Effects of Phytosterol Ester-Enriched Margarine on Plasma Lipoproteins in Mild to Moderate Hypercholesterolemia Are Related to Basal Cholesterol and Fat Intake

Marcus J. Mussner, Klaus G. Parhofer, Klaus von Bergmann, Peter Schwandt, Uli Broedl, and Carsten Otto

Dietary phytosterols have been reported to lower total and low-density lipoprotein (LDL) cholesterol. However, less is known about the influence of cholesterol and fat intake on the cholesterol-lowering effect of esterified phytosterols in mild to moderate hypercholesterolemia. Sixty-three healthy subjects (38 women, 25 men, 42 ± 11 years, LDL cholesterol > 130 mg/dL) were investigated in a randomized, double-blind, placebo-controlled, cross-over study. A total of 20 g/d of a phytosterol ester-enriched margarine (1.82 g/d of phytosterols) was compared with a control margarine (0.06 g/d of phytosterols). After 3 weeks of intake, participants crossed over to the other margarine. A 3-day dietary recall was performed at the beginning and at the end of the study to assess cholesterol, fat, and energy intake. Phytosterol ester-enriched margarine significantly changed total cholesterol (-3.4%, $P < .005$), LDL cholesterol (-5.4%, $P < .001$, 144 ± 28 *v* 154 ± 26 mg/dL), high-density lipoprotein (HDL) cholesterol (+3.4%, $P < .05$), apolipoprotein B (-4.0%, $P < .005$), and LDL/HDL cholesterol ratio (-7.8%, $P < .001$) compared with the control margarine. In the tertiles with the highest dietary intake of cholesterol, energy, total fat, and saturated fatty acids, and with the highest baseline proportion of campesterol to cholesterol, LDL cholesterol reduction was 11.6% ($P < .001$), 9.5% ($P = .001$), 9.4% ($P = .001$), 8.4% ($P = .005$), and 6.2% ($P = .014$), respectively. Triglycerides, plasma viscosity, and fibrinogen concentration did not change significantly. The improvements of LDL, HDL, total cholesterol, apolipoprotein B concentrations, and LDL/HDL cholesterol ratio during the daily consumption of a phytosterol ester-enriched margarine were most marked in those subjects with a high dietary intake of cholesterol, energy, total fat, and saturated fatty acids and with high baseline cholesterol absorption.

HYPERCHOLESTEROLEMIA is a well-established risk factor for atherosclerosis. Several drugs are available for cholesterol-lowering therapy, which reduce the risk for coronary heart disease (CHD) and mortality from CHD.[1] However, in mild to moderate hypercholesterolemia, dietary interventions may be sufficient.

Plant-derived sterols (phytosterols) are known to lower plasma cholesterol[2,3] and have therefore been added to fat-containing foods (eg, margarines, salad dressings, mayonnaise).[4] Phytosterols are structurally related to cholesterol and inhibit the absorption of dietary and the reabsorption of biliary cholesterol from the intestine[5,6] due to their higher affinity for mixed micelles compared with cholesterol.[7]

The effects of phytosterols were evaluated in subjects with moderate or severe hypercholesterolemia,[6,8,9-11] in patients with diabetes mellitus,[12] or in patients on a low-fat diet.[6,8,9-11,13,14] However, little is known about how phytosterols affect lipoprotein concentrations in patients with mild to moderate hypercholesterolemia continuing their regular diet, the most likely situation in which phytosterol-enriched food will be used.

It is also controversial whether the effects of phytosterols on lipoproteins depend on the composition of the diet, particularly on the intake of cholesterol and fat. While some investigators reported that low levels of dietary cholesterol intake attenuate the effectiveness of phytostanols,[11] others showed that phytosterols lower cholesterol concentrations even in subjects with a strong restriction of cholesterol intake.[15]

Therefore, we investigated the effects of a phytosterol ester-enriched margarine on lipoprotein concentrations in mild to moderate hypercholesterolemia in a cross-over, double-blind, placebo-controlled, randomized study, in which participants continued their regular diet, and in which dietary habits were registered by 2 dietary recalls, each for 3 days.

SUBJECTS AND METHODS

A total of 206 healthy subjects, all employees of our Medical Department or their relatives, were screened. Subjects were included if total cholesterol was between 200 and 300 mg/dL, low-density lipoprotein (LDL) cholesterol between 130 and 200 mg/dL, triglycerides less than 160 mg/dL, high-density lipoprotein (HDL) cholesterol greater than 35 mg/dL, and body mass index less than 30 kg/m^2. Subjects with abnormal liver (elevated γ-glutaryl transferase [γ-GT] of more than 2-fold of the upper limit) or renal function (creatinine greater than 1.2 mg/dL) were excluded. Sixty-three subjects were included in the study. Of these, 62 finished the study (38 women, 24 men, aged 42 ± 11 years), 1 person dropped out because he developed pneumonia while he was on the phytosterol ester-enriched margarine. According to the Declaration of Helsinki, all subjects gave written informed consent before entering the study.

Subjects were randomly assigned to consume either the phytosterol ester-enriched margarine or a control margarine for 3 weeks. Thereafter, they were crossed over to consume the other margarine for another 3 weeks. Both margarines were delivered in identical-looking 10-g containers. Subjects were advised to replace 20 g of their usual daily spread (eg, butter or conventional margarine) by the study spread and to eat 1 container, ie, 10 g, in the morning and 1 container in the evening. Apart from this, the subjects should not change their usual

From the Medical Department II, Klinikum Grosshadern, University of Munich, Munich; and the Department of Clinical Pharmacology, University of Bonn, Bonn, Germany.

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Address reprint requests to Carsten Otto, MD, Medical Department II, Klinikum Grosshadern, Marchioninistrasse 15, Ludwig-Maximilians-University, 81377 Munich, Germany.

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dietary habits. They were advised to complete 2 dietary recalls, 1 at the beginning (during the first week of consuming the first margarine) and 1 at the end of the study (during the third week of consuming the second margarine), each for 3 representative days, to register their dietary habits. Subjects were questioned about adverse events after each study period.

The phytosterol ester-enriched margarine contained 9.1 g phytosterols (as phytosterol esters)/100 g margarine, the control margarine contained 0.3 g phytosterols/100 g margarine, resulting in a daily intake of 1.82 g and 0.06 g phytosterols/day, respectively (corresponding to about 2.9 g and 0.09 g of phytosterol esters daily, respectively). The main phytosterols of the phytosterol ester-enriched margarines were sitosterol (0.86 g/d), campesterol (0.45 g/d), and stigmasterol (0.34 g/d) (Table 1). The margarines were very similar in the composition of individual fatty acids, and the amount of poly-, monounsaturated, and saturated fatty acids (Table 1). Compliance was controlled by measurement of phytosterol concentrations in plasma at weeks 3 and 6. Lipoprotein concentrations (total, LDL, HDL cholesterol and triglyceride concentration) were determined every week, γ-GT concentration and rheologic parameters (plasma viscosity and fibrinogen concentration) at weeks 0, 3, and 6, and apolipoprotein B concentration at weeks 3 and 6. Blood was drawn from an antecubital vein in the sitting position in EDTA tubes at each visit after an overnight fast.

Plasma viscosity was measured at 37°C using a Contraves 30 low shear rotation viscosimeter (Contraves AG, Zurich, Switzerland) at a shear rate of 117 s^{-1} with a variability in repeated measurements of less than 1%. Fibrinogen and apolipoprotein B concentrations were measured nephelometrically using the Behring Laser Nephelometer (Behringwerke AG, Marburg, Germany) with specific antibodies against human fibrinogen (OSCA, Behringwerke AG) and against human apolipoprotein B (OSAN, Behringwerke AG), respectively. Aliquots for the determination of fibrinogen and apolipoprotein B concentrations were stored at −80°C, determination was performed at the end of the study in 1 assay to exclude assay drifts.

For the determination of phytosterols, 2 μg of epicoprostanole was added to 200 μL of plasma as internal standard. After alkaline hydrolysis, extraction with cyclohexane, the plasma sterols were derivatisized to their trimethylsilyl (TMSi)-ethers by adding 1.5 mL TMSi-reagent (pyridine/-hexamethyldisilazan/-trimethylchlorosilane 9:3:1, vol/vol/vol). After incubation for 1 hour at 65°C, the solvent was evaporated under N_2 at 65°C. The residue was dissolved in 200 μL n-decane and transferred into microvials for gas-chromatography/mass-spectrometry (Hewlett Packard GC 5890II [Böblingen, Germany] combined with an 5971 quadropule MS). The chromatographic separation was performed on a 30 m DB-XLB capillary column (J&W Scientific, Folsom, CA) with a 250-μm film of cross-linked methyl silicone. Selected ion monitoring was performed with the ions m/z 370 for epicoprostanole, m/z 306 for cholestanol, m/z 458 for lathosterol, m/z 472 for campesterol, m/z 474 for campestanol, m/z 484 for stigmasterol, m/z 486 for sitosterol, m/z 488 for sitostanol, m/z 393 for lanosterol, m/z 441 for desmosterol, and m/z 458 for cholesterol. With each run (20 samples), a calibration curve of all sterols was added. The concentration of the sterols was then calculated from the calibration curve.

Total cholesterol, HDL cholesterol, and triglyceride concentrations were determined enzymatically using commercially available kits (Boehringer Mannheim GmbH Diagnostica, Mannheim, Germany) and an automated photometer (Autoanalyzer EPOS, Eppendorf, Hamburg, Germany). LDL cholesterol concentration was calculated using the formula of Friedewald et al.[16] Cholesterol absorption was estimated by the subjects' baseline proportion of campesterol to cholesterol[17] by using the campersterol and cholesterol concentrations at the end of the control margarine period.

The dietary recalls were evaluated using the software Prodi 4.5/02 expert (Wissenschaftliche Verlagsgesellschaft mbH, Stuttgart, Germany). The values for the food-composition database in Prodi 4.5/02 expert were taken from the Bundeslebensmittelschlüssel (Bundesinstitut für gesundheitlichen Verbraucherschutz und Veterinärmedizin, 1994), from nutrition value tables of Souci et al,[18] and from the food industry.

Statistical analyses were performed with SPSS 9.0 (SPSS, Chicago, IL) using Wilcoxon's signed rank test for paired observations to show statistical differences between the values after 3 weeks of control margarine and the values after 3 weeks of phytosterol ester-enriched margarine. *P* values less than .05 were considered to indicate statistical significance. The results are reported as mean values ± SD.

Table 1. Sterols and Fatty Acids in Control Margarine and in Phytosterol Ester-Enriched Margarine

	Control Margarine (%)	Phytosterol Ester-Enriched Margarine (%)
Total sterols		
(mg sterols/100 g margarine)	300 (100)	9100 (100)
Major sterols		
(mg sterols/100 g margarine)		
Sitosterol	160 (53)	4284 (47)
Campesterol	64 (21)	2248 (25)
Stigmasterol	13 (4)	1686 (19)
Sitostanol	—	169 (2)
Brassicasterol	14 (5)	142 (2)
Delta-5-avenasterol	9 (3)	136 (1)
Campestanol	4 (1)	116 (1)
Delta-7-stigmasterol	16 (5)	51 (0,5)
Cholesterol	3 (1)	39 (0,4)
Total fat as fatty acids (g/100 g)	60	60
PUFA	52.1	53.1
MUFA	24.8	24.7
SFA	22.7	21.6
TRANS	0.4	0.6

Abbreviations: PUFA, polyunsaturated fatty acids; MUFA, monounsaturated fatty acids; SFA, saturated fatty acids; TRANS, trans fatty acids.

RESULTS

Subjects had a body mass index of 24.0 ± 3.0 kg/m^2 (range, 18.2 to 29.7 kg/m^2), which did not change significantly during the study. Their average baseline concentrations of total cholesterol, LDL cholesterol, HDL cholesterol, and triglycerides were 233 ± 29 mg/dL, 152 ± 26 mg/dL, 58 ± 14 mg/dL, and 115 ± 35 mg/dL, respectively. Both margarines were well tolerated, no adverse side effects were reported. Nutritional habits were not significantly different during the 2 study periods (Table 2). Measurement of plasma sitosterol concentration showed that the subjects were compliant in consuming the margarines, 60 subjects showed increased plasma sitosterol concentrations during the phytosterol ester-enriched margarine period, while in 2 subjects, plasma sitosterol concentrations were higher during the intake of the control margarine. Randomization had no effect on the study results. Therefore, for the statistical analysis, the 2 randomization groups were analyzed together. After phytosterol ester-enriched margarine, plasma levels of sitosterol, campesterol, and stigmasterol, as well as their ratios to cholesterol, were significantly increased ($P < .001$) (Table 3). Two of the three cholesterol precursors (latho-

Table 2. Composition of Diets During Consumption of Control Margarine and Phytosterol Ester-Enriched Margarine (including 20 g of the margarines/d)

Nutrients	Control Margarine	Phytosterol Ester-Enriched Margarine
Energy (kJ/d)	8,571 ± 2138	8,537 ± 2213
Protein (% of energy)	14.5 ± 3.1	13.9 ± 2.9
Fat (% of energy)	41.4 ± 7.2	40.6 ± 6.1
Carbohydrate (% of energy)	42.4 ± 7.0	43.3 ± 6.9
Alcohol (% of energy)	1.5 ± 2.5	2.2 ± 2.9
Fiber (total) (g/d)	17.6 ± 7.1	16.6 ± 6.1
Fiber (water-soluble) (g/d)	5.5 ± 1.7	5.4 ± 1.8
Fiber (water-unsoluble) (g/d)	12.0 ± 5.8	11.2 ± 4.5
Cholesterol (mg/d)	258.6 ± 118.9	256.5 ± 156.6
Polyunsaturated fatty acids (g/d)	14.6 ± 4.7	14.8 ± 6.3
Monounsaturated fatty acids (g/d)	34.9 ± 12.1	33.4 ± 11.7
Saturated fatty acids (g/d)	34.9 ± 13.1	33.8 ± 12.8

NOTE. There were no significant differences between the 2 periods. Data from 2 dietary recalls, each for 3 days (1 recall in the control margarine period, 1 recall in the phytosterol ester-enriched margarine period).

Table 4. Lipoprotein Concentrations and Hemorrheological Parameters After Three Weeks of Consumption of Control Margarine and Phytosterol Ester-Enriched Margarine

	Control Margarine	Phytosterol Ester-Enriched Margarine
Total cholesterol (mg/dL)	235 ± 31	226 ± 34*
LDL cholesterol (mg/dL)	154 ± 26	144 ± 28*
HDL cholesterol (mg/dL)	57 ± 15	59 ± 15†
Triglycerides (mg/dL)	117 ± 43	112 ± 39
LDL/HDL cholesterol	2.9 ± 0.9	2.6 ± 0.8*
Apolipoprotein B (mg/dL)	116 ± 20	110 ± 19*
Plasma viscosity (mPas)	1.34 ± 0.06	1.35 ± 0.06
Fibrinogen (mg/dL)	288 ± 61	282 ± 52

*$P < .005$ *v* control margarine.
†$P < .05$ *v* control margarine.

sterol, desmosterol) and their ratios to cholesterol also increased significantly ($P < .001$) (Table 3). The γ-GT values during consumption of the control margarine and the phytosterol ester-enriched margarine did not differ significantly (15.4 ± 12.5 U/L *v* 15.6 ± 12.0 U/L).

Lipoprotein concentrations were significantly different between the control margarine and phytosterol ester-enriched margarine period (Table 4). Total cholesterol, LDL cholesterol, and apolipoprotein B were 3.4% ($P < .005$), 5.4% ($P < .001$), and 4.0% ($P < .005$) lower after 3 weeks of consumption of the phytosterol ester-enriched margarine compared with the control margarine. HDL cholesterol concentration increased by 3.4% ($P < .05$) after consumption of the phytosterol ester-enriched margarine, resulting in a reduction in the LDL/HDL cholesterol ratio by 7.8% ($P < .001$) after 3 weeks. Triglyceride concentrations, plasma viscosity, and fibrinogen concentrations were not affected by the ingestion of the phytosterol ester-enriched margarine. After just 1 week and 2 weeks of consuming the phytosterol ester-enriched margarine, LDL cholesterol concentration was 3.0% ($P < .05$), respectively, 3.1% ($P < .05$) lower compared with the control margarine. Total cholesterol, triglyceride, and HDL cholesterol concentrations did not change significantly after weeks 1 and 2.

When subjects were stratified according to the intake of cholesterol, energy, fat, and saturated fatty acids (Table 5), significant differences between control and phytosterol ester-enriched margarine were only observed in the high-intake groups (cholesterol, fat, saturated fatty acids) or in the average and high-intake group (energy). Further analysis showed that

Table 3. Sterol Plasma Concentrations and Sterol/Cholesterol Ratios After Three Weeks of Consumption of Control Margarine and Phytosterol Ester-Enriched Margarine

	Control Margarine	Phytosterol Ester-Enriched Margarine	Percent Change
Cholestanol (mg/dL)	0.192 ± 0.064	0.182 ± 0.059	−4.1*
Cholestanol/cholesterol (μg/mg)	0.745 ± 0.223	0.732 ± 0.212	−0.6
Lathosterol (mg/dL)	0.249 ± 0.109	0.289 ± 0.133	+22†
Lathosterol/cholesterol (μg/mg)	0.971 ± 0.446	1.173 ± 0.554	+25†
Campesterol (mg/dL)	0.357 ± 0.147	0.636 ± 0.223	+88†
Campesterol/cholesterol (μg/mg)	1.381 ± 0.527	2.564 ± 0.831	+93†
Campestanol (μg/dL)	1.638 ± 0.739	1.614 ± 0.682	+6.9
Campestanol/cholesterol (ng/mg)	6.459 ± 3.202	6.515 ± 2.561	+10
Stigmasterol (μg/dL)	11.078 ± 3.066	19.137 ± 5.215	+79†
Stigmasterol/cholesterol (ng/mg)	43.567 ± 13.521	77.923 ± 22.365	+87†
Sitosterol (mg/dL)	0.184 ± 0.083	0.249 ± 0.098	+42†
Sitosterol/cholesterol (μg/mg)	0.710 ± 0.286	1.000 ± 0.356	+46†
Sitostanol (μg/dL)	2.464 ± 0.788	2.486 ± 0.722	+4.5
Sitostanol/cholesterol (ng/mg)	9.604 ± 3.022	10.148 ± 3.132	+8.0
Lanosterol (mg/dL)	1.218 ± 0.496	1.310 ± 0.478	+20
Lanosterol/cholesterol (μg/mg)	4.740 ± 2.024	5.291 ± 1.837	+23*
Desmosterol (μg/dL)	15.006 ± 3.900	16.371 ± 4.481	+12*
Desmosterol/cholesterol (ng/mg)	58.173 ± 13.118	66.075 ± 15.852	+15†

*$P < .005$ *v* control margarine.
†$P < .001$ *v* control margarine.

Table 5. LDL Cholesterol Concentration After Three Weeks of Consumption of Control Margarine and Phytosterol Ester-Enriched Margarine Stratified in Tertiles According to Dietary Intake of Cholesterol, Energy, Fat, and Saturated Fatty Acids

Tertiles	Dietary Intake Level	LDL Cholesterol (mg/dL)		
		Control Margarine	Phytosterol Ester-Enriched Margarine	Percent Change
Cholesterol intake (mg/d)				
Low (n = 21)	61-175	158 ± 30	154 ± 31	−2.4
Average (n = 21)	175-272	153 ± 29	148 ± 30	−2.6
High (n = 20)	273-843	149 ± 21	131 ± 18	−11.6*
Energy intake (kJ/d)				
Low (n = 21)	4,407-7,348	156 ± 27	157 ± 34	−0.7
Average (n = 21)	7,386-9,290	153 ± 28	140 ± 27	−7.7†
High (n = 20)	9,345-15,548	152 ± 26	136 ± 18	−9.5*
Total fat intake (g/d)				
Low (n = 21)	36-74	159 ± 31	153 ± 35	−3.9
Average (n = 21)	75-104	149 ± 21	143 ± 28	−3.2
High (n = 20)	105-195	153 ± 27	136 ± 18	−9.4*
Intake of SFA (g/d)				
Low (n = 21)	11-27	164 ± 27	156 ± 33	−4.2
Average (n = 21)	28-37	145 ± 22	140 ± 30	−3.8
High (n = 20)	38-74	151 ± 28	136 ± 16	−8.4*

Abbreviation: SFA, saturated fatty acids.

*$P < .005$ *v* control margarine.

†$P < .05$ *v* control margarine.

men had a considerably higher intake of cholesterol than women (299 ± 156 *v* 230 ± 153 mg/d, $P < .05$). When subjects were stratified according to sex, a striking difference was observed: in men the phytosterol ester-enriched margarine resulted in a significant ($P < .001$) 9.8% reduction in LDL cholesterol concentration (144 ± 26 *v* 160 ± 24 mg/dL), while the reduction was not significant in women (-2,7%, $P = .11$, 145 ± 30 *v* 150 ± 28 mg/dL).

In the groups with high and average proportion of campesterol to cholesterol, reflecting high and average cholesterol absorption efficiency, the LDL cholesterol concentration decreased by 6.2% ($P = .01$) and 6.9% ($P = .03$), respectively. However, in the group with the low proportion of campesterol to cholesterol, LDL cholesterol concentration did not change significantly (Table 6). Body mass index, baseline LDL cholesterol concentration, and intake of unsaturated fatty acids had no impact on the response to phytosterol ester-enriched margarine.

DISCUSSION

The consumption of a phytosterol ester-enriched margarine (1.82 g/d) improves total cholesterol, LDL cholesterol, HDL cholesterol, apolipoprotein B, and LDL/HDL cholesterol ratio and was particularly beneficial in subjects with a high intake of cholesterol, energy, total fat, saturated fatty acids, and a high baseline cholesterol absorption.

The reduction in total (-3.4%) and LDL cholesterol (-5.4%) concentration is in the range of the results of other studies with phytosterols, which found reductions of total and LDL cholesterol concentrations from 0.5%[11] to 26%[6] and from 2%[11] to 33%,[6] respectively. Differences between studies may be related to the different forms of phytosterols used in these studies (free or esterified forms of saturated or unsaturated phytosterols). Free stanols, which represent the saturated forms of free sterols with no double bond in the ring structure, have been shown to inhibit cholesterol absorption more effectively than free sterols.[5] However, esterified sterols (which were used in our study) and esterified stanols inhibit cholesterol absorption equally[19,20] and lower blood cholesterol levels similarly.[20,21] Esterification of sterols and stanols seems to result in a higher reduction of cholesterol absorption efficiency, although the esters have to be hydrolyzed by pancreas enzymes before replacing cholesterol from mixed micelles. Differences in blood cholesterol reduction observed in published studies may

Table 6. LDL Cholesterol Concentration After Three Weeks of Consumption of Control Margarine and Phytosterol Ester-Enriched Margarine Stratified in Tertiles According to Campesterol/Cholesterol Ratio (indicating the basal cholesterol absorption)

Tertiles	Campesterol/ Cholesterol Ratio (μg/mg)	Campesterol Concentration (mg/dL)	LDL Cholesterol (mg/dL)		
			Control Margarine	Phytosterol Ester-Enriched Margarine	Percent Change
Low (n = 21)	0.486-1.128	0.214 ± 0.054	152 ± 21	147 ± 27	−3.1
Average (n = 21)	1.139-1.663	0.369 ± 0.117	147 ± 24	136 ± 30	−6.9*
High (n = 20)	1.684-2.697	0.494 ± 0.098	162 ± 33	150 ± 27	−6.2*

*$P < .05$ *v* control margarine.

also be related to the simultaneous consumption of different types of diets, eg, low in cholesterol,[11,22,23] different intake levels of phytosterols, or the administration of phytosterols in capsules, which may reduce effectiveness.[11] After weeks 1 and 2 of consumption of the phytosterol ester-enriched margarine, LDL cholesterol concentration was already significantly lower compared with the control margarine (-3.0% and -3.1%, respectively) and further decreased until week 3 (-5.4%). Thus, no plateau was reached within the first 3 weeks after initiation of the treatment. This is in good agreement with published studies in which phytosterols reached the maximal LDL cholesterol reduction 2 to 3 weeks after initiation of the treatment.[24]

Dividing the subjects in tertiles according to their dietary intakes of cholesterol, energy, fat, and saturated fatty acids showed that subjects with a high dietary intake of these components particularly benefit from consuming the phytosterol ester-enriched margarine. In those subjects with high dietary intake of cholesterol (273 to 843 mg/d, average 426 ± 166 mg/d), LDL cholesterol concentration decreased by 11.6% compared with a 5.4% reduction of LDL cholesterol concentration in the whole group. This observation supports the supposition of Denke[11] that a low level of dietary cholesterol attenuates the effectiveness of a low-dose phytostanol therapy, although other studies found that phytosterols can lower serum cholesterol concentrations even in subjects with a low cholesterol intake.[15] In Denke's[11] study, low-dose sitostanol therapy did not significantly lower LDL cholesterol concentration in 33 mildly hypercholesterolemic men who were consuming a diet in which dietary cholesterol was restricted to less than 200 mg/d. The influence of the dietary intake of cholesterol on the reduction of LDL cholesterol concentration can be explained by the fact that phytosterols inhibit the absorption of dietary and biliary cholesterol from the intestine.[5-7] This could explain the difference in response between women and men in our study, because the average dietary cholesterol intake of men was 30% higher ($P < .05$) compared with women. On the other hand, different compliance rates were not the reason for the sex differences, because the increase of serum sitosterol concentration during the test margarine compared with control margarine was not significantly ($P = .57$) different between women (+44%) and men (+40%).

Not only cholesterol intake, but also the rate of cholesterol absorption, had an impact on the effectiveness of phytosterol esters in our study. Subjects with a high or average baseline proportion of campesterol to cholesterol showed a significant reduction in LDL cholesterol concentrations, whereas in subjects with low baseline ratios, no significant change in LDL cholesterol concentrations was found. A recent study indicates that subjects with high baseline absorption of cholesterol benefit less from long-term simvastatin treatment than subjects with a lower baseline absorption.[25] These subjects with high baseline cholesterol absorption may be prime candidates for a combined treatment with a statin and phytosterols.[26]

Although some studies reported no change[9,11,14,21,27,28] or a decrease in HDL cholesterol concentrations[6] during consumption of phytosterols, we found a significant increase of 3.4%. This supports results of Gylling and Miettinen,[12] who observed an increase of HDL cholesterol concentrations by 11% in 1 study and 4% in another study.[29] The reason for these differences in HDL cholesterol concentration might be the fact that the subjects in Gylling and Miettinen's and in our study did not change their dietary habits concerning fat. However, a low-fat diet, which was performed in many other studies, may lower HDL cholesterol concentrations.[30]

Measurement of plasma sterol concentrations showed that plasma concentrations of the phytosterols sitosterol, campesterol, and stigmasterol, as well as their ratios to cholesterol, increased during the consumption of phytosterol ester-enriched margarine. Other studies also found an increase in plasma phytosterol concentrations during administration of phytosterols.[6,8,13] The observed increase in blood plant sterol levels was within the normal variation reported in the population (0.3 to 1.7 mg/dL).[2,31] Two of the cholesterol precursors also showed an increase in their plasma concentrations and their ratios to cholesterol, indicating an elevated synthesis of cholesterol during the test margarine,[17] most likely as a reaction to the reduced intestinal absorption of cholesterol caused by phytosterols.

In the present study, the consumption of the phytosterol ester-enriched margarine had no effect on hemorrheologic parameters, despite a 3.4% lower total cholesterol concentration after consumption of the phytosterol ester-enriched margarine. Although Koenig et al[32] found a positive linear association of plasma viscosity with total cholesterol and apolipoprotein B in an epidemiologic study, no effect of lowering LDL cholesterol on hemorrheologic parameters was found in most of the interventional studies. Although it was shown that high LDL cholesterol concentrations cause elevated plasma viscosity in vitro,[33] the achieved reduction of LDL cholesterol concentration in this study was most likely too small to have a detectable effect on plasma viscosity. In contrast to total cholesterol concentration, the fibrinogen concentration, a more important determinant of plasma viscosity,[34,35] remained unchanged during consumption of the phytosterol ester-enriched margarine.

In summary, the consumption of low doses of phytosterol esters improves the lipid status in mild to moderate hypercholesterolemia and is of particular benefit in subjects with a high dietary intake of cholesterol, energy, total fat, and saturated fatty acids and in subjects with a high intestinal cholesterol absorption efficiency. The findings imply that the daily consumption of a phytosterol ester-enriched margarine may lower the risk of atherosclerosis in subjects with mild to moderate hypercholesterolemia.[36]

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