completed the study.
Interventions: Subjects consumed three rapeseed oil-based test margarines (STAEST, STEEST and control (no added stanols or sterols)) as part of a low-fat diet each for 4 weeks.
Results: Mean daily intake of total plant sterols plus stanols was 2.01 – 2.04 g during the two test margarine periods. In reference to control, serum total cholesterol was reduced by 9.2 and 7.3% with the STAEST and STEEST margarine, respectively ($P<0.001$ for both). The respective reductions for low-density lipoprotein (LDL) cholesterol were 12.7 and 10.4% ($P<0.001$). The cholesterol-lowering effects of the test margarines did not differ significantly. The presence of apolipoprotein E4 allele had a significant effect on LDL cholesterol response during the STAEST margarine only. Serum sitosterol and campesterol increased by 0.83 and 2.77 mg/l with the STEEST ($P<0.001$), respectively and decreased by 1.18 and 2.60 mg/l with the STAEST margarine ($P<0.001$). Increases of serum sitostanol and campestanol were 0.11 and 0.19 mg/l with the STAEST margarine ($P<0.001$), repsectively. No significant changes were found in serum fat-soluble vitamin and carotenoid concentrations when related to serum total cholesterol.
Conclusions: STAEST and STEEST margarines reduced significantly and equally serum total and LDL cholesterol concentrations as part of a low-fat diet.
Sponsorship: Grant to the University of Kuopio by Raisio Benecol Ltd, Raisio, Finland.
Descriptors: cholesterol-lowering diet; hypercholesterolaemic; plant sterols; plant stanols; carotenoids; vitamins; apolipoprotein E
European Journal of Clinical Nutrition (2000) **54,** 715–725

Introduction

Great interest has been focused on the cholesterol-lowering properties of plant sterols and stanols (Jones *et al*, 1997; Pollak & Kritchevsky, 1981), and there are now commercial products available aimed at helping to reduce elevated serum total cholesterol concentrations. Based on previous studies 2.0 – 3.0 g/day plant stanols from full-fat sitostanol ester margarine or mayonnaises as part of a moderately rich or high-fat diet significantly reduces serum total and low-density lipoprotein (LDL) cholesterol concentrations without affecting high-density lipoprotein (HDL) cholesterol or triglyceride concentrations (Gylling *et al*, 1995,1997; Gylling & Miettinen, 1994; Miettinen *et al*, 1995; Niinikoski *et al*, 1997; Vanhanen *et al*, 1994). In our own study (Hallikainen & Uusitupa, 1999), low-fat stanol ester margarines, consumed as part of a recommended low-fat, low-cholesterol diet, reduced serum LDL cholesterol by 18.4 – 23.6% as compared to the high-fat baseline diet, the additional effect of stanol ester margarine being 8.6 – 10.6%. Sitostanol has been suggested to have greater hypocholesterolaemic activity than sitosterol (Becker *et al*, 1993; Heinemann *et al*, 1986). In one single study with normolipidaemic subjects, a soybean sterol ester margarine, based on unhydrogenated soysterols with an esterification degree of 65%, was found to be as effective as a stanol ester margarine in lowering plasma cholesterol concentrations (Weststrate & Meijer, 1998). In that trial, subjects followed their own habitual diet except that the habitually used spreads were replaced by test margarines. However, efficacy of plant sterol ester margarine in comparison to stanol ester margarine with matching fatty acid compositions, equal esterification degree (>98.5%) and equal daily intake of total

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Contributors: MAH was responsible for the study design, gave nutrition counselling, analysed and interpreted the data and wrote the manuscript. MIJU and ESS contributed to the planning of study design and diets, interpreting the data and writing the manuscript. HG analysed serum cholesterol precursors and plant sterols, and ATE analysed fatty acid composition of serum lipids and, in addition, both also contributed to interpreting the data and writing the manuscript.

sterols (unsaturated + saturated sterols) has not been studied in connection with a low-fat, cholesterol-lowering diet.

Plant stanols have been suggested to be almost nonabsorbable (Hassan & Rampone, 1979; Lütjohann *et al*, 1995), but recent studies indicate that they may be poorly absorbed from the small intestine (Gylling *et al*, 1999a; Gylling & Miettinen, 1999). Plant sterols are absorbed from 5% (sitosterol) to 15% (campesterol) (Heinemann *et al*, 1993; Salen *et al*, 1970). Furthermore, plant sterols and stanols may interfere with the absorption of carotenoids, as indicated by reduced serum carotenoid concentrations (Gylling *et al*, 1999b; Hallikainen *et al*, 1999, 2000; Hallikainen & Uusitupa, 1999; Hendriks *et al*, 1999; Weststrate & Meijer, 1998). This has to be taken into account in long-term use of plant sterol- and stanol-containing products emphasizing the use of a generally recommended diet.

Therefore, in the present study we investigated whether or not the two margarines enriched with plant sterol esters or stanol esters are equal in lowering serum total and LDL cholesterol concentrations and to what extent these two margarines would reduce serum cholesterol concentrations as part of a low-fat diet in reference to the low-fat diet alone. Additionally serum plant sterol and stanol concentrations were studied. As a *post-hoc* analysis we also investigated the effects of the main apolipoprotein E genotypes on lipid responses.

Methods

Subjects

Altogether 42 subjects (20 men/22 women) with mild to moderate hypercholesterolaemia were recruited to the study from the former studies carried out at the Department of Clinical Nutrition, University of Kuopio and from the occupational health care system. In addition, employees of the city of Kuopio were recruited to the study. The main inclusion criteria were as follows: serum total cholesterol 4.8–7.0 mmol/l and total triglycerides below 2.5 mmol/l at screening. Other inclusion criteria were age 30–65 y, normal liver, kidney and thyroid function, no lipid lowering medication, no unstable coronary heart disease, no diabetes, no gastrointestinal diseases, no alcohol abuse (>45 g ethanol/day) and no irregular eating habits. Eight subjects dropped out during the study: one at the beginning of the pre-trial period due to poor compliance; four at the end of the pre-trial period due to personal reasons or poor compliance; and three during the first period due to personal reasons. Altogether 34 subjects completed the study. In the beginning of the study their mean age was 48.8 ± 8.1 y (mean $\pm$ s.d.), their mean body mass index (BMI) was $24.9 \pm 2.4\,kg/m^2$, and their mean systolic and diastolic blood pressure was 124 ± 14 and 81 ± 9 mmHg, respectively. Baseline total-, LDL-, HDL- and VLDL-cholesterol and triglyceride concentrations were 6.24 ± 0.83, 4.43 ± 0.81, 1.60 ± 0.31, 0.22 ± 0.21 and 1.11 ± 0.52 mmol/l, respectively.

One subject used low-oestrogen oral contraceptives, two subjects had hormone-releasing intra-uterine devices (IUD), one subject used hormone replacement therapy, three subjects used postmenopausal oestrogen therapy, one had thyroxin therapy for hypothyroidism, one used a calcium channel blocker, one used a renin–angiotensin system-affecting medication and two used anti-thrombolytic medication. Two subjects were smokers. The subjects were requested to maintain their medication, weight, alcohol consumption, smoking habits and physical activity constant during the study.

The subjects gave written consent for the study and the study protocol was approved by the Ethics Committee of the University of Kuopio.

Study design

The study was carried out from September to December 1998 applying a randomized double-blind repeated measures design with three test spreads (stanol ester (STAEST), sterol ester (STEEST) and control margarine). Each period lasted for 4 weeks. The randomization was made according to the model of Latin square design. Before randomization during the first 2 weeks the subjects followed a standardized low-fat diet and their diet was monitored. The pre-trial period made it possible to evaluate the inclusion criteria and compliance with the study.

Routine laboratory measurements were taken to ensure normal health status at the first and last visits of the study. In addition, previous and present diseases, current medication, alcohol and tobacco consumption, physical activity, use of vitamins or other nutrient supplements were interviewed by a structured questionnaire at the first visit of the study. Alcohol and tobacco consumption and physical activity were reviewed also at the last visit. Furthermore, possible changes in health, medication, use of vitamin or nutrient supplements were recorded during the study. Fasting blood samples were taken at the beginning of the pre-trial period (−2 weeks), at the beginning of the first experimental period (0 weeks), at the middle and the end of each period (2, 4, 6, 8, 10 and 12 weeks). Body weight was recorded at each visit. The possible adverse effects and symptoms were interviewed based on a structured questionnaire at the end of each period.

Diet

The composition of low erucic acid rapeseed oil (LEAR)-based margarines (Raisio Group, Raisio, Finland) is presented in Table 1. The total amount of fat was 80% and 71%, and amount of absorbable fat excluding sterols and stanols was 70% and 71% in test margarines and control margarine, respectively. STAEST margarine was prepared from wood and vegetable sterols (DRT, Les Derives Resiniques & Terpeniques Granel S.A. Dax Cedex, France and Archer Daniels Midland Co, Decatur, IL, respectively) by recrystallization, hydrogenation to form plant stanols, and esterification to produce low erucic acid rapeseed oil based fatty acid esters of plant stanols. In turn STEEST margarine was prepared from vegetable oil based sterols (Archer Daniels Midland Co, Decatur, IL) by recrystallization, and esterification with low erucic acid rapeseed oil based fatty acid esters to produce fatty acid esters of plant sterols. The daily dose of the test margarine was 20 g taken in two to three portions with meals. The theoretical daily amount of total sterols and stanols was 2.02 g (0.10 g total sterols and 1.92 g total stanols) in the STAEST margarine and 2.06 g (1.98 g total sterols and 0.09 g total stanols) in the STEEST margarine. The control margarine and the margarine consumed during the pre-trial period contained naturally small amounts of sterols (about 0.09 g/daily dose of margarine). All three margarines were fortified with vitamin A (870 μg RE/100 g) and vitamin D

Table 1 Composition of daily dosage (20 g) of test margarines

Nutrients (g/20 g margarine)	*Control margarine*	*STAEST margarine*	*STEEST margarine*
Total fat	14.2	16.2	16.0
Absorbable fat[a]	14.2	14.0	14.0
Fatty acids:			
Saturated	3.2	3.3	3.3
Lauric (C 12:0)	0.3	0.4	0.5
Myristic (C 14:0)	0.2	0.2	0.3
Palmitic (C 16:0)	3.3	3.3	3.3
Stearic (18:0)	0.6	0.5	0.5
Monounsaturated	7.1	7.0	6.9
Oleic (C 18:1 *cis*)	10.2	10.1	9.9
Polyunsaturated	3.1	3.1	3.2
Linoleic (C 18:2 *cis*)	3.2	3.3	3.3
Linolenic (C 18:3 *cis*)	1.3	1.3	1.4
Total stanols	0	1.92	0.09
Sitostanol	0	1.43	0.06
Campestanol	0	0.49	0.02
Total sterols	0.09	0.10	1.98
Brassicasterol	0.01	—	0.06
Campesterol	0.03	0.04	0.57
Sitosterol	0.04	0.06	1.00
Stigmasterol	—	—	0.34
Total sterols and stanols	0.09	2.02	2.06

STAEST = stanol ester margarine and STEEST = sterol ester margarine.
[a]Absorbable fat = total fat − total sterol and stanols.

(7 μg/100 g). This kind of fortification of margarines is a normal procedure in Finland.

The subjects received the coded tubs of test margarines when visiting the study unit. To verify the precise daily dose of test spread, 142 g, ie weekly dose of the test and control margarines, was packed into one tub and subjects were advised to mark the fat spread with a knife into seven equal parts in advance. One tub of test spread per each week was delivered and one extra tub for occasions of unexpected loss or damage of test products. The subjects were asked to record the use of test fats daily, and to return the empty and partly empty tubs and the extra tub of test spread to the study unit at the end of each period. The packages and the test spread left over were weighed and recorded.

Subjects followed a low-fat diet (step 1) of the National Cholesterol Education Program (1994) throughout the study. The planned composition of the diet was: <30 of energy percent (E%) from fat including 8–10 E% saturated, 12–14 E% monounsaturated and 5–7 E% polyunsaturated fat, and <300 mg/day dietary cholesterol. The diet was composed of normal Finnish food items. All subjects received individual oral and written instructions on the diet, including the precise amounts and quality of foods as main food groups: fats, dairy products, meat and meat products, cereals, fruits and berries, and vegetables and roots. The diet plan was made for eight energy levels: 6.7–12.6 MJ/day. The energy requirement of a subject was estimated according to the Harris Benedict formula with the energy requirement due to physical activity added (Alpers *et al*, 1986). If necessary the energy intake level was changed in order to ensure unchanged body weight during the study. The feasibility of the diet was improved by providing test margarines, rapeseed oil, salad dressing and low-fat cheese for the participants free of charge.

Adherence to the low-fat diet was monitored by 4-day food records kept at the end of each period, four times altogether during the study. One of the recording days was a weekend day or the person's day off from work. The subjects recorded their food consumption using a portion-size booklet with photos to estimate the portion size (Haapa *et al*, 1985). At study visits the amounts and qualities of foods in the records were checked by the nutritionist for completion, filling in data that were lacking. Fatty acid composition of serum lipids was determined as an objective marker of dietary adherence.

The diet was planned and the nutrients in the food records were calculated using the Micro-Nutrica® dietary analysis program (version 2.0, Finnish Social Insurance Institute, Turku, Finland). The food composition database is based on analyses of the Finnish food and international food composition tables (Rastas *et al*, 1993). In addition, the database was updated for the purposes of the present study.

Laboratory measurements

Systolic and diastolic blood pressure was measured by a mercury sphygmomanometer (Mercurius Stator, Spiedel + Keller, Germany) after subjects had rested for 5–10 min. Two measurements were taken and the mean of them used in the analyses. Body weight was measured with a digital scale. All measurements were done and venous blood samples were obtained after a 12 h overnight fast using standardized methods. Since the phase of the menstrual cycle may have an effect on serum cholesterol concentration (Cullinane *et al*, 1995), the end measurements were performed at days 5–10 of the cycle in those women with the menstrual cycle.

Routine laboratory samples were analysed with standardized methods at the Kuopio University Hospital. Plasma glucose was analysed by enzymatic photometric method using reagent Granutest 100 (Merck, Damstadt, Germany) with a Kone Specific Clinical Analyser (Kone Ltd, Espoo, Finland).

Lipoproteins were separated by ultracentrifugation for 18 h at density 1.006 to remove very low density lipoprotein (VLDL). HDL in the infranatant was separated from LDL by precipitation of LDL with dextran sulphate and magnesium chloride (Penttilä *et al*, 1981). LDL cholesterol was calculated as a difference between the mass of cholesterol in the infranatant and HDL, and VLDL cholesterol was calculated as a difference between the whole serum and the infranatant. Enzymatic photometric methods were used for the determination of cholesterol and triglycerides from whole serum and separated lipoproteins using commercial kits (Monotest® Cholesterol and Triglyceride GPO-PAP, Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany) with a Kone Specific Clinical Analyser (Kone Ltd, Espoo, Finland). The coefficient of variance (CV) between measurements for serum total cholesterol was 1.3–1.4%, for triglycerides 1.7–1.9% and for HDL cholesterol 1.1–1.2%.

Serum samples for analysis of apolipoprotein (apo) A-I and B, carotenoids and fat soluble vitamins, cholesterol precursors, plant sterols and cholestanol, and fatty acid composition were stored at −70°C until analysed at the end of the study. Analyses of apolipoproteins were based on the measurement of immunoprecipitation enhanced by polyethylene glycol (PEG) at 340 nm. A Kone Specific Clinical Analyser and apo A-I and apo B reagents from Kone Corporation (Espoo, Finland) were used. Serum carotenoids, retinol and tocopherols were analysed by the high-performance liquid chromatography system (Perkin-Elmer, Norwalk, CT) on a C_{18} column (Waters, Milford, MA)

(Kaplan *et al*, 1987; Parviainen, 1983) using an external standard. Serum 25-hydroxyvitamin D_3 was analysed with a radioimmunoassay method (25-Hydroxyvitamin D I125 RIA KIT, DiaSorin, Stillwater, MN) using an external standard.

Serum cholesterol precursors (Δ8-cholestenol, Δ7-lathosterol, desmosterol and squalene), plant sterols (sitosterol, sitostanol, campesterol, campestanol and avenasterol) and cholestanol, a metabolite of cholesterol, were quantified from nonsaponifiable serum materials by capillary gas–liquid chromatograph (GLC, HP 5890 Series II, Hewlett Packard, Delaware) equipped with a 50 m long Ultra 1 capillary column (methyl-polysiloxane) (Hewlett Packard, USA) for cholestanol, squalene, Δ8-cholestenol, Δ7-lathosterol, campesterol and sitosterol, and equipped with a 50 m long Ultra 2 capillary column (5% phenyl-methyl siloxane, Hewlett Packard, USA) for sitostanol and campestanol (Miettinen 1988, Miettinen & Koivisto 1983). Serum cholesterol precursors, plant sterols and cholestanol were determined in duplicate from the same samples and the mean value of two measurements was used in the statistical analyses.

In the analysis of serum fatty acid composition lipids were extracted with chloroform–methanol (2 : 1) (Ågren *et al*, 1992), and lipid fractions (cholesteryl esters, triglycerides and phospholipids) were separated with an amino-propyl column. Fatty acids were analysed with a gas-chromatograph (Hewlett-Packard 5890 series II, Hewlett-Packard Company, Waldbronn, Germany) equipped with FFAP-column (length 25 m, inner diameter 2 mm and film thickness 0.3 μm). Fatty acids are presented as molar percentage of total fatty acids.

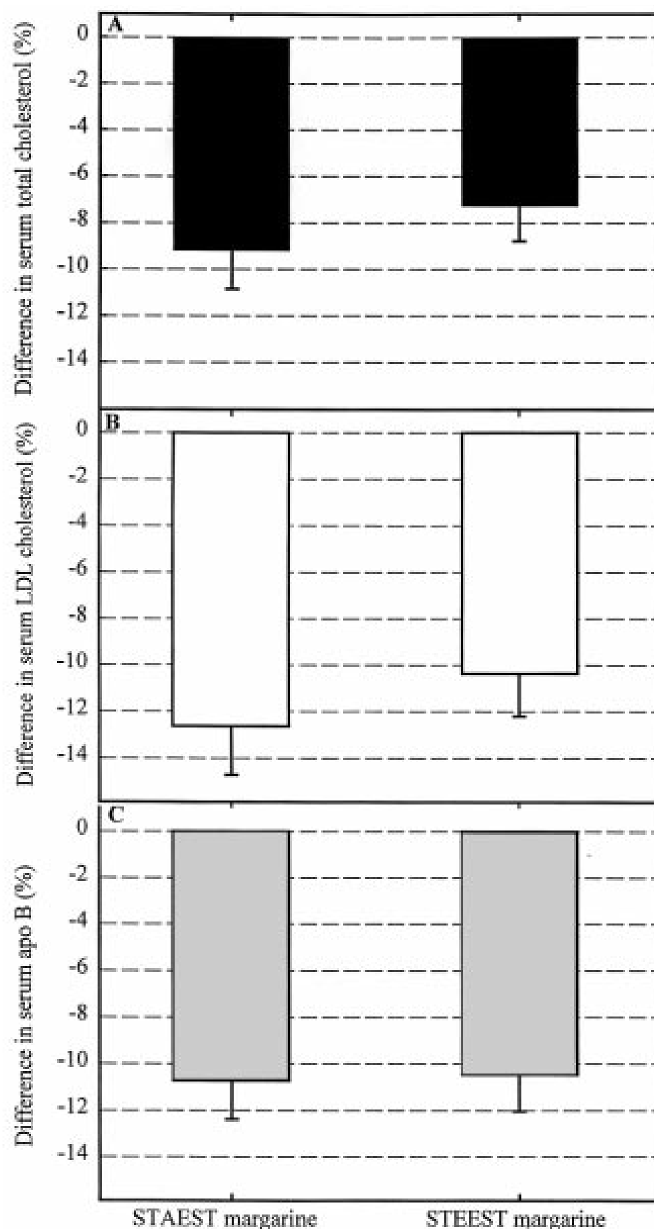

Figure 1 Differences in serum total (A) and LDL cholesterol (B), and apo B (C) concentrations (%) in reference to the control margarine period; $n = 34$, values are means ± s.e.m. There were no significant differences in serum lipid and lipoprotein responses between the two test margarine periods analysed with analysis of variance for repeated measurements (GLM). STAEST = stanol ester margarine and STEEST = sterol ester margarine.

Apolipoprotein E genotypes were determined by polymerase chain reaction (PCR) using primers described previously by Tsukamoto *et al* (1993). PCR amplification of apolipoprotein E polymorphism was conducted in a 16 µl volume containing 50 ng of genomic DNA, 0.4 pmol/l of each primer, 10 mmol/l Tris–HCl (pH 8.8), 50 mmol/l KCl, 1.5 mmol/l of $MgCl_2$, 0.1% Triton X-100, 113 µmol/l dNTPs, 0.7 units of DNA polymerase (DynaZyme DNA polymerase, Finnzymes, Espoo, Finland), and 8.5% glycerol to enhance amplification and annealing of GC-rich primers. Conditions for amplification were denaturation at 96°C for 4 min, followed by 35 cycles of denaturation at 96°C for 45 s, annealing at 64°C for 45 s, and extension at 72°C for 60 s with final extension at 72°C for 4 min. The PCR product was digested with *Hha*I (New England Biolabs, Beverly, MA). The digested DNA fragments were separated on 12% polyacrylamide gel. Finally, separated DNA fragments were visualized by ethidium bromide staining.

Statistical analyses
All statistical analyses were performed with SPSS for Windows 7.5 statistics program (SPSS, Chicago, IL, USA). The results are given as means ± s.d., except in Figure 1, where they are given as means ± s.e.m.

The main comparison was made among the mean values at the end of each experimental period. In the results and discussion sections only the end measurements, and their absolute or percentage changes, are presented. The percentage changes were computed comparing the end measurements of both test margarine periods to the end measurement of the control margarine period. To eliminate the effects of changes in lipoprotein concentrations, serum carotenoid, tocopherol, cholesterol precursor, plant sterol and cholestanol values are given, besides the crude concentrations, also in terms mmol/mol of cholesterol or 10^{-3} mg/mg of cholesterol, which express the ratios to total cholesterol.

Normal distribution was checked with Shapiro Wilks test, and homogeneity of variance was checked with analysis of variance for repeated measurement (GLM) before further analyses. If a variable was not normally distributed the statistical analysis was made after logarithmic transformation. GLM was used to compare the overall changes and the effect of the order of spread consumption periods, carry-over effect and gender on the main end-point variables among the different experimental margarine periods. GLM was also used in the further analyses. Confidence intervals (CI) presented in the text for the percentage changes of total and LDL cholesterol and apo B were not corrected for the above-mentioned factors.

Intake of nutrients and serum fatty acid composition among the experimental periods was analysed with GLM and a paired *t*-test, or if nutrient data or fatty acid data was not normally distributed even after the logarithmic transformation Friedman two-tailed ANOVA test was used. To control the overall α level, Bonferroni adjustment was used. The Wilcoxon matched-paired signed rank test was used to compare the change of alcohol consumption, smoking habits and physical activity. Routine laboratory examinations were tested with paired *t*-test or Wilcoxon matched-paired signed rank test.

The number of subjects recruited for the study was based on an assumption of having 0.2 mmol/l difference in serum LDL cholesterol response among the test spreads significant with an α level of 0.05 ($P<0.05$), and with 0.80 statistical power (Cohen, 1988).

Results

Baseline characteristics
There were no significant changes in BMI (24.9 ± 2.3, 24.8 ± 2.4 and 24.8 ± 2.4 kg/m^2 during the control, STAEST and STEEST margarine periods, respectively) or systolic and diastolic blood pressure during the study. Physical activity, alcohol consumption and smoking habits remained stable. A woman who used low-oestrogen oral contraceptives stopped use at the end of the first study period. Excluding her from the statistical analyses did not affect the results.

To ensure normal health status during the study, samples for routine laboratory measurements were drawn in the beginning (−2 weeks) and at the end (12 weeks) of the study. There was a small but significant decrease in the mean fasting plasma glucose (from 5.53 ± 0.41 to 5.35 ± 0.41 mmol/l) and serum γ-glutamyltransferase (from 25.8 ± 20.3 to 21.3 ± 12.5 U/l) values during the study. The mean serum TSH concentration increased slightly (from 1.7 ± 1.3 to 1.9 ± 1.2 mU/l, $P<0.05$). One subject had a marginally elevated serum TSH concentration (7.3 and 6.1 mU/l, at the beginning and end of the study, respectively). Excluding him from statistical analyses did not affect the results. The changes in routine laboratory measurements cannot be connected with the test margarines. In men the mean blood haemoglobin concentration decreased slightly (from 144.4 ± 8.1 to 141.2 ± 6.3 g/l) but significantly due to the fact that seven of the men also participated in a fat-loading test with many blood samples at the end of all test margarine periods. In women blood haemoglobin concentration remained unchanged during the entire study. Other routine measurements, blood thrombocytes, serum alanine aminotransferase and creatinine, did not change significantly during the study.

Feasibility of the diet
The mean daily consumption of margarine according to the weighed returned tubs was 19.2–19.9 g during the different margarine periods. The actual mean daily intake of total sterols and stanols was 0.09 ± 0.03 g (0.09 ± 0.03 g total sterols and 0 g total stanols), 2.01 ± 0.06 g (0.10 ± 0.03 g total sterols and 1.91 ± 0.05 g total stanols) and 2.04 ± 0.14 (1.96 ± 0.13 g total sterols and 0.09 ± 0.06 g total stanols) during the control, STAEST margarine and STEEST margarine periods, respectively.

The actual composition of the diet during the different test margarine periods is presented in Table 2. The goal for the composition of the low-fat diet was well achieved (Table 2). Generally, there were no significant differences in the nutrient intakes among the periods.

The results of the fatty acid composition of serum cholesteryl esters paralleled with the food records (Table 3). In general, there were no major differences in the fatty acid composition during the different test margarine periods. However, the proportion of oleic acid was slightly, but

Table 2 Actual composition of the diets during the different test margarine periods

Nutrients	Period: Control margarine (n = 34)	STAEST margarine (n = 34)	STEEST margarine (n = 34)	P-values[a]
Energy (MJ/day)	8.3 ± 1.9	8.3 ± 1.8	8.1 ± 1.7	0.318
Fat (percentage total energy intake, E%)	30.0 ± 3.7	30.5 ± 3.2	31.1 ± 3.9	0.100
Saturated fatty acids (E%)	8.2 ± 1.9	8.4 ± 1.8	8.8 ± 2.0	0.347
Monounsaturated fatty acids (E%)	12.4 ± 1.6	12.5 ± 1.5	12.7 ± 1.9	0.648
Polyunsaturated fatty acids (E%)	6.7 ± 0.9	6.6 ± 0.8	6.9 ± 0.9	0.069
Proteins (E%)	16.1 ± 2.3	16.9 ± 2.1	17.2 ± 2.7	0.045
Carbohydrates (E%)	49.6 ± 5.4	49.1 ± 5.2	48.3 ± 4.8	0.916
Alcohol (E%)	2.9 ± 4.1	2.1 ± 3.3	2.0 ± 2.5	0.642
Cholesterol (mg/d)	166 ± 88	173 ± 66	179 ± 66	0.098
Cholesterol (mg/MJ)	19.6 ± 7.6	20.5 ± 4.9	22.2 ± 6.6	0.061
Fibre (g/day)	30.3 ± 8.4	30.1 ± 7.5	28.9 ± 9.5	0.443
Fibre (g/MJ)	3.7 ± 1.0	3.7 ± 0.9	3.6 ± 0.9	0.445
Vitamin A (μg RE/day)[b]	1337 ± 878	1188 ± 544	1139 ± 736	0.452
β-carotene (μg/day)	4155 ± 2732	4726 ± 3120	4056 ± 2638	0.413
Vitamin D (μg/day)	4.4 ± 2.7	5.7 ± 3.8	6.1 ± 4.9	0.318
Vitamin E (mg/day)	12.3 ± 3.3	12.5 ± 2.8	12.0 ± 3.2	0.434

Values are means ± s.d. STAEST = stanol ester margarine and STEEST = sterol ester margarine.
[a]Variables were analysed with Friedman two-way ANOVA test (energy, saturated fatty acid, carbohydrates, alcohol, vitamin A and vitamin D) or analysis of variance for repeated measurements (GLM) and paired *t*-test with Bonferroni correction. In pairwise comparisons after Bonferroni correction there were no significant differences in protein intake between any two periods.
[b]RE = retinol equivalents.

significantly lower at the end of the STEEST margarine period than at the end of the control period. In addition, the proportion of stearic acid was significantly lower at the end of both test margarine periods than at the end of the control period. There were no significant differences in the fatty acid composition of triglycerides or phospholipids during the study (data not shown).

Serum lipids and lipoproteins
The concentrations of serum lipids and lipoproteins at the end of each experimental periods are shown in Table 4. Figure 1 presents the percentage differences in serum total and LDL cholesterol and apo B compared to the control margarine period.

No significant differences were found in the concentrations of serum lipids between genders ($P = 0.095$) or among orders of spread consumption ($P > 0.1$). In addition, no carry-over effect was found ($P > 0.1$).

Serum total and LDL cholesterol concentrations were significantly lower at the end of STAEST and STEEST margarine periods than at the end of the control period (Table 4). Compared to the control period the mean decreases in serum total cholesterol were 9.2 ± 9.7% (CI −12.6, −5.8) and 7.3 ± 8.9% (CI −10.4, −4.2) during the STAEST and STEEST margarine periods, respectively. For LDL cholesterol the mean decreases were 12.7 ± 12.2% (CI −16.9, −8.4) and 10.4 ± 10.6% (CI −14.1, −6.7), respectively. There were no significant differences in the decreases of serum total and LDL cholesterol concentrations between the two test margarine periods (difference 1.9 ± 8.4% and 2.3 ± 11.7%, respectively).

Serum HDL and VLDL cholesterol, and triglyceride concentration did not change significantly during the entire study (Table 4).

There were no significant changes in serum apo AI concentration (Table 4) during the study. The changes in

Table 3 Serum fatty acid composition of cholesteryl esters during the different test margarine periods

Fatty acid (mol %)	Period: Control margarine (n = 34)	STAEST margarine (n = 34)	STEEST margarine (n = 34)	P values[a]
Myristic acid 14:0	1.17 ± 0.33	1.14 ± 0.36	1.22 ± 0.42	0.526
Palmitic acid 16:0	12.46 ± 0.87	12.37 ± 0.86	12.45 ± 0.89	0.699
Palmitoleic acid 16:1	3.80 ± 0.88	3.68 ± 0.91	3.58 ± 0.80	0.233
Stearic acid 18:0	0.78 ± 0.15	0.67 ± 0.13*	0.67 ± 0.13*	<0.001
Oleic acid 18:1 n-9 + n-7	21.22 ± 1.50	21.07 ± 1.49	20.66 ± 1.52†	0.007
Linoleic acid 18:2 n-6	51.45 ± 3.21	52.27 ± 3.27	52.20 ± 3.37	0.142
γ-linolenic acid 18:3 n-6	0.65 ± 0.29	0.63 ± 0.32	0.59 ± 0.26	0.313
α-linolenic acid 18:3 n-3	1.07 ± 0.20	1.00 ± 0.16	0.99 ± 0.23	0.282
Dihomo-γ-linolenic acid 20:3 n-6	0.52 ± 0.15	0.49 ± 0.10	0.53 ± 0.17	0.360
Arachidonic acid 20:4 n-6	4.58 ± 0.95	4.50 ± 0.89	4.63 ± 0.82	0.374
Eicosapentanoic acid 20:5 n-3	1.68 ± 1.00	1.57 ± 0.66	1.86 ± 1.04	0.065
Docosahexanoic acid 22:6 n-3	0.63 ± 0.18	0.60 ± 0.19	0.63 ± 0.20	0.486

Values are means ± s.d. STAEST = stanol ester margarine and STEEST = sterol ester margarine.
[a]The significance of the differences for overall changes during the test margarine periods analysed with analysis of variance for repeated measurements (GLM) or Friedman two-tailed ANOVA (α-linolenic acid and eicosapentanoic acid).
*$P < 0.001$; †$P < 0.01$, significant difference between either of the test margarine periods and control margarine period analysed with paired *t*-test with Bonferroni correction. There were no significant differences in serum fatty acid composition of cholesteryl esters between two test margarine periods.

Table 4 Serum lipids and lipoproteins at the end of the test margarine periods

Variables	Period			P-values[a]
	Control margarine (n = 34)	STAEST margarine (n = 34)	STEEST margarine (n = 34)	
Total cholesterol (mmol/l)	6.10 ± 0.69	5.52 ± 0.75*	5.64 ± 0.71*	<0.001
LDL cholesterol (mmol/l)	4.19 ± 0.61	3.65 ± 0.69*	3.74 ± 0.58*	<0.001
HDL cholesterol (mmol/l)	1.50 ± 0.27	1.50 ± 0.29	1.55 ± 0.31	0.079
VLDL cholesterol (mmol/l)	0.40 ± 0.30	0.37 ± 0.26	0.35 ± 0.14	0.768
Triglycerides (mmol/l)	1.13 ± 0.45	1.10 ± 0.53	1.03 ± 0.33	0.221
Apo AI (g/l)	1.59 ± 0.22	1.55 ± 0.23	1.59 ± 0.27	0.143
Apo B (g/l)	1.01 ± 0.18	0.90 ± 0.18*	0.90 ± 0.13*	<0.001
Apo AI/apo B	1.62 ± 0.41	1.79 ± 0.46*	1.81 ± 0.43*	<0.001

Values are means ± s.d. STAEST = stanol ester margarine and STEEST = sterol ester margarine.
[a]Significance of the difference for overall changes during the test margarine periods analysed with analysis of variance for repeated measurements (GLM).
*P<0.001 denotes the significance of the difference between either of the test margarine periods and control period analysed with GLM with Bonferroni correction. There were no significant differences in serum lipid and lipoprotein concentrations between the two test margarine periods.

serum apo B concentration and in apo AI/apo B lipoprotein ratio were parallel with the changes in serum LDL cholesterol concentration (Table 4). The mean decreases in apo B concentrations were 10.7 ± 9.7% (CI −14.1, −7.3) and 10.4 ± 9.3% (CI −13.7, −7.2) during the STAEST and STEEST margarine periods, respectively, compared with the control period.

In a secondary analysis we also examined whether apolipoprotein E genotype group (E4/3, $n = 12$ *vs* E3/3, $n = 22$) has an effect on the LDL cholesterol response during the STAEST and STEEST margarine periods. This analysis revealed that the overall reduction in LDL cholesterol was greater in subjects with apolipoprotein E4/3 than in those with apolipoprotein E3/3 ($P = 0.024$, interaction term of genotype with test margarine periods, GLM). This was entirely due to a greater effect of STAEST margarine on the LDL cholesterol response in subjects having apolipoprotein E4 allele (16.8 ± 13.9% *vs* 10.4 ± 10.8%, apolipoprotein E4/3 *vs* E3/3, $P = 0.141$, ANOVA). The LDL cholesterol response was almost the same in the two apolipoprotein E groups (9.6 ± 13.5% *vs* 10.8 ± 8.9%, apolipoprotein E4/3 *vs* E 3/3, $P = 0.707$, ANOVA) during the STEEST margarine period.

Cholesterol precursors and plant sterols

The concentrations of serum cholesterol precursors and plant sterols during the different test margarine periods are shown in Table 5.

As expected, serum Δ8-cholestenol and Δ7-lathosterol concentrations, which are indicators of cholesterol synthesis, increased during the STAEST and STEEST margarine periods in reference to the control period (Table 5). When those concentrations were related to serum total cholesterol

Table 5 Serum cholesterol precursors, plant sterols and cholestanol (mg/l), and ratios of serum cholesterol precursors, plant sterols and cholestanol to cholesterol (10^{-3} mg/mg of cholesterol) at the end of the test margarine periods

	Period			P-values[a]
	Control margarine (n = 34)	STAEST margarine (n = 34)	STEEST margarine (n = 34)	
Cholestanol	2.68 ± 0.48	2.36 ± 0.52*	2.28 ± 0.42*	<0.001
Δ8-cholestenol	0.33 ± 0.12	0.36 ± 0.13	0.37 ± 0.12†	0.010
Desmosterol	1.22 ± 0.35	1.22 ± 0.29	1.17 ± 0.24	0.850
Δ7-lathosterol	3.28 ± 1.11	3.58 ± 1.21	3.60 ± 1.24†	0.008
Campesterol	7.70 ± 1.92	5.09 ± 1.58*	10.46 ± 2.44*‡	<0.001
Sitosterol	3.40 ± 0.88	2.21 ± 0.73*	4.23 ± 1.02*‡	<0.001
Squalene	0.66 ± 0.14	0.71 ± 0.17	0.70 ± 0.17	0.292
Campestanol	0.06 ± 0.03	0.16 ± 0.04*	0.05 ± 0.02‡	<0.001
Sitostanol	0.08 ± 0.04	0.27 ± 0.07*	0.06 ± 0.04‡	<0.001
Avenasterol	1.00 ± 0.22	0.70 ± 0.15*	0.83 ± 0.16*‡	<0.001
Cholestanol/TC[b]	1.36 ± 0.23	1.30 ± 0.24	1.25 ± 0.24*§	<0.001
Δ8-Cholestenol/TC	0.16 ± 0.06	0.20 ± 0.06*	0.20 ± 0.06*	<0.001
Desmosterol/TC	0.61 ± 0.15	0.66 ± 0.13†	0.64 ± 0.12¶	0.001
Δ7-lathosterol/TC	1.64 ± 0.50	1.95 ± 0.61*	1.95 ± 0.59*	<0.001
Campesterol/TC	3.88 ± 0.91	2.78 ± 0.74*	5.70 ± 1.11*‡	<0.001
Sitosterol/TC	1.72 ± 0.43	1.21 ± 0.33*	2.30 ± 0.47*‡	<0.001
Squalene/TC	0.33 ± 0.07	0.39 ± 0.10¶	0.39 ± 0.10¶	0.003
Campestanol/TC	0.03 ± 0.01	0.09 ± 0.02*	0.03 ± 0.01‡	<0.001
Sitostanol/TC	0.04 ± 0.02	0.15 ± 0.04*	0.03 ± 0.02‡	<0.001
Avenasterol/TC	0.50 ± 0.10	0.39 ± 0.06*	0.45 ± 0.06*‡	<0.001

Values are means ± s.d. STAEST = stanol ester margarine and STEEST = sterol ester margarine.
[a]Significance of the differences for overall changes during the test margarine periods analysed with analysis of variance for repeated measurements (GLM).
[b]TC = total cholesterol.
*P<0.001, †P<0.01, ¶P<0.05, significant difference between the either the test margarine period and the control margarine period; ‡P<0.001, §P<0.05, significant difference between the STAEST margarine and the STEEST margarine periods analysed with GLM with Bonferroni correction.

concentration the increases were more pronounced (Table 5). Serum cholestanol concentration, which reflects cholesterol absorption, decreased significantly during both test margarine periods (Table 5).

Serum campesterol and sitosterol concentrations decreased significantly during the STAEST margarine period and increased significantly during the STEEST margarine period compared to the control period (Table 5). Furthermore, serum campesterol and sitosterol concentrations were significantly higher at the end of the STEEST margarine period than at the end of the STAEST margarine period. In reference to control period, the reduction in serum campesterol and sitosterol concentrations was 2.60 ± 1.03 mg/l (33.8%) and 1.18 ± 0.47 mg/l (34.8%), respectively, during the STAEST margarine period. During the STEEST margarine period the increase in serum campesterol and sitosterol concentrations was 2.77 ± 1.49 mg/l (38.3%) and 0.83 ± 0.62 mg/l (26.6%), respectively. Changes in the ratios of serum campesterol and sitosterol to serum total cholesterol were parallel with the changes in their absolute concentrations (Table 5).

Serum campestanol (0.11 ± 0.04 and 0.11 ± 0.05 mg/l, change in reference to control and STEEST margarine period) and sitostanol concentrations (0.19 ± 0.07 mg/l and 0.21 ± 0.68 mg/l, change in reference to control and STEEST margarine period) were slightly but significantly higher at the end of STAEST margarine period than at the end of the control and the STEEST margarine periods (Table 5). There were no significant differences in serum campestanol or sitostanol concentrations between the STEEST margarine period and the control margarine period. Serum sitostanol concentration was significantly higher in women than in men at the end of the STAEST margarine period (0.24 ± 0.07 *vs* 0.30 ± 0.05 mg/l men *vs* women), but there were no differences in serum sitostanol concentrations between the genders at the end of the two other periods.

There were no significant differences in percentage changes in either serum cholesterol precursors or plant sterols between two apolipoprotein E genotype groups (3/3 and 4/3) among the different test margarine periods (data not shown).

Carotenoids and fat soluble vitamins

There were no significant changes in serum 25-hydroxyvitamin D_3, retinol, α-carotene or lycopene concentrations nor their ratios to the serum total cholesterol during the study (Table 6). Serum β-carotene concentration was significantly lower at the end of the STAEST and STEEST margarine periods than at the end of the control period. The serum $\alpha + \beta$-carotene concentration was significantly lower at the end of the STEEST margarine period as compared to the control period, but the difference between the control and the STAEST margarine periods was not significant. However, there were no significant differences in serum β-carotene/total cholesterol ratio or $\alpha + \beta$-carotene/total cholesterol ratio among the periods.

Serum γ-tocopherol concentration did not change significantly during the study, but serum α-tocopherol concentrations was significantly lower at the end of both test margarine periods than at the end of the control period (Table 6). After relating the serum α- and $\alpha + \gamma$- tocopherol to the serum total cholesterol there were no significant differences among the different periods.

Discussion

In the present study the STAEST and STEEST margarines reduced significantly serum total (9.2% and 7.3%, respectively) and LDL cholesterol (12.7% and 10.4%, respectively) concentration as part of a low-fat diet compared to a low-fat diet alone in the subjects with mild to moderate hypercholesterolaemia, but the cholesterol-lowering effects of the test margarines did not differ significantly from each other. The decreases of serum apo B were parallel with the decreases of serum LDL cholesterol concentration.

On the basis of the food records the adherence to the low-fat diet was good. The intake of saturated fatty acids achieved the goal of step 1 diet of the National Cholesterol Education Program (1994) (actual mean intake 8–9 E% *vs*

Table 6 Serum carotenoids and fat-soluble vitamins at the end of the test margarine periods

	Period			
	Control margarine (n = 34)[a]	*STAEST margarine (n = 34)*[a]	*STEEST margarine (n = 34)*[a]	*P-values*[b]
Retinol (μmol/l)	2.80 ± 1.00	2.71 ± 1.04	2.70 ± 1.01	0.494
α-Carotene (μmol/l)	0.66 ± 0.43	0.64 ± 0.42	0.61 ± 0.36	0.129
β-Carotene (μmol/l)	1.39 ± 1.03	1.23 ± 0.99*	1.16 ± 0.82*	0.004
α + β-Carotene (μmol/l)	2.05 ± 1.43	1.87 ± 1.38	1.77 ± 1.16*	0.022
Lycopene (μmol/l)	0.73 ± 0.37	0.71 ± 0.37	0.69 ± 0.39	0.677
α-Tocopherol (μmol/l)	43.55 ± 7.56	40.08 ± 6.83†	40.55 ± 7.01†	0.001
γ-Tocopherol (μmol/l)	3.00 ± 0.98	2.98 ± 0.81	2.87 ± 0.89	0.592
α + γ-Tocopherol (μmol/l)	46.55 ± 7.95	43.07 ± 7.15†	43.42 ± 7.41†	0.001
25-Hydroxyvitamin D_3 (nmol/l)	50.33 ± 23.52	49.38 ± 24.39	52.26 ± 25.18	0.659
α-Carotene/TC	0.11 ± 0.08	0.12 ± 0.08	0.11 ± 0.07	0.161
β-Carotene/TC	0.23 ± 0.17	0.23 ± 0.19	0.21 ± 0.15	0.251
α + β-Carotene/TC	0.34 ± 0.24	0.35 ± 0.26	0.32 ± 0.22	0.563
Lycopene/TC	0.12 ± 0.07	0.13 ± 0.07	0.13 ± 0.08	0.505
α-Tocopherol/TC	7.14 ± 0.89	7.27 ± 0.91	7.21 ± 1.03	0.382
γ-Tocopherol/TC	0.49 ± 0.15	0.55 ± 0.15	0.51 ± 0.15	0.086
α + γ-Tocopherol/TC	7.63 ± 0.91	7.82 ± 0.96	7.72 ± 1.06	0.249

Values are means ± s.d. STAEST = stanol ester margarine and STEEST = sterol ester margarine.

[a] $n = 33$ for results concerning lycopene and lycopene/TC (TC = total cholesterol).

[b] Significance of the difference for overall changes during the test margarine periods analysed with analysis of variance for repeated measurements (GLM).

*$P<0.05$, †$P<0.001$, significant difference between the either test margarine period and control period analysed with GLM with Bonferroni correction. There were no significant differences in serum fat-soluble and carotenoid concentrations between two test margarine periods.

goal 8–10 E%) and the mean intake of fat (30–31 E%) was close to the goal (<30 E%) in all experimental periods. In addition, the mean intake of dietary cholesterol met the goal well. The results of the fatty acid composition of cholesteryl esters paralleled with the results of the food records. There were no major differences in serum fatty acid composition among the experimental periods. Furthermore, the fatty acid composition of the STAEST and STEEST margarine, the fatty acid composition of the sterol and stanol fatty acid esters, the esterification degree of stanols and sterols, and the actual daily intake of total sterols (unsaturated and saturated) in the STAEST and STEEST margarine were identical. No significant changes in BMI, physical activity or other living habits were found during the study. Thus, the differences in lipid responses between two test margarine periods can be ascribed to the stanol and sterol fatty acid esters rather than differences in background diet or other background variables.

In the present study the subjects consumed test margarines in a randomized order according to the model of Latin square design, and each subject worked as his/her own control. The benefit of the present study design is that it eliminates the between-individual variation and the effect of time. In earlier studies it has been shown that plant sterols reduce cholesterol concentrations within 2–3 weeks of the initiation of treatment, and on the other hand, that the serum cholesterol concentration return to initial value within 2–3 weeks, upon cessation the ingestion of plant sterols (Farquhar *et al*, 1956; Heinemann *et al*, 1986; Jones *et al*, 1997; Weststrate & Meijer, 1998). Therefore, the 4 week study period can be considered long enough to demonstrate the cholesterol-lowering effect of the test margarines.

Our findings are parallel with the findings of our earlier study (Hallikainen & Uusitupa, 1999) and strengthen the view that stanol ester margarine decreases serum total and LDL cholesterol concentrations as part of a low-fat diet. Furthermore, our findings are in accordance with the study of Weststrate and Meijer (1998), in which a soybean oil sterol ester margarine based on unhydrogenated soysterols was equally effective as a stanol ester margarine (Benecol®) in lowering plasma total and LDL cholesterol concentration. The finding that plant stanols or sterols can reduce serum cholesterol concentrations even after a markedly low dietary cholesterol intake indicates that plant stanols and sterols must inhibit not only the absorption of dietary but also that of biliary cholesterol. This is supported by the findings of the earlier plant stanols/sterols studies, in which the faecal excretion of neutral sterols was increased despite the constant dietary cholesterol intake (Becker *et al*, 1993; Gylling *et al*, 1997; Gylling & Miettinen 1994).

The subjects with apolipoprotein E4 allele have been found to have a higher cholesterol absorption rate (Kesäniemi *et al*, 1996; Ordovas, 1999) and therefore those subjects might have more benefit from plant stanols and sterols. In a secondary analysis the subjects with apolipoprotein E4/3 genotype had a greater percentage reduction in LDL cholesterol during the STAEST margarine period (16.8%) than during the STEEST margarine period (9.6%). There are some studies in which the effects of sitosterol, sitostanol and sitostanol esters after combining different treatment groups (Miettinen & Vanhanen, 1994), sitostanol esters (Vanhanen *et al*, 1994) or plant stanol esters (Uusitupa & Hallikainen, 1999) on lipid responses in different apolipoprotein E phenotype or genotype groups have been investigated. Miettinen and Vanhanen (1994) and Vanhanen *et al* (1994) found that serum total and LDL cholesterol concentrations were reduced more effectively in subjects with the apolipoprotein E allele 4 than those with allele 2 or 3. However, we did not find this difference with stanol ester-enriched low-fat margarines (Uusitupa & Hallikainen, 1999). As far as we know there are no previous studies in which the effects of plant stanols on serum cholesterol concentrations in different apolipoprotein E genotype groups have been compared to that of plant sterols. Our present results are interesting, but these results need to be confirmed in a prospective study design where equal numbers of subjects with different apolipoprotein E genotypes should be allocated to different diet groups.

Serum plant sterols in very high concentrations have been found to be atherogenic (Glueck *et al*, 1991). Under normal conditions the plant sterol concentration is only on an average 3–17 mg/l in serum being roughly only 1/1000 of serum cholesterol concentration, although typical dietary intake of plant sterols is almost equal to dietary intake of cholesterol (about 160–360 mg/day, Ling & Jones 1995). Furthermore, dietary intake of plant stanols and their serum concentrations are very low compared to that of the plant sterol concentrations. In the present study serum campesterol and sitosterol concentrations were significantly higher at the end of the STEEST margarine period compared to the end of the control and STAEST margarine periods. Respectively, serum sitostanol and campestanol concentrations were significantly higher at the end of the STAEST margarine period than at the end of the control and STEEST margarine periods. However, during the entire study serum plant sterol and stanol concentrations remained very low, indicating that the absorbed amounts were very small in relation to the daily intake of plant sterols or stanols from the test margarines. Although STAEST margarine also contained a small amount of plant sterols, their serum concentrations reduced significantly when that margarine was consumed owing to the ability of the plant stanols to inhibit the absorption of plant sterols. All these findings are in line with the findings of earlier studies (Gylling *et al*, 1999a; Gylling & Miettinen 1999; Hallikainen & Uusitupa, 1999; Hallikainen *et al*, 2000; Jones *et al*, 1997,1999; Weststrate & Meijer, 1998). Besides the negligible absorption of plant stanols, the low serum concentrations could also result from fast and effective clearance of absorbed stanols (Salen *et al*, 1970).

The increased serum Δ7-lathosterol concentration and Δ7-lathosterol/total cholesterol ratio can be ascribed to compensatorily increased endogenous cholesterol synthesis due to cholesterol malabsorption during the test margarine periods. Also, in previous studies the synthesis of Δ7-lathosterol has been found to be stimulated by plant stanol esters (Gylling *et al*, 1995,1997,1999a; Gylling & Miettinen, 1994,1996; Vanhanen *et al*, 1993).

During the test margarine periods there were no significant changes in concentrations of serum 25-hydroxyvitamin D_3, retinol or in concentrations of serum lycopene, α-carotene, β-carotene and tocopherols related to the serum total cholesterol concentration. The findings of the present study are in agreement with the findings of our earlier studies (Hallikainen *et al*, 1999, 2000; Hallikainen & Uusitupa, 1999), in which we found only small effects on serum carotenoid concentrations when changes in serum carotenoids were related to the changes in serum total cholesterol. However, there are plant stanol and plant

sterol studies (Gylling *et al*, 1999b; Gylling & Miettinen 1999; Hendriks *et al*, 1999; Weststrate & Meijer, 1998), in which serum β- or $\alpha+\beta$-carotene concentrations were found to decrease significantly even after relating to the changes in serum lipid concentrations. The differences in the results might be a consequence of variability in composition of background diets. The diets in the studies by Gylling *et al* (1999b), Gylling and Miettinen (1999), Hendriks *et al* (1999) and Weststrate and Meijer (1998) were not standardized the way the diets were in our earlier studies (Hallikainen *et al*, 1999, 2000; Hallikainen & Uusitupa, 1999) and in the present one. In our studies subjects received detailed written and oral instructions about the low-fat diets, specifying the amounts and quality of food by main food groups, including vegetables. According to our findings the effects of plant sterols and stanols on serum carotenoid concentrations are minor.

In conclusion, as part of a low-fat diet the STAEST and STEEST margarines reduce serum total and LDL cholesterol concentrations significantly and there is no significant difference in their cholesterol-lowering efficacy in subjects with mild to moderate hypercholesterolaemia. In a secondary analysis the subjects with apolipoprotein E4 allele seemed to have greater effect on LDL cholesterol response with the STAEST margarine than with the STEEST margarine, but this result should be confirmed in a prospective study. Serum plant stanol and sterol concentrations increased with the STAEST and STEEST margarine, respectively, but their concentrations in serum remained very low. However, our study indicates that both plant stanols and sterols are absorbable in small amounts from the intestine.

Acknowledgements—The authors wish to thank Soili Lehtomäki for nutrition counselling, Virpi Lindi for analysis of apolipoprotein E genotypes, and Leena Kaipiainen, Pia Hoffström, Ritva Nissilä, Irja Lyytikäinen, Erja Kinnunen and Kaija Kettunen for excellent laboratory work. In addition, the authors wish to thank statistician Pirjo Halonen for valuable advice on study planning and data analyses.

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