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ORIGINAL CONTRIBUTION

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Cholesterol-lowering effect of spreads enriched with microcrystalline plant sterols in hypercholesterolemic subjects

Summary *Background* Plant sterols have been shown to reduce serum lipid concentrations. The effectiveness is highly dependent on the physical state of the plant sterols. By means of a new crystallizing method, plant sterols can be added into dietary fats and oils homogeneously. In this fat ingredient, plant sterols are in a microcrystalline form. *Aims of the study* We investigated the cholesterol-lowering effect and possible side effects of vegetable oil-based spreads fortified with two different doses of microcrystalline plant sterols. *Methods:* This double-blind randomized, placebo-controlled study consisted of a 6-wk run-in and a 6-month experimental period. During the run-in period, all 155 hypercholesterolemic subjects received rapeseed oil-based control spread. In the beginning of the experimental period subjects were randomly assigned into one of three experimental groups. The control group continued to use control spread, and the two test groups used spreads with added plant sterols of either 1.5 g/d or 3.0 g/d. The subjects consumed test spreads as a part of their normal diet without any restrictions in lifestyle and diet. *Results* Plasma total- and LDL-cholesterol concentrations were significantly reduced by 7.5–11.6 % (0.46–0.62 mmol/l) in groups consuming margarine enriched with free plant sterols, compared with the control group. The effects were similar between the two groups consuming either 1.5 g or 3.0 g plant sterols per day. No effect on HDL-cholesterol or triacylglycerol concentrations occurred. The test spreads did not induce any adverse effects in blood clinical chemistry, hematology or decreases in serum concentrations of lipid soluble vitamins. *Conclusions* Microcrystalline plant sterols are effective in lowering serum total- and LDL-cholesterol concentrations without obvious side effects. The daily dose of 1.5 g plant sterols is enough to reach the maximum effect.

Key words Plant sterols – Cholesterol – LDL-cholesterol – Crystalline form – Clinical study

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Introduction

Plant sterols are naturally occurring components of plants. They reduce serum cholesterol levels by inhibiting cholesterol absorption in the small intestine. In the lumen of the intestine, dietary fat including sterols is distributed between the oil and micellar phases [1]. When the solubility of sterols is exceeded, sterols may also occur as solid sterol monohydrated crystals [2]. The solubilities of cholesterol and plant sterols are not independent, but are mutually limiting [3, 4]. The presence of plant sterols decreases the solubility of cholesterol in the oil phase with the consequent precipitation of solid cholesterol monohydrate, which is not absorbed. According to the mechanism presented by Mattson et al [2] the absorbability of cholesterol is determined by the total sterol concentration (cholesterol + plant sterols) in the

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fat. Similarly, large cholesterol doses are known to reduce the percent of cholesterol absorption [5]. It is likely that the effective form of plant sterols is the free form, not the ester form, as the free form will predominate in the intestinal lumen [1].

The maximum effectiveness of the plant sterols can be obtained only if they are present in the intestine simultaneously with the cholesterol [6]. The preferred carrier for plant sterols would be dietary fat, which is also a carrier of dietary cholesterol. Enrichment of food products with plant sterols is difficult from a production technology and food quality point of view since plant sterols are insoluble in water and only poorly soluble in dietary fats. Esterification of the plant sterols and stanols with fatty acids increases their lipid solubility and thus facilitates their incorporation into fat containing foods [4].

By means of a new crystallizing method, up to 30 % of plant sterols can be added to food fats and oils without any chemical reactions or additives such as emulsifying agents. The resulting fat ingredient is homogeneous and stable, and plant sterols exist as the free sterols in both the dissolved and microcrystalline form.

The purpose of the present study was to investigate the cholesterol-lowering effect of the microcrystalline plant sterol ingredient in hypercholesterolemic subjects as a part of a normal Finnish diet. In addition the effect of 6 months consumption of the ingredient on serum concentrations of plant sterols, lipid soluble vitamins, clinical chemistry and hematological parameters were measured.

Subjects and methods

The study protocol was approved by the Human Ethical Committee of the Faculty of Agriculture and Forestry, University of Helsinki and by the Ethical Committee of the Oulu Deaconess Institute, in Finland.

Subjects

Subjects were recruited through advertising in the local newspaper. Altogether, 270 volunteers were screened for the study. To be included in the study, subjects had to have a total serum cholesterol concentration ≥ 5.8 mmol/l, to have serum triacylglycerol concentration < 3 mmol/l, to be aged 25–64 y, to be willing to participate and not to be an abuser of alcohol. The following subjects were excluded: persons with a diagnosis of type I diabetes mellitus, myocardial infarction within the previous 3 months, malignancy, psychosis, malabsorption, chronic liver or renal disease or homozygous familiar hypercholesterolemia; subjects receiving lipid-lowering drugs or dietary regimen or using corticosteroids, oral anticoagulants, immunosuppressants; pregnant women, women who were breast feeding and women of child-bearing potential who were not using chemical or mechanical contraception. Subjects who had stable medication for hypothyreosis, type II diabetes, hypertension or other CVD were included. A total of 155 subjects participated in the study. All subjects received both written and oral information regarding the trial and gave written consent.

Study design

During the pre-screening visit medical history, alcohol consumption and use of drugs, including lipid-lowering therapy, were recorded. Weight and height were measured and recorded. The first blood samples were drawn for cholesterol and triacylglycerol concentration screening. The pre-screening visit also included a routine physical examination. Subjects were asked to confirm their agreement by signing a consent form and they were told that they could withdraw from the study at any time.

This double-blind randomized, placebo-controlled study consisted of a 6-wk run-in period and a 6-month experimental period. All subjects received a control spread during the run-in period. At the end of the 6-wk run-in period, the subjects were randomly assigned to one of three groups: the control group continued to use the control margarine, the second group used the margarine with 1.5 g/d added plant sterols and the third group used margarine with 3.0 g/d added plant sterols. The double-blind dietary testing period lasted for 6 months and there were three control visits during that period (0, 3 and 6 month's visits). Six weeks after subjects had finished the margarine-eating study and had returned to their habitual diet, the subjects were invited to attend the last control visit.

Test spreads and diet

The subject consumed test spreads as a part of their normal diet and they were advised not to make any dietary changes during the study. Subjects received 25 g per day of the test-spread and were advised to replace 25 g of their normal dietary fat by the test spread. The subjects were advised to use the daily margarine in at least two doses.

The three different spreads included a control spread (rapeseed oil based margarine) and two test spreads fortified with two different concentrations of ingredient-containing plant sterols. These test spreads provided 1.5 g/d and 3.0 g/d of plant sterols. The plant sterol-containing ingredient was a microcrystalline suspension of plant sterols in rapeseed oil. Wood-based plant sterols

(DRT, Les dérivés résiniques et terpéniques, France) were dissolved in oil by heating. During cooling, the suspension was stabilized by rapid addition of water. Plant sterols were partly in the dissolved and partly in the microcrystalline form. The wood-based plant sterol was used composed of β-sitosterol (75–80 %), β-sitostanol (10–14 %), campesterol and campestanol (6–11 %).

The test and control spreads were packed into identical small containers, containing 25 g spread each. Products were stored in refrigerators and they were dispensed to the study subjects at 2 to 4 week intervals. Compliance was checked by weighing the returned containers, and by structured interview about the use of the test spreads.

Seven-day food diaries were kept by half of the participating subjects. The first was at the beginning of the study (home diet), the second one during the run-in period before randomization, and the third was during the experimental period. Portion sizes for food were estimated using the validated [7] portion size picture booklet [8]. Nutrient intakes were calculated by using the MICRO-NUTRICA dietary analysis program (Finnish Social Insurance Institute, Turku, Finland).

Laboratory measurements

Blood samples were drawn after subjects had been fasting for at least 12 hours. Blood samples were taken at the beginning of the run-in and the experimental diet periods at 3 and 6 months after randomization. The post-treatment blood samples were taken 6 weeks after the study was ended.

Serum total (CHOL), low-density lipoprotein (LDL) and high-density lipoprotein (HDL) cholesterol and triacylglycerol (TG) concentrations were determined by enzymatic colorimetric methods (Roche Diagnostics, Rotkreutz, Switzerland) with an automatic analyzer (Cobas Integra 700, F. Hoffman-La Roche Ltd., Basel, Switzerland).

Routine blood chemistry samples were performed at the beginning of the run-in period and at the end of the experimental period to assess possible adverse effects of margarine consumption. Hemoglobin concentration, white blood cell count, red blood cell count, platelet count and calculation of hematocrit were performed using an automatic bloodcell counter (Cell-Dyn 4000 System, Abbott Diagnostics, Illinois, USA). Serum tyrotropin (TSH) was measured by using an immunofluorometric method and gamma-glutamyl transferace (γ-GT) by using the European recommended method for determination of enzymes. Serum glucose (GLU) was analyzed by the enzymatic hexocinase method and creatinine (CREA) by the enzymatic PAP-method.

Serum concentrations of lipid soluble vitamins (retinol and α-tocopherol) and carotenoids (α- and β-carotene) were analyzed at the beginning and at the end of the experimental period. Vitamin and carotenoids were analyzed by HPLC (System Gold, Beckman Instruments, USA) equipped with an ODS–2 column (4.6 mm, 150 mm; Inertsil, Tokio, Japan) for carotenoids and an SC–04 column (125 mm, 4 mm; Bischoff, Leonberg, Germany) for vitamins.

Serum plant sterol and ubiquinol-10 concentrations, the amount of baseline diene conjugation in circulating LDL (LDL-BDC) and the antioxidant potential of LDL (LDL-TRAP) [9, 10] were analyzed in 60 randomly selected persons. LDL-BDC presents the actual amount of LDL conjugated dienes *in vivo*, but not diene conjugation during or after chemically induced oxidation of LDL *ex vivo* [11]. The antioxidant potential of LDL samples (LDL-TRAP) was estimated *in vitro* by their potency in resisting ABAP-induced peroxidation [12]. Campesterol and β-sitosterol concentrations in serum were determined by GC-MS using a Hewlett-Packard (HP) 5890 GC equipped with a NB–54 fused-silica capillary column (15 m, 0.20 mm I. D.; Nordion, Helsinki, Finland) interfaced with an HP-5970A MS detector. Sterols were analyzed as their trimethylsilyl derivatives using the selected ion monitoring (SIM) technique and m/z 129 as the specific fragment. Serum concentrations of ubiquinone-10 were analyzed by standard HPLC procedures with UV detection (Beckman, Fullerton, CA, USA).

Other observations and measurements

Body weight (0.1 kg precision) in light indoor clothing without shoes, blood pressure and resting pulse were recorded at every visit. Height (0.5 cm precision) was measured only at the beginning of the study. During each study visit, compliance and health status, medication and deviations from the normal lifestyle were registered by a questionnaire. Subjects were also asked if they had experienced any side effects.

Statistical analyses

Statistical analyses were performed with SPSS for Windows 6.0 statistics program (SPSS Inc, Chicago). Analysis of variance (ANOVA) for repeated measurements was used for the hypothesis testing. P-values are given for the differences of cholesterol levels between different study groups according to the time. P-values less than 0.05 for the differences between the mean values are considered statistically significant. The observed mean values were compared by using a 95 % confidence level to illustrate possible actual differences between study groups or points of time. The results are expressed as mean ± SD.

Results

General

The study started with 155 volunteers, 55 males and 100 females. Of the subjects, 21 voluntarily withdrew from the study: 7 persons from the control group, 5 from group receiving 1.5 g plant sterols per day and 9 from group receiving 3 g plant sterols per day. The reasons for withdrawl were that the daily consumption of margarine was found to be too much, pregnancy in spite of IUD device, traveling, *herpes zoster* infection, bad taste of the test margarine, desire to lose much weight and poor compliance.

The mean age of subjects was 50.7 years ranging from 25–64 years. At the beginning of the study the mean body mass indices (BMIs) of control group and groups consuming 1.5 and 3.0 plant sterols per day were 26.5 ± 2.9, 24.9 ± 3.8 and 25.2 ± 2.7 kg/m^2, respectively. The BMIs did not change significantly during the study.

Dietary intake and daily test margarine consumption

Mean estimated test margarine intake was 25 ± 3 g for all three groups. No statistically significant changes during the study were observed. Thus, the estimated daily plant sterol intakes in the two test groups were 1.5 ± 0.2 g and 3.0 ± 0.4 g.

Dietary intakes of energy and nutrients are presented in Table 1. The intakes of dietary cholesterol, total fat and hard fat, monounsaturated (MUFA), and polyunsaturated (PUFA) fatty acids were similar in the three study groups and resembled closely that of a normal Finnish diet [13]. During the run-in period the intake of total fat and MUFA and PUFA increased in all study groups but did not change statistically significantly from those of the home diet.

Tab. 1 Daily intakes of energy and nutrients during the study (mean ± SD)

	Control (n=17)	1.5 g/d plant sterols (n=19)	3.0 g/d plant sterols (n=16)
Energy (MJ)			
Home diet	7.5 ± 1.9	7.8 ± 2.2	6.8 ± 1.3
Run-in period	8.0 ± 2.7	7.6 ± 2.5	7.6 ± 2.1
Experimental period	7.9 ± 1.9	7.2 ± 2.0	7.0 ± 1.7
Cholesterol (mg)			
Home diet	208 ± 97	255 ± 113	228 ± 105
Run-in period	261 ± 139	216 ± 94	248 ± 95
Experimental period	239 ± 125	220 ± 104	190 ± 72
Total fat (E %)			
Home diet	32.8 ± 5.1	35.6 ± 5.8	33.4 ± 6.5
Run-in period	35.9 ± 7.1	36.8 ± 6.7	36.7 ± 6.2
Experimental period	35.7 ± 4.8	36.5 ± 5.1	35.5 ± 5.2
Hard fat (E %)			
Home diet	15.0 ± 3.1	17.3 ± 3.5	15.4 ± 3.1
Run-in period	15.7 ± 3.6	15.7 ± 3.4	16.1 ± 3.7
Experimental period	15.8 ± 2.3	15.1 ± 3.3	14.7 ± 3.6
MUFA (E %)			
Home diet	11.8 ± 2.3	12.5 ± 2.4	11.9 ± 2.9
Run-in period	13.6 ± 3.3	14.2 ± 3.0	14.1 ± 3.0
Experimental period	13.3 ± 2.3	14.4 ± 2.4	13.8 ± 1.9
PUFA (E %)			
Home diet	5.9 ± 0.9	5.7 ± 1.5	6.1 ± 1.6
Run-in period	6.6 ± 1.3	7.0 ± 1.6	6.5 ± 1.1
Experimental period	6.6 ± 1.1	6.9 ± 1.6	7.0 ± 1.0
Protein (E %)			
Home diet	16.1 ± 2.6	16.4 ± 2.9	18.6 ± 3.6
Run-in period	16.7 ± 3.2	16.3 ± 3.1	17.1 ± 5.2
Experimental period	16.3 ± 3.0	15.7 ± 2.3	16.8 ± 3.6
Carbohydrates (E %)			
Home diet	49.7 ± 5.9	46.0 ± 8.5	46.4 ± 5.6
Run-in period	45.0 ± 9.2	44.4 ± 8.5	45.5 ± 5.7
Experimental period	46.5 ± 7.9	44.2 ± 7.1	45.4 ± 6.6
Alcohol (E %)			
Home diet	1.4 ± 4.2	3.0 ± 4.0	1.5 ± 4.1
Run-in period	1.5 ± 3.4	2.4 ± 4.3	1.9 ± 2.9
Experimental period	1.6 ± 2.4	2.3 ± 2.5	2.3 ± 3.2

No statistically significant differences between control and test groups (ANOVA).

Blood lipids

At the beginning of the experimental period the average serum total-, LDL- and HDL-cholesterol levels were 6.66 ± 0.82 mmol/l, 4.29 ± 0.75 mmol/l and 1.75 ± 0.39 mmol/l, respectively. Statistically, the baseline lipid concentrations of the three study groups did not differ significantly. During the run-in period no significant changes in lipid concentration occurred.

Total and LDL-cholesterol concentrations decreased significantly after the consumption of the plant sterol enriched spreads, but not after consumption of the control spread (Fig. 1). In the control group, the total cholesterol concentration increased slightly (0.17 mmol/l) during the experimental period, but not significantly. This was probably due to seasonal variation, as the study started in June and the 6 month time point was in December/January. According to Samman [14], the cholesterol concentrations are about 0.2 mmol/l higher in December/January than in June/July (Northern Hemisphere). After 3 month of consuming the test spreads, the serum total cholesterol levels were 0.58 mmol/l (8.6 %) and 0.51 mmol/l (7.5 %) lower with 1.5 and 3.0 g of plant sterols as compared with control group (p=0.003; difference in mean values compared to control), respectively. After 6 months of consumption, corresponding cholesterol levels were 0.62 mmol/l (8.9 %) and 0.58 mmol/l (8.3 %) lower than those of control group (p=0.001), respectively. LDL-cholesterol serum levels were 0.46 mmol/l (10.5 %) and 0.50 mmol/l (11.5 %) lower than those of control group after 3 months of consumption (p=0.004) and 0.49 mmol/l (11.3 %) and 0.46 mmol/l (10.6 %) lower after 6 months of consumption (p=0.002) of 1.5 and 3.0 g plant sterols

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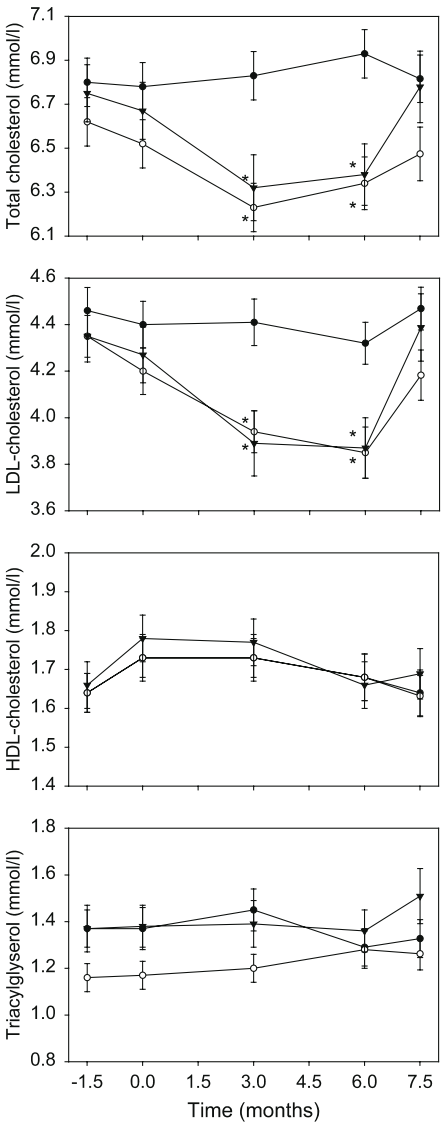

Fig. 1 Mean (± SEM) concentrations in serum total-, LDL-, HDL-cholesterol and triacylglycerol during the trial. During the run-in period (–1.5–0 months) all the subjects consumed control spread. During the experimental period (0–6 months) groups consumed either control spread (black circle), spread fortified with 1.5 g (open circle) or 3.0 g (triangle) added plant sterols and then followed-up for 1.5 months consuming a normal habitual diet. In the HDL-cholesterol figure, the control group and the 1.5 g/d group overlap on all but the last timepoint. *Significantly different from the control group, $p < 0.01$.

per day, respectively. Statistically, the total and LDL cholesterol levels of the two test groups consuming plant sterol enrichment did not differ significantly.

When subjects resumed their habitual diet after the study, the total cholesterol and LDL-cholesterol concentrations returned nearly to the initial levels and no significant differences were observed between the test and control groups.

Serum HDL-cholesterol and triacylglycerol concentrations were not affected by the consumption of test spreads as no statistically significant changes occurred during the trial.

Plant sterols

The plant sterol levels were similar among the three groups at the beginning of the run-in and experimental periods (Table 2). Serum β-sitosterol concentrations decreased in all groups during the run-in period. The concentration increased after 3 or 6 months consumption of test spreads fortified with plant sterols and after 6 months consumption of the control spread. Statistically, the β-sitosterol levels of the test groups were significantly higher compared with the control group after 3 and 6 months consumption. The serum β-sitosterol concentrations did not differ between the two groups receiving the two different doses of plant sterols.

Serum campesterol concentrations decreased in all groups during the run-in period. After 3 months consumption of test spreads serum campesterol concentrations were lower and after 6 months consumption higher than at the beginning of the experimental period. However, there were no statistically significant differences between different groups at different points of time.

LDL-cholesterol oxidation

LDL baseline diene conjugation (LDL-BDC) decreased during the run-in period in all the three groups (Table 3). No further changes occurred during the experimental period. The decrease was statistically significant in the test groups, but not in the control group. There were

Tab. 2 Serum β-sitosterol and campesterol concentrations in the three study groups (mean ± SD)

Month	Control (n=17) (n=19)	1.5 g/d plant sterols (n=16)	3.0 g/d plant sterols
β-sitosterol (µmol/l)			
$-1^1/_2$	3.54 ± 1.28	4.29 ± 2.40	3.49 ± 1.15
0	2.59 ± 0.94[1)]	3.03 ± 1.38[1)]	2.83 ± 0.76[1)]
3	2.42 ± 0.90	3.83 ± 1.46[2,3)]	3.68 ± 1.14[2,3)]
6	4.03 ± 1.23[2)]	7.07 ± 3.03[2,3)]	6.34 ± 1.69[2,3)]
Campesterol (µmol/l)			
$-1^1/_2$	8.91 ± 3.26	9.60 ± 4.73	8.20 ± 2.84
0	6.34 ± 2.97[1)]	7.48 ± 3.97[1)]	6.83 ± 1.85
3	5.41 ± 2.80[2)]	5.46 ± 2.52[2)]	4.38 ± 1.23[2)]
6	9.72 ± 3.84[2)]	9.59 ± 4.09[2)]	7.74 ± 1.62[2)]

[1)] = significantly different ($p < 0.05$) from the value at the beginning of the run-in period ($-1^1/_2$ months)
[2)] = significantly different ($p < 0.05$) from the value at the beginning of the experimental period (0 months)
[3)] = significantly different ($p < 0.05$) from the value of control group

no significant differences between the LDL-BDC values between different groups at any time points.

Statistically, free-radical trapping capacity of circulating LDL (LDL-TRAP) was increased significantly during consumption of test spreads fortified with plant sterols, but not during consumption of the control spread. Although, there were no statistically significant differences between groups at different time points.

Carotenoids and fat-soluble vitamins

The minor changes in serum concentrations of retinol, α-tocopherol, α-, and β-carotene during the study were not significant (Table 4). There were no significant differences between the control and test groups.

Tab. 3 LDL baseline diene conjugation (LDL-BDC) and free-radical trapping capacity of LDL (LDL-TRAP) in the three study groups (mean ± SD)

Month	Control (n=17)	1.5 g/d plant sterols (n=19)	3.0 g/d plant sterols (n=16)
LDL-BDC (µmol/l)			
−1½	50.3 ± 17.2	48.5 ± 11.5	53.4 ± 11.6
0	37.5 ± 14.7	34.9 ± 10.3[1)]	38.8 ± 13.2[1)]
3	36.4 ± 10.2[1)]	38.0 ± 14.8[1)]	35.1 ± 8.6[1)]
6	42.3 ± 10.7	42.4 ± 15.5	37.8 ± 12.1[1)]
LDL-TRAP (µmol/mmol)			
−1½	17.7 ± 4.6	17.8 ± 3.1	17.1 ± 2.8
0	20.0 ± 3.3	18.6 ± 2.9	19.2 ± 2.9
3	21.1 ± 4.1	23.5 ± 3.6[1,2)]	23.3 ± 3.2[1,2)]
6	22.6 ± 6.0	23.5 ± 3.5[1,2)]	22.5 ± 4.0[1,2)]

No statistically significant differences between groups.
[1)] = value significantly different ($p < 0.05$) from the value at the beginning of the run-in period (−1.5 months)
[2)] = significantly different ($p < 0.05$) from the value at the beginning of the experimental period (0 months)

Tab. 4 Retinol, α-tocopherol, α- and β-carotene concentrations in the three study groups at the beginning and the end of the experimental period (mean ± SD)

	Control (n=46)	1.5 g/d plant sterols (n=46)	3.0 g/d plant sterols (n=41)
Retinol (µmol/l)			
Before	2.08 ± 0.42	2.05 ± 0.39	2.07 ± 0.43
After	1.90 ± 0.31	1.84 ± 0.35	1.86 ± 0.43
α-tocopherol (µmol/l)			
Before	33.2 ± 6.0	31.2 ± 4.6	33.5 ± 6.6
After	31.8 ± 4.6	29.2 ± 4.5	30.1 ± 6.8
α-carotene (µmol/l)			
Before	0.15 ± 0.08	0.15 ± 0.13	0.14 ± 0.10
After	0.23 ± 0.14	0.19 ± 0.15	0.21 ± 0.15
β-carotene (µmol/l)			
Before	0.59 ± 0.33	0.61 ± 0.34	0.58 ± 0.31
After	0.64 ± 0.35	0.55 ± 0.33	0.53 ± 0.35

No statistically differences between control and test groups (ANOVA)

Tab. 5 Routine hematological parameters in the three study groups at the beginning of the run-in period (before) and at the end of the experimental period (after) (mean ± SD)

	Control (n=46)	1.5 g/d plant sterols (n=46)	3.0 g/d plant sterols (n=43)
Thyrotropin (mU/l)			
Before	2.21 ± 1.08	1.94 ± 0.95	1.90 ± 0.90
After	2.34 ± 0.81	2.21 ± 1.10	2.46 ± 1.31
Creatinine (µmol/l)			
Before	78 ± 9	81 ± 11	81 ± 9
After	80 ± 11	82 ± 11	81 ± 9
γ-GT (U/l)			
Before	26 ± 16	26 ± 17	23 ± 12
After	30 ± 24	27 ± 19	26 ± 17
Glucose (mmol/l)			
Before	5.41 ± 0.45	5.38 ± 0.72	5.39 ± 0.65
After	5.40 ± 0.46	5.31 ± 0.86	5.28 ± 0.84
White blood cell count (x10⁹/l)			
Before	5.43 ± 1.41	5.79 ± 1.76	5.28 ± 1.30
After	5.27 ± 1.41	5.54 ± 1.22	5.10 ± 1.09
Red blood cell count (x 10¹²/l)			
Before	4.66 ± 0.36	4.61 ± 0.39	4.64 ± 0.34
After	4.63 ± 0.38	4.57 ± 0.50	4.61 ± 0.38
Hemoglobin concentration (g/l)			
Before	138 ± 10	137 ± 10	138 ± 10
After	143 ± 11	141 ± 12	143 ± 10
Platelet count (x 10⁹/l)			
Before	251 ± 56	260 ± 46	272 ± 58
After	251 ± 54	262 ± 53	265 ± 61
Hematocrit (l/l)			
Before	0.428 ± 3.4x10⁻²	0.423 ± 2.9x10⁻²	0.425 ± 2.9x10⁻²
After	0.427 ± 3.3x10⁻²	0.423 ± 4.1x10⁻²	0.424 ± 3.1x10⁻²
Q10 (mmol/l)	(n=17)	(n=19)	(n=16)
Before	1.33 ± 0.34	1.36 ± 0.29	1.28 ± 0.31
After	1.42 ± 0.39	1.30 ± 0.56	1.28 ± 0.37

No statistically differences between control and test groups (ANOVA)

Other

Blood/serum parameters describing liver, kidney and thyroid function, serum ubiquinone concentration and hematological variables were all within normal ranges at the beginning of the run-in period and at the end of the experimental period (Table 5). No significant changes occurred during the trial.

Discussion

The cholesterol-lowering effects of esterified plant sterols and stanols in humans have been shown in several studies [15–19]. Esterification of the plant sterols with fatty acids increases their lipid solubility and thus facilitates their incorporation into fat-containing foods. However, only a few studies have been done with nonesterified crystalline plant sterols, and even less in a free-living setting. Jones et al [20] studied the effect of free tall oil plant sterols as a part of a strictly controlled diet

during a 30 d period. Plant sterols were suspended in margarine, which was divided equally into three meals.

In the present study, the subjects consumed the test spreads as a part of their normal diet and lifestyle. They were advised to use the daily test spread in at least two doses. Plant sterols in a microcrystalline form, reduced both serum total and LDL-cholesterol concentrations significantly. In agreement with earlier studies, plant sterols did not affect serum HDL-cholesterol and triacylglycerol concentrations [15–20]. Routine screening on a number of clinical chemistry and hematological variables showed that plant sterol enrichments did not have any adverse effects of clinical importance.

According to previous dose-response studies with either plant sterol or stanol esters, with a dose of 1.6 g plant sterols/stanols per day, a significant reduction in serum total and LDL-cholesterol was reached [17, 21]. The increasing dose provided a slight increase in the effect. In this study, the cholesterol lowering effects of the two doses: 1.5 g and 3.0 g plant sterols a day, did not differ from each other. These findings indicate that plant sterols in a microcrystalline form are as effective as in a fat soluble ester form. Due to the microcrystalline structure of this ingredient, the effective surface area of the plant sterol crystals is large and thus achieves a highly effective trapping of cholesterol molecules in the intestinal lumen. Because plant sterols are in the free from, no hydrolyzing is required before this effect can be achieved. Further dose response studies are needed to determine the smallest possible dose of microcrystalline plant sterols with a cholesterol-lowering effect.

The cholesterol concentrations did not differ between 3 and 6 months consumption of plant sterol enrichments. According to earlier studies, dietary plant sterols reduce plasma cholesterol concentrations within a few weeks of initiation of treatment and maintains these reduced levels over 12 months of continued plant sterol ingestion [15, 22, 23]. Cholesterol concentrations returned to baseline within a few weeks after consumption of plant sterols was stopped.

The plant sterol mixture was composed mainly of β-sitosterol, but contained also smaller amounts of β-sitostanol, campesterol and campestanol. Absorption of β-sitosterol in humans is only about 5 % and the excretion is more rapid than that of cholesterol [24]. Thus a several hundred-fold increase in dietary β-sitosterol causes only about a twofold increase in serum concentration, such as in studies with plant sterol esters [16]. Also in the present study with free plant sterols, an almost twofold increase in serum β-sitosterol concentration was observed. The absorption was probably saturated, as there was no difference between serum concentrations of the groups receiving either 1.5 or 3.0 g/d plant sterols. β-sitostanol is virtually unabsorbable and it restricts the absorption of other sterols, including cholesterol and other plant sterols [25, 26]. Campesterol and its saturated form, campestanol, are absorbed at about 10 % of the ingested amount, but the absorption is restricted by both β-sitosterol and β-sitostanol. The mean serum campesterol concentrations of the test groups did not differ from those of the control group. Thus, the changes in serum campesterol concentrations could be due to seasonal or other nutritional variations during the study.

Plant sterols and stanols may interfere with the absorption of fat-soluble vitamins and carotenoids while reducing cholesterol absorption [17, 27]. In this study, the plant sterols enrichments did not cause statistically significant changes in serum retinol, α-tocopherol, ubiquinone, α-, or β-carotene concentrations.

The serum and LDL fatty acid compositions reflect dietary habits [28, 29]. Thus dietary fatty acids affect LDL oxidation by changing its fatty acid composition. Monounsaturated fatty acids seem to protect LDL against oxidation. In this study the consumption of mono- and polyunsaturated fatty acids increased in all the three study groups during the run-in period, although the differences were not statistically significant. The increase could be explained by replacing a part of the habitual dietary fat with rapeseed oil-based margarine, which is relatively rich in monounsaturated fatty acids. The decreases during the consumption of both control and test margarines in baseline diene conjugation in LDL lipids, as an indicator for circulating oxidized LDL, was probably mainly due to the rapeseed oil margarine base. In addition, the antioxidant potential of LDL was increased in all groups, not significantly in the control group, during the experimental period.

In conclusion, plant sterols in a microcrystalline form reduced serum cholesterol concentrations significantly when used as part of a normal diet. The daily dose of 1.5 g was enough to reach the maximum effect. In the test groups consuming plant sterol enrichments either 1.5 or 3.0 g/day, the serum β-sitosterol concentration increased almost twofold during the study. The sterol enrichments did not affect the serum concentrations of lipid soluble vitamins or carotenoids or have any other obvious adverse effects.

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