

## Short Communication

# Effects of long-term plant sterol or stanol ester consumption on lipid and lipoprotein metabolism in subjects on statin treatment

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Consumption of plant sterol- or stanol-enriched margarines by statin users results in an additional LDL-cholesterol reduction of approximately 10%, which may be larger than the average decrease of 3–7% achieved by doubling the statin dose. However, whether this effect persists in the long term is not known. Therefore, we examined in patients already on stable statin treatment the effects of 85 weeks of plant sterol and stanol ester consumption on the serum lipoprotein profile, cholesterol metabolism, and bile acid synthesis. For this, a double-blind randomised trial was designed in which fifty-four patients consumed a control margarine with no added plant sterols or stanols for 5 weeks (run-in period). For the next 85 weeks, seventeen subjects continued with the control margarine and the other two groups with either a plant sterol ( $n$  18) or plant stanol ( $n$  19) (2.5 g/d each) ester-enriched margarine. Blood was sampled at the end of the run-in period and every 20 weeks during the intervention period. Compared with the control group, plant sterol and stanol ester consumption reduced LDL-cholesterol by 0.28 mmol/l (or 8.7%;  $P=0.08$ ) and 0.42 mmol/l (13.1%;  $P=0.006$ ) respectively after 85 weeks. No effects were found on plasma concentrations of oxysterols or 7 $\alpha$ -hydroxy-4-cholesten-3-one, a bile acid synthesis marker. We conclude that long-term consumption of both plant sterol and stanol esters effectively lowered LDL-cholesterol concentrations in statin users.

**Plant sterols: Plant stanols: Bile acid synthesis: Statins**

Patients on statin treatment often do not reach the goal for LDL-cholesterol concentrations as set by the National Cholesterol Education Program<sup>(1)</sup>. Since increasing the statin dose is not always effective enough, combination treatments that act by different mechanisms may be more appropriate. In that respect, products enriched with plant sterol or stanol esters can be useful<sup>(2)</sup>. Statins lower cholesterol synthesis by inhibiting 3-hydroxy-3-methylglutaryl-CoA reductase, whereas plant sterols and stanols inhibit the absorption of dietary and biliary cholesterol in the intestine<sup>(2)</sup>. Therefore, these two mechanisms complement each other and short-term interventions have shown that the cholesterol-lowering effects of plant sterols and stanols (2.5 g/d) are indeed additional to those of statins. In this way, an extra LDL-cholesterol reduction of approximately 10% can be achieved<sup>(2)</sup>. Although the longer-term efficacy of plant sterols and stanols has been demonstrated in non-statin users<sup>(3,4)</sup>, longer-term studies in statin users are lacking. Therefore, we examined the effects of plant sterol or stanol consumption on the serum lipoprotein profile, plant sterol and stanol concentrations, and markers for cholesterol metabolism in subjects on stable statin treatment for a period of 85 weeks.

## Subjects and methods

### Subjects

Subjects were included when they met the following criteria: stable statin treatment, age 18–65 years, BMI  $\leq 32$  kg/m<sup>2</sup>, no proteinuria or glucosuria, diastolic blood pressure  $\leq 95$  mmHg and systolic blood pressure  $\leq 200$  mmHg. Subjects with clinical manifestations of liver disorders, CVD ( $< 6$  months) or type 2 diabetes mellitus were excluded. After screening, fifty-nine patients could start the study. Two subjects, however, decided not to participate. One subject from the plant sterol group dropped out in week 40, because of diagnosis of type 2 diabetes. Another subject from the plant stanol group withdrew in week 48, because her statin treatment was stopped due to side effects. Finally, one subject from the plant stanol group had to stop in week 70, because she started a weight-loss programme. Thus, fifty-four subjects (thirty-two males and twenty-two females) completed the study (see Table 1 for subject characteristics). All women, except one, were postmenopausal. The Ethics Committee of the Maastricht University had approved the protocol and all subjects had signed an informed consent.

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**Table 1.** Baseline characteristics of the subjects  
(Mean values and standard deviations)

	Control group		Plant sterol ester group		Plant stanol ester group	
	Mean	SD	Mean	SD	Mean	SD
Subjects ( <i>n</i> )						
Male		11		10		11
Female		6		8		8
Age (years)	59.9	7.6	59.2	10.8	58.9	7.2
BMI (kg/m <sup>2</sup> )	25.5	2.6	25.9	4.7	26.5	2.9
SBP (mmHg)	144	18	143	17	145	16
DBP (mmHg)	87	11	88	10	88	11
Total cholesterol (mmol/l)	5.16	0.73	5.38	0.87	5.61	0.65
HDL-cholesterol (mmol/l)	1.36	0.26	1.34	0.33	1.32	0.25
LDL-cholesterol (mmol/l)	3.17	0.63	3.14	0.69	3.40	0.72
TAG (mmol/l)	1.41	0.76	1.69	0.73	1.76	0.69
Type of statin ( <i>n</i> )						
Pravastatin		7		8		9
Atorvastatin		5		4		5
Simvastatin		4		5		4
Rosuvastatin		0		1		1
Fluvastatin		1		0		0

SBP, systolic blood pressure; DBP, diastolic blood pressure.

### Diets and design

Subjects were asked to replace their own spread with the experimental 'light' margarines (40% fat). For the first 5 weeks, subjects used a control margarine without added plant sterols or stanols (run-in period). At the end of the run-in period subjects were randomly allocated to one of the three experimental groups, stratified for sex and age. For the following 85 weeks, the first group continued with the control margarine, the second group with a plant sterol (2.5 g/d)-enriched margarine and the third group with a plant stanol (2.5 g/d)-enriched margarine. In the margarines, both the plant sterols (sitosterol (49%), campesterol (31%) and stigmasterol (16%)) (Unilever, Vlaardingen, The Netherlands) and the plant stanols (sitostanol (70%) and campestanol (29%)) were esterified with sunflower-seed oil (Walter Rau Neusser Öl und Fett AG, Neuss, Germany). The margarines were packed in tubs of 250 g and subjects were advised to weigh the tubs daily to ensure a daily consumption of 30 g. All products were numerically coded to blind the subjects. In weeks 5, 50 and 90, subjects had to return the used tubs of the previous 8 weeks to be weighed back to calculate weekly margarine intake. At the same time points subjects completed an FFQ<sup>(5)</sup> to record their food intake from the previous 4 weeks. The dietitian checked these questionnaires immediately with the subjects, and energy and nutrient intakes were calculated using the Dutch food composition table. In a diary, subjects had to write down daily any signs of illness, change of medication, and the amount of margarine used. Finally, subjects were asked not to change their habitual diet, level of physical exercise, smoking habits or use of alcohol during the study.

### Blood sampling

Fasting blood samples were taken in the antecubital vein with a vacutainer system and 0.8 × 38 mm sterile needles in weeks

0, 4, 5, 9, 10, 29, 30, 49, 50, 69, 70, 89 and 90. Blood was sampled in 10 ml serum separator tubes for analysis of lipids and (apo)lipoproteins, serum plant sterol and stanols, and cholesterol precursors. Serum was obtained by centrifugation at 2000 g for 30 min at 4°C, minimally 1 h after blood sampling. A 4 ml heparin tube was used to obtain plasma for analysis of haematological parameters. All tubes and needles were from Becton, Dickinson and Company (Franklin Lakes, NJ, USA). Serum samples were snap-frozen in small samples and stored at -80°C.

### Lipids and apolipoproteins

Total cholesterol, HDL-cholesterol and TAG concentrations were analysed in all serum samples as described<sup>(5)</sup>. LDL-cholesterol was calculated using the Friedewald formula<sup>(6)</sup>. For apoA-1 and apoB analyses<sup>(5)</sup>, samples of weeks 4 and 5, 49 and 50, and weeks 89 and 90 were pooled. All (pooled) samples from one subject were analysed within the same run.

### Plant sterols and cholesterol precursors, oxysterols and 7 $\alpha$ -hydroxy-4-cholesten-3-one

Serum plant sterol (sitosterol, campesterol), plant stanol (sitostanol, campestanol), cholesterol precursor (lathosterol) and oxysterol (7 $\alpha$ -OH-cholesterol and 24S- and 27-OH-cholesterol) concentrations were analysed by GLC-MS as described<sup>(7)</sup>. Serum samples of weeks 4 and 5, 49 and 50, and weeks 89 and 90 were pooled before analyses. All samples from one subject were analysed within the same sequence. The marker 7 $\alpha$ -hydroxy-4-cholesten-3-one for cholesterol was determined by HPLC<sup>(8)</sup>.

### Statistics

Fifty-four subjects completed the study. Results of six subjects were excluded from the statistical analyses because they

changed their type or dose of cholesterol-lowering medication during the study. In addition, the serum total cholesterol concentration of one subject decreased by more than 5 SD of the group mean. These results were therefore considered as an outlier and excluded. Thus, statistical analyses were performed on the results of forty-seven subjects, twenty-nine males and eighteen females. Responses to treatment were calculated for each subject as the difference between values obtained half-way (means of weeks 49 and 50) and the run-in period (means of weeks 4 and 5), and the difference at the end of the experimental period (means of weeks 89 and 90) and the run-in period. Differences between the treatment groups were analysed by one-way ANOVA. When a significant diet effect was found, results of the plant sterol and stanol ester groups were compared with those of the control group using Dunnett's *t* test ( $\alpha = 0.05$ ). The Dunnett's *t* test was chosen because the study was not designed to examine differences between the plant sterol and stanol ester groups, but to compare their results with those of the control group. The study was powered ( $\beta = 0.8$ ) to find a decrease in LDL-cholesterol of 10% between the plant sterol or stanol group *v.* the control group. Influence of baseline cholesterol concentrations on the cholesterol-lowering effect was determined by univariate ANOVA. All statistical analyses were performed with SPSS 11.0 for Mac Os X (SPSS Inc., Chicago, IL, USA).

## Results

### *Dietary intakes, margarine consumption and body weight*

Baseline characteristics were not significantly different between the three groups (Table 1). Energy intake and the proportions of energy from macronutrients, as well as cholesterol intake, were the same in the three groups and did not change significantly during the study. Also body weight did not change during the study. Calculated daily plant sterol or stanol intake was 2.5 g. Inspection of the diaries did not reveal any deviations from the protocol that might have affected the results.

### *Serum lipids and apolipoproteins*

As shown in Table 2, after 45 weeks, total cholesterol decreased significantly in the plant sterol group by  $-0.49$  (95% CI  $-0.91, -0.07$ ) mmol/l (8.8%) as compared with the control group ( $P=0.019$  for the difference in absolute changes). After 85 weeks, this reduction was  $-0.29$  (95% CI  $-0.62, 0.04$ ) mmol/l or 5.1% ( $P=0.092$ ). For the plant stanol group, these reductions were  $-0.39$  (95% CI  $-0.82, 0.03$ ) mmol/l (6.9%) after 45 weeks ( $P=0.072$ ) and  $-0.50$  (95% CI  $-0.84, -0.16$ ) mmol/l (9.4%) after 85 weeks ( $P=0.003$ ).

LDL-cholesterol was decreased by  $-0.40$  (95% CI  $-0.79, -0.02$ ) mmol/l (11.6%) after 45 weeks in the plant sterol group ( $P=0.037$ ) and by  $-0.28$  (95% CI  $-0.59, 0.03$ ) mmol/l (8.7%) after 85 weeks ( $P=0.080$ ). For the plant stanol ester group, these decreases were respectively  $-0.31$  (95% CI  $-0.70, 0.07$ ) mmol/l (8.7%) after 45 weeks ( $P=0.117$ ), while LDL-cholesterol was significantly lowered by  $-0.42$  (95% CI  $-0.73, -0.11$ ) mmol/l (13.1%) after 85 weeks ( $P=0.006$ ). These LDL-cholesterol-lowering effects

**Table 2.** Effects of plant sterol and stanol ester consumption on serum concentrations of lipids and lipoproteins†  
(Mean values and standard deviations)

	Control group (n 17)		Plant sterol group (n 18)		Plant stanol group (n 19)	
	Mean	SD	Mean	SD	Mean	SD
<b>Total cholesterol (mmol/l)</b>						
Run-in	5.20	0.73	5.27	0.82	5.49	0.64
Halfway	5.36	0.85	4.95	0.78	5.26	0.64
End	5.34	0.77	5.12	0.68	5.14	0.66
Change halfway	0.16	0.42	-0.33*	0.57	-0.23	0.55
Change end	0.14	0.41	-0.15	0.46	-0.36*	0.36
<b>LDL-cholesterol (mmol/l)</b>						
Run-in	3.20	0.64	3.23	0.61	3.32	0.63
Halfway	3.32	0.71	2.95	0.64	3.13	0.65
End	3.28	0.60	3.04	0.59	2.99	0.59
Change halfway	0.12	0.32	-0.28*	0.52	-0.19	0.54
Change end	0.09	0.38	-0.19	0.42	-0.33*	0.32
<b>HDL-cholesterol (mmol/l)</b>						
Run-in	1.34	0.26	1.36	0.34	1.35	0.28
Halfway	1.37	0.27	1.39	0.32	1.37	0.34
End	1.38	0.28	1.45	0.30	1.47	0.43
Change halfway	0.03	0.12	0.03	0.14	0.02	0.15
Change end	0.04	0.14	0.10	0.13	0.12	0.25
<b>TAG (mmol/l)</b>						
Run-in	1.45	0.76	1.70	0.76	1.81	0.75
Halfway	1.50	0.77	1.51	0.54	1.69	0.70
End	1.53	0.85	1.42	0.61	1.57	0.70
Change halfway	0.04	0.52	-0.19	0.39	-0.13	0.36
Change end	0.08	0.65	-0.28	0.67	-0.24	0.34
<b>ApoA-1 (g/l)</b>						
Run-in	1.46	0.20	1.49	0.20	1.51	0.25
Halfway	1.51	0.20	1.52	0.18	1.53	0.26
End	1.52	0.20	1.56	0.18	1.54	0.30
Change halfway	0.05	0.11	0.03	0.09	0.01	0.11
Change end	0.06	0.13	0.06	0.10	0.03	0.16
<b>ApoB (g/l)</b>						
Run-in	1.08	0.18	1.10	0.19	1.14	0.17
Halfway	1.11	0.22	1.03	0.17	1.06	0.19
End	1.09	0.19	1.03	0.14	1.04	0.17
Change halfway	0.03	0.11	-0.07	0.14	-0.07	0.14
Change end	0.01	0.09	-0.06	0.14	-0.10*	0.12

\* Mean value is significantly different from that of the control group ( $P<0.05$ ).

† For details of subjects and procedures, see Table 1 and Subjects and methods.

were not related to baseline LDL-cholesterol concentrations ( $P=0.815$ ). In the plant sterol group, apoB concentrations did not change significantly after 45 ( $-0.1$  (95% CI  $-0.20, 0.01$ ) g/l or 8.6%;  $P=0.080$ ) or 85 weeks ( $-0.1$  (95% CI  $-0.17, 0.02$ ) g/l or 6.3%;  $P=0.138$ ). In the plant stanol ester group, apoB concentrations tended to decrease after 45 weeks by  $-0.07$  (95% CI  $-0.21, 0.0$ ) mg/l (5.8%;  $P=0.058$ ) and were significantly decreased after 85 weeks by  $-0.11$  (95% CI  $-0.21, -0.01$ ) g/l (10.0%;  $P=0.022$ ). No changes were found in HDL-cholesterol, TAG and apoA-1 concentrations.

### *Plant sterols and stanols, cholesterol precursors, oxysterols and 7 $\alpha$ -hydroxy-4-cholesten-3-one*

As expected, plant sterol ester consumption increased cholesterol-standardised sitosterol and campesterol concentrations as compared with the control group (see Table 3). Both cholesterol-standardised sitostanol and campestanol

**Table 3.** Effects of plant sterol and stanol ester consumption on serum concentrations of plant sterols, plant stanols, lathosterol and oxysterols†

(Mean values and standard deviations)

	Control group (n 17)		Plant sterol group (n 18)		Plant stanol group (n 19)	
	Mean	SD	Mean	SD	Mean	SD
<b>Sitosterol (10<sup>2</sup> × μmol/mmol cholesterol)</b>						
Run-in	174	64	212	109	159	85
Halfway	170	62	307	138	124	67
End	178	75	297	122	133	62
Change halfway	-5	18	95***	53	-35	48
Change end	4	43	84***	48	-26	49
<b>Campesterol (10<sup>2</sup> × μmol/mmol cholesterol)</b>						
Run-in	266	112	331	178	249	139
Halfway	247	108	684	294	180	97
End	242	100	686	288	180	73
Change halfway	-19	48	353***	156	-69	89
Change end	-24	56	355***	168	-70	104
<b>Sitostanol (10<sup>2</sup> × μmol/mmol cholesterol)</b>						
Run-in	3	1	3	1	3	1
Halfway	3	1	3	1	20	12
End	3	1	3	1	23	16
Change halfway	0	0	0	1	17***	11
Change end	0	1	-1	1	20***	15
<b>Campestanol (10<sup>2</sup> × μmol/mmol cholesterol)</b>						
Run-in	3	1	4	1	3	1
Halfway	3	1	4	1	18	10
End	8	2	9	2	47	28
Change halfway	0	1	0	1	15***	9
Change end	5	1	5	1	43***	28
<b>Lathosterol (10<sup>2</sup> × μmol/mmol cholesterol)</b>						
Run-in	66	23	66	30	64	29
Halfway	63	25	78	33	66	22
End	58	17	81	40	68	24
Change halfway	-3	12	12	19	2	20
Change end	-8	12	15*	14	4	23
<b>7α-OH-cholesterol (μg/mmol serum)</b>						
Run-in	14	5	15	6	14	4
Halfway	13	3	16	5	14	4
End	12	5	13	5	14	4
Change halfway	-1	3	1	3	0	4
Change end	-2	4	-1	4	0	3
<b>24S-OH-cholesterol (μg/mmol serum)</b>						
Run-in	11	1	13	3	12	4
Halfway	11	2	12	2	12	4
End	11	2	13	3	12	4
Change halfway	0	1	-1	1	0	2
Change end	0	1	0	2	0	2
<b>27-OH-cholesterol (μg/mmol serum)</b>						
Run-in	45	13	38	8	42	12
Halfway	44	16	38	7	41	12
End	44	15	40	7	40	11
Change halfway	-1	7	0	7	-1	7
Change end	-1	7	2	6	-2	10
<b>7α-Hydroxy-4-cholesten-3-one (μg/mmol serum)</b>						
Run-in	5	3	5	3	5	2
Halfway	4	2	4	3	5	2
End	4	3	5	4	5	2
Change halfway	-1	1	0	2	0	2
Change end	0	1	1	4	0	3

Mean value is significantly different from that of the control group: \**P*<0.05, \*\*\**P*<0.001.

† For details of subjects and procedures, see Table 1 and Subjects and methods.

concentrations were significantly increased in the stanol ester group as compared with the control group. Surprisingly, serum cholesterol-standardised campestanol concentrations further increased after 45 weeks in the plant stanol group. Changes

in cholesterol-standardised lathosterol concentrations were not significantly different between the three groups (*P*=0.126) after 45 weeks. However, after 85 weeks cholesterol-standardised lathosterol was only significantly increased in the plant sterol group by 32% as compared with the control group (*P*=0.009).

Cholesterol-standardised serum concentrations of 7α-OH-cholesterol, 24S-OH-cholesterol, 27-OH-cholesterol and 7α-hydroxy-4-cholesten-3-one did not change significantly.

### Discussion

In the present 85-week study we found that in patients on stable statin treatment, margarines enriched with plant sterol or stanol esters lowered LDL-cholesterol by 8.7 and 13.1% respectively. In a meta-analysis based on shorter-term studies<sup>(2)</sup>, an LDL-cholesterol-lowering effect of 9.7% was reported for a plant sterol dose of 2.3 g/d and of 10.1% for a plant stanol dose of 2.5 g/d. Since the decreases we observed during the course of the study fluctuated about these values, the present study showed for the first time that also in statin users, plant sterol or stanol ester consumption effectively lowers LDL-cholesterol concentrations during a period of 1.5 years.

So far, only two studies have examined the longer-term effects (52 weeks) of plant sterol or stanol ester consumption on lipid and lipoprotein metabolism. We can therefore compare those results with our own findings after 45 weeks. At that time point, the 11.6% LDL reduction in the plant sterol ester group was higher as compared with the 6.0% reduction in the study of Hendriks *et al.*<sup>(3)</sup>. This difference in effect may be related to the higher intake of plant sterols (2.5 v. 1.6 g/d) in the present study. In addition, the effects on LDL-cholesterol in the study of Hendriks *et al.*<sup>(3)</sup> were less than expected<sup>(2)</sup>. Mietinnen *et al.*<sup>(4)</sup> showed an LDL reduction of 13% after 52 weeks of plant stanol consumption (2.6 g/d), which was more pronounced than the 8.7% reduction after 45 weeks in the present study for which we do not have an explanation.

The mechanism underlying the LDL-cholesterol-lowering effect of plant sterol and stanol esters is probably identical: both compounds lower the absorption of intestinal cholesterol<sup>(9)</sup>. However, O'Neill *et al.*<sup>(10)</sup> speculated that the LDL-cholesterol-lowering effect of plant sterols may attenuate over time, due to a gradual decreased cholesterol excretion via the bile. Others have also reported a reduced bile acid synthesis in healthy volunteers after consumption of plant sterols<sup>(11)</sup> or in sitosterolaemic patient after consumption of plant stanols<sup>(12)</sup>. However, in the present study markers for bile acid synthesis were not changed. Moreover, at least two other studies in non-statin users found no effects of plant sterol consumption on bile acid synthesis<sup>(13,14)</sup>. Therefore, effects on plant sterols and stanols on bile acid metabolism remain controversial.

In this long-term dietary intervention study, compliance was good, as indicated by the amount of margarine consumed and the changes in serum plant sterol and plant stanol concentrations during the intervention period. Plant stanol consumption significantly lowered cholesterol-standardised serum plant sterol concentrations and increased those of plant sitostanol as shown before<sup>(4,15)</sup>, and these changes remained

stable during the entire study. Although the initial increase in serum cholesterol-standardised campestanol concentrations is in line with earlier observations, serum cholesterol-standardised campestanol concentrations further increased after 45 weeks. This effect was not only observed in the plant stanol group, but also in the control and the plant sterol groups, although to a lesser extent. Since all samples of one subject were analysed in the same analytical run and samples from the three groups were randomly distributed over the runs, these increases cannot be attributed to technical problems and warrant further investigation.

In conclusion, both plant sterol and stanol ester consumption effectively lowered serum LDL-cholesterol concentrations over a period of 85 weeks in patients on stable statin treatment. This implies that also in the longer term, products enriched with plant sterol or stanol esters can be useful for statin users to further optimise their serum LDL-cholesterol concentrations.

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A. de J. conducted the study and statistically analysed the data. R. P. M. and J. P. designed and supervised the study. D. L. was responsible for the analyses of serum plant sterols and stanols, cholesterol precursors and oxysterols. All authors contributed to the writing of the paper. None of the authors had a conflict of interest.

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