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ORIGINAL CONTRIBUTION

Serum and lipoprotein sitostanol and non-cholesterol sterols after an acute dose of plant stanol ester on its long-term consumption

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Abstract
Purpose Chronic inhibition of cholesterol absorption with large doses of plant stanol esters (staest) alters profoundly cholesterol metabolism, but it is unknown how an acute inhibition with a large staest dose alters the postprandial serum and lipoprotein cholesterol precursor, plant sterol, and sitostanol contents.
Methods Hypercholesterolemic subjects, randomly and double-blind divided into control ($n = 18$) and intervention groups ($n = 20$), consumed experimental diet without and with staest (plant stanols 8.8 g/day) for 10 weeks. Next morning after a fasting blood sample (0 h), the subjects had a breakfast without or with staest (4.5 g of plant stanols). Blood sampling was repeated 4 h later. Lipoproteins were separated with ultracentrifugation, and sterols were measured with gas–liquid chromatography.
Results In 0-h chylomicrons and VLDL, plant sterols were lower in staest than in controls. Postprandially, cholestenol (cholesterol synthesis marker) was reduced in chylomicrons in staest compared with controls (-0.13 ± 0.04 μg/dL vs. 0.01 ± 0.08 μg/dL, $P < 0.05$). Staest decreased postprandially avenasterol in chylomicrons ($P < 0.05$ from 0 h). Sitostanol was high at 0 h by chronic staest in serum and VLDL but not in chylomicrons. Postprandial sitostanol was increased by staest in VLDL only.
Conclusions Chronic cholesterol absorption inhibition with large amount of plant stanol esters decreases plant sterols in triglyceride-rich lipoproteins. Acute plant stanol ester consumption increases sitostanol content in triglyceride-rich lipoproteins but suggests to decrease the risk of plant sterol and plant stanol accumulation into vascular wall by chylomicrons.

Keywords Plant stanol ester · Postprandium · Sitostanol · Sitosterol · Cholesterol synthesis · Cholesterol absorption

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Background

Long-term consumption of food enriched with plant stanols decreases cholesterol absorption and serum plant sterols and increases cholesterol synthesis and serum cholesterol precursor sterols as compared with controls [1–6]. However, less is known about the effect of phytosterol consumption on postprandial lipoproteins, markers of atherosclerotic burden, and on non-cholesterol sterols, markers of cholesterol metabolism. There are two earlier studies dealing with postprandial effects of chronic or acute

use of plant stanols [7, 8]. After consumption of 3 g of plant stanols as esters for 2 weeks, the postprandial lipoproteins were reduced even though serum cholesterol level was inconsistently decreased during this short treatment period [7]. An acute plant stanol ester supplementation with 1 g of plant stanols added to the test meal in subjects with no previous plant stanol consumption had no effect on postprandial lipoproteins [8]. However, serum campesterol-to-cholesterol ratio was reduced after 6 h postprandially suggesting inhibited absorption of campesterol from its intestinal pool already with one small dose. Lowering the postprandial lipoprotein concentration is considered to decrease the atherosclerotic burden. In addition, lowering of plant sterol contents together with cholesterol might be beneficial regarding the arterial wall. For example, in phytosterolemia, it is well known that the serum and arterial wall phytosterol levels are markedly increased, coronary artery disease is manifested early in life, but serum cholesterol levels are not usually prominent. It has been shown that increasing the plant stanol ester intake from the regular 2–3 g of plant stanols/day to 8.8 g/day enhanced serum cholesterol lowering [9] and altered the synthesis and absorption markers of cholesterol metabolism [6]. Accordingly, we assumed that long-term consumption with a large dose would also affect the postprandial lipids and non-cholesterol sterols. We were interested to find out how an extensive inhibition of cholesterol absorption with a large amount of plant stanols could alter postprandial sterol contents of chylomicrons and very low-density lipoproteins (VLDL), focusing especially to their plant sterol and sitostanol contents. So, after consuming a large amount of plant stanol ester in foods (8.8 g of plant stanols/d) for 10 weeks, we took a fasting blood sample next morning (0 h) and administered a low-fat breakfast with plant stanol esters (4.5 g of plant stanols), followed the subjects up to 4 h, and separated lipoproteins and analyzed squalene and non-cholesterol sterols including sitostanol from the 0- and 4-h blood samples. Control subjects used the same test products without plant stanol esters.

Methods

Study population

Eighty-four subjects were screened for the study from announcements in a local newspaper in North Savo, Finland. The inclusion criteria were as follows [6, 9]: serum cholesterol concentration 4.5–7.5 mmol/L, age 18–75 years, and normal liver, kidney, and thyroid function. The presence of inflammatory gastrointestinal disease, diabetes, lipid-lowering medication, or consumption of plant sterol or plant stanol products excluded subjects from the study. Fifty-one subjects were selected for the study. One subject in the intervention group and one in the control group dropped out at the beginning of the study because of infeasibility to use the test drink and because of start of cholesterol-lowering medication. Altogether, 49 subjects with a mean age of 62 years completed the 10-week study, of which the main results have been presented earlier [6, 9]. Of these subjects, 40 volunteered to the postprandial test performed immediately after the first study, 20 from both study groups. However, two controls were not able to participate in the test due to personal reasons. Altogether, 38 subjects (16 men and 22 women) completed the postprandial study.

Four subjects used hormone replacement therapy. One subject had beta-blocking agents, two had calcium channel blockers, three had angiotensin converting enzyme- or angiotensin receptor-blocking agents for hypertension, and two had beta-blocking agents for arrhythmia. One was a smoker. The subjects were requested to maintain their medication, weight, alcohol consumption, smoking habits, and physical activity constant during the study.

The subjects gave their written informed consent for the study. The study was performed according to the Declaration of Helsinki. The study protocol was approved by the Ethics Committee of the Kuopio University Hospital.

Study design

The 10-week study was carried out as a randomized, double-blind, parallel design with an intervention and a control group. The detailed study design and composition of the test products and the diet are presented earlier [6, 9]. In brief, the intervention group consumed vegetable oil-based spread and oat milk-based drink enriched with plant stanol ester (staest) containing 8.8 g of plant stanols/day, and the control group consumed the same products without added plant stanols for 10 weeks.

The present postprandial study started next morning after 10 weeks on staest and control products, so that the last dose of the 10-week study was consumed on the day before the postprandial study. A 12-h fasting blood sample was obtained (0 h or baseline sample of the present study) followed by ingestion of a low-fat breakfast meal containing 1.25 dL of oat drink, two slices of white bread, 10 g of experimental spread, and 20 g of cheese. The breakfast meal contained 14 g of fat, and the intervention diet, but not the control one, in addition 4.5 g of plant stanols (from experimental spread and oat drink) as fatty acid esters. Blood sampling was repeated at 4 h (the postprandial samples).

Laboratory methods

Chylomicrons and VLDL were separated with ultracentrifugation into density classes. Fasting and postprandial cholesterol, squalene, and non-cholesterol sterols and sitostanol in chylomicrons and in VLDL were assayed with gas–liquid chromatography (GLC). After addition of 5-alpha cholestane as an internal standard to the saponified lipid extracts, they were analyzed with GLC. Thus, cholesterol, cholesterol precursors (squalene, cholestenol, desmosterol, lathosterol), plant sterols and stanols (sitosterol, sitostanol, avenasterol, campesterol), and cholestanol, a metabolite of cholesterol, were quantified from the samples by a GLC instrument (Agilent 6890N Network GC System, Agilent Technologies Inc., Wilmington, DE) equipped with a 50-m long Ultra 2 capillary column (5% Phenyl-methyl siloxane) (Agilent Technologies, Wilmington, DE, USA) principally according to a method developed earlier [10]. Squalene and non-cholesterol sterol values were expressed as concentrations (μg/dL), but also in terms of $10^2 \times$ mmol/mol of cholesterol (called ratio in the text) by dividing the squalene and non-cholesterol sterol values with the cholesterol value of the same GLC run in order to eliminate most of the effects of the changing concentrations of sterol transporter lipoproteins. Cholesterol precursors, especially as ratios, reflect cholesterol synthesis, while those of cholestanol and plant sterols relay cholesterol absorption [11, 12].

Statistical analyses

All statistical analyses were performed using SPSS for Windows 14.0 statistics program (SPSS, Chicago, IL, USA).

Normality and homogeneity of variance assumptions were checked before further analyses. Univariate analysis of variance was used to compare the baseline values and the differences from 0 to 4 h between the groups. The analysis of variance for repeated measurements (GLM) was used to analyze the interaction of time and group, changes over time, and the effect of gender in between-group comparisons followed by post hoc comparisons with Bonferroni corrections. Non-continuous variables were tested using Fisher's exact test. A P value of <0.05 was considered statistically significant. The results are given as mean ± SEM.

Results

Characteristics of the study population

Mean age was 64.7 ± 1.6 (SEM) in the staest and 62.4 ± 2.9 years in the control group, respectively. Of the 20 subjects in the staest group, 12 were women and 8 men, and the corresponding figures in the control group were 10 and 8. BMI was higher (27.7 ± 0.7 kg/m^2 vs. 24.9 ± 0.8 kg/m^2, $P = 0.013$) in the staest group compared with controls, so that all results were BMI adjusted. There were no differences in any of the clinical variables or in the frequencies of diseases, medications, dietary variables, smoking habits, physical activity, or alcohol consumption between the study groups in the 10 week [9] or in the present study. Postprandial serum triglycerides increased significantly and similarly by 12–15% from 0 to 4 h within both groups, indicating that the postprandial test was successful in spite of the low-fat meal. The change was no longer significant after adjusting with BMI.

Serum cholesterol, squalene, and non-cholesterol sterols (Table 1)

Cholesterol synthesis marker concentrations or ratios did not differ between the groups in the fasting 0-h samples. Plant sterol concentrations and ratios were lower in the staest than in the control group, but cholestanol concentrations and ratios were similar between the groups.

Postprandial serum concentrations of cholesterol increased similarly from 0 to 4 h within both groups (+4.49 ± 1.11 mg/dL in the staest and +2.52 ± 1.96 mg/dL in the control group) ($P = 0.003$ for both). Lathosterol decreased similarly in the two groups ($P < 0.01$ for both), and the decrease was exceptionally high as ratios in both groups. Also, the desmosterol ratio was decreased in both groups ($P < 0.01$ for both). Absorption markers were unchanged in controls, but avenasterol concentration and ratio were decreased by staest compared with the controls ($P < 0.05$). The cholestanol concentration was increased in both groups ($P < 0.01$ for both).

Chylomicron cholesterol, squalene, and non-cholesterol sterols (Table 1)

Chylomicron cholesterol levels were similar between the groups at 0 h, and they did not significantly change postprandially in either of the groups. The 0-h synthesis markers were similar in the two groups. Campesterol and sitosterol ratios and concentrations were lower ($P < 0.05$) in the staest than in the control group.

Postprandially, of the synthesis markers, only cholestenol was significantly decreased by staest, and the decrease differed from controls ($P < 0.05$). Sitosterol and avenasterol ratios were significantly decreased from 0 to 4 h within the staest group, but these changes did not differ from the control group.

Table 1 Baseline and absolute change in response of non-cholesterol sterol concentrations and ratios of cholesterol in serum and lipoproteins before and after the test meal in the two study groups

	Control group ($N = 18$)		Staest group ($N = 20$)	
	0 h	4–0 h	0 h	4–0 h
Serum				
μg/dL				
Squalene	39.1 ± 4.9	−5.56 ± 2.39	33.6 ± 2.2	−3.90 ± 1.42
Cholestenol	46.9 ± 3.9	−1.94 ± 0.89	51.8 ± 3.8	−1.10 ± 1.25
Desmosterol	178.9 ± 10.8	−2.28 ± 1.71	188.7 ± 9.4	2.00 ± 2.34
Lathosterol	288.4 ± 25.2	−29.11 ± 2.95	322.6 ± 22.4	−27.00 ± 4.54
Cholestanol	303.5 ± 16.9	3.28 ± 3.60	258.3 ± 12.1	8.45 ± 2.16
Campesterol	644.0 ± 65.6	13.44 ± 9.20	258.4 ± 21.1[a]	3.45 ± 2.74
Sitosterol	322.3 ± 36.0	2.83 ± 4.03	130.8 ± 8.3[a]	2.25 ± 1.18
Avenasterol	102.9 ± 8.5	−0.06 ± 1.84	59.6 ± 2.5[a]	−3.80 ± 0.60[b,c]
10^2 mmol/mol of cholesterol				
Squalene	19.9 ± 2.6	−3.24 ± 1.47	18.2 ± 1.1	−2.53 ± 0.76
Cholestenol	23.8 ± 2.0	−1.29 ± 0.42	27.7 ± 1.5	−1.14 ± 0.67
Desmosterol	90.6 ± 5.9	−2.81 ± 0.86	102.0 ± 4.1	−1.56 ± 1.00
Lathosterol	147.0 ± 14.0	−16.47 ± 1.98	175.0 ± 11.4	−18.14 ± 2.43
Cholestanol	152.6 ± 7.6	−0.64 ± 0.51	139.8 ± 5.1	0.98 ± 0.43
Campesterol	320.3 ± 27.7	3.28 ± 2.19	142.0 ± 12.9[a]	−1.43 ± 1.05
Sitosterol	159.9 ± 15.1	−0.36 ± 0.69	71.7 ± 5.1[a]	−0.44 ± 0.47
Avenasterol	51.2 ± 3.4	−0.61 ± 0.74	32.3 ± 1.0[a]	−2.75 ± 0.38[b,c]
Chylomicron				
mg/dL				
Cholesterol	1.13 ± 0.15	0.23 ± 0.09	0.95 ± 0.08	0.41 ± 0.09
μg/dL				
Squalene	1.53 ± 0.46	−0.10 ± 0.30	1.07 ± 0.14	−0.08 ± 0.13
Cholestenol	1.80 ± 0.16	0.01 ± 0.08	1.66 ± 0.18	−0.13 ± 0.04[b,c]
Desmosterol	1.29 ± 0.25	0.17 ± 0.10	1.09 ± 0.07	0.36 ± 0.07
Lathosterol	1.99 ± 0.29	0.21 ± 0.19	1.99 ± 0.18	0.59 ± 0.17
Cholestanol	1.82 ± 0.29	0.27 ± 0.12	1.30 ± 0.11	0.41 ± 0.10
Campesterol	3.49 ± 0.34	0.89 ± 0.37	1.44 ± 0.17[a]	0.44 ± 0.12
Sitosterol	2.09 ± 0.16	0.29 ± 0.18	1.09 ± 0.09[a]	0.15 ± 0.07
Avenasterol	1.10 ± 0.10	0.09 ± 0.08	0.88 ± 0.04	0.05 ± 0.06
10^2 mmol/mol of cholesterol				
Squalene	120.4 ± 23.6	−18.26 ± 22.75	122.2 ± 19.1	−42.05 ± 21.04
Cholestenol	192.7 ± 22.5	−20.73 ± 17.26	181.4 ± 19.4	−62.94 ± 12.93[b,c]
Desmosterol	116.4 ± 9.6	−5.33 ± 5.61	118.5 ± 4.8	−5.91 ± 5.83
Lathosterol	176.9 ± 16.9	−9.27 ± 8.34	210.2 ± 13.0	−19.47 ± 7.03
Cholestanol	165.2 ± 9.5	−8.72 ± 7.77	142.9 ± 9.2	−9.72 ± 3.65
Campesterol	337.6 ± 28.8	5.70 ± 7.91	153.8 ± 12.2[a]	−7.79 ± 6.78
Sitosterol	211.3 ± 18.3	−17.18 ± 7.13	123.2 ± 11.1[a]	−21.61 ± 5.73[c]
Avenasterol	110.7 ± 10.7	−12.98 ± 7.10	102.4 ± 9.7	−26.77 ± 6.30[c]
VLDL				
mg/dL				
Cholesterol	12.4 ± 2.0	1.59 ± 0.61	14.2 ± 1.5	2.6 ± 0.52
μg/dL				
Squalene	12.2 ± 3.7	−3.42 ± 1.84	12.9 ± 1.9	−3.79 ± 1
Cholestenol	4.4 ± 0.5	−0.22 ± 0.25	5.0 ± 0.5	0.06 ± 0.13

Table 1 continued

	Control group ($N = 18$)		Staest group ($N = 20$)	
	0 h	4–0 h	0 h	4–0 h
Desmosterol	10.7 ± 2.8	0.54 ± 0.52	11.6 ± 1.4	1.18 ± 0.42
Lathosterol	25.3 ± 4.7	−1.39 ± 1.49	33.5 ± 4.2	−0.24 ± 0.86
Cholestanol	15.9 ± 3.7	1.74 ± 0.79	15.5 ± 1.6	3.14 ± 0.59
Campesterol	35.9 ± 7.3	4.35 ± 1.86	17.5 ± 2.1[a]	3.2 ± 0.67
Sitosterol	13.7 ± 2.6	1.57 ± 0.67	7.1 ± 0.7[a]	1.26 ± 0.25
Avenasterol	7.2 ± 1.2	−0.12 ± 0.33	6.3 ± 0.7	0.06 ± 0.21
10^2 mmol/mol of cholesterol				
Squalene	90.7 ± 13.8	−36.46 ± 10.53	88.7 ± 7.1	−32.34 ± 5.72
Cholestenol	41.4 ± 3.8	−4.52 ± 3.86	36.4 ± 2.2	−5.86 ± 1.01
Desmosterol	74.0 ± 5.4	−2.81 ± 1.92	79.4 ± 3.3	−5.35 ± 1.32
Lathosterol	187.8 ± 16.6	−26.27 ± 3.45	231.8 ± 15.8	−37.81 ± 4.84
Cholestanol	123.0 ± 7.2	0.07 ± 1.58	111.0 ± 4.0	1.76 ± 1.16
Campesterol	298.0 ± 25.0	4.48 ± 3.31	132.2 ± 11.7[a]	−0.61 ± 2.22
Sitosterol	115.5 ± 9.9	1.45 ± 2.41	53.7 ± 3.8[a]	−0.80 ± 0.82
Avenasterol	60.4 ± 3.5	−7.23 ± 1.32	45.1 ± 1.8[a]	−6.84 ± 1.07

Values shown are mean ± SEM

[a] $P < 0.05$ denotes a significant difference at the baseline (0 h) between the groups. If the baseline significantly differed between groups, it was taken into account as covariance

[b] $P < 0.05$ denotes a significant difference at the absolute change (4–0 h) between the groups

[c] $P < 0.05$ denotes a significant change from 0 to 4 h within a group. Analysis was done after group-by-time interaction was significant

VLDL cholesterol, squalene, and non-cholesterol sterols (Table 1)

In VLDL, cholesterol, squalene, and the synthesis markers did not differ between the groups at 0 h. However, both the concentrations and ratios of campesterol and sitosterol and the ratio of avenasterol were from 25 to 56% lower from controls ($P < 0.05$ for all).

Postprandially, the lathosterol ratio was similarly decreased in both groups ($P < 0.05$ for both), but no changes were observed in other postprandial sterols.

Sitostanol in lipoproteins (Table 2)

Serum sitostanol concentration and ratio of cholesterol increased during the 10-week staest treatment, so that at 0 h

Table 2 Baseline and 4–0 h postprandial change in sitostanol in serum and lipoproteins before and after the test meal in the two study groups

	Control group ($N = 18$)		Staest group ($N = 20$)	
	0 h	4–0 h	0 h	4–0 h
Serum				
Sitostanol (μg/dL)	18.2 ± 1.5	1.11 ± 0.65	38.5 ± 1.7[a]	−0.25 ± 1.04
Sitostanol (10^2 mmol/mol of cholesterol)	9.1 ± 0.7	0.41 ± 0.35	21.3 ± 1.2[a]	−0.51 ± 0.56
Chylomicron				
Sitostanol (μg/dL)	0.24 ± 0.03	0.02 ± 0.03	0.27 ± 0.04	0.12 ± 0.03
Sitostanol (10^2 mmol/mol of cholesterol)	26.7 ± 4.7	−0.34 ± 3.49	27.7 ± 2.8	1.33 ± 2.20
VLDL				
Sitostanol (μg/dL)	1.3 ± 0.2	−0.03 ± 0.13	2.0 ± 0.2[a]	0.8 ± 0.29[b]
Sitostanol (10^2 mmol/mol of cholesterol)	10.6 ± 0.7	−1.01 ± 0.94	15.1 ± 0.9[a]	2.12 ± 1.11

Values shown are mean ± SEM

[a] $P < 0.05$ denotes a significant difference at the baseline (0 h) between the groups. If the baseline significantly differed between groups, it was taken into account as covariance

[b] $P < 0.05$ denotes a significant change from 0 to 4 h within a group. Analysis was done after group-by-time interaction was significant

they were about twofold compared with controls ($P < 0.05$). The 4-h postprandial changes were not significant between the groups.

The 0-h sitostanol values were similar in chylomicrons in both groups, and the 4-h postprandial concentrations and ratios were unchanged in both groups.

In VLDL, the 0-h sitostanol concentrations and ratios were higher in staest than in controls ($P < 0.05$). The postprandial sitostanol concentration, but not ratio, was increased significantly from the 0-h value, and the 4-h values differed from controls.

Discussion

A novel finding was that inhibition of cholesterol absorption with adding a relatively high dose of plant stanol esters (4.5 g of plant stanols) to a low-fat breakfast on the next day after long-term consumption of a large daily plant stanol (8.8 g/day) ester dose appeared to reduce the chylomicron concentration and ratio of cholestenol, a synthesis marker of cholesterol, and the serum concentration and ratio and chylomicron ratio of avenasterol, a plant sterol. The changes occurred during reduced baseline absorption of sterols caused by chronic staest consumption. Accordingly, plant sterols were lower by chronic staest consumption in serum, chylomicrons, and VLDL compared with controls.

Owing to the complicated methods of evaluating cholesterol metabolism, new approaches have been developed using the measurement of serum non-cholesterol sterols. The initial determination of the cholesterol precursors with gas–liquid chromatography suggested that they were related to cholesterol synthesis in normal situation as well as in many clinical conditions, and later on, additional determinations indicated that serum plant sterol and cholestanol levels, especially their ratios of serum cholesterol, were positively related to absolute cholesterol absorption [11, 12]. We have recently reviewed the role of serum non-cholesterol sterols as surrogate markers of cholesterol metabolism in different populations and under different occasions [13], if cholesterol homeostasis is preserved. However, individual cholesterol precursors and markers of cholesterol absorption behave according to their own individual metabolism, and using only one single marker may give erroneous information. Moreover, the cholesterol-standardized values were more sensitive surrogate markers of cholesterol metabolism than their respective concentrations.

What is then the effect of acute sterol absorption inhibition with plant stanol ester on serum sterols during the 4-h postprandial period? Serum synthesis marker ratios were not different from controls. Unexpected observations were that the 4-h level of postprandial avenasterol was further significantly decreased by acute additional staest. Why only avenasterol of the other plant sterols reaches a statistically significant reduction remains open, but this may reflect the fact that individual plant sterols have their own metabolism in humans. In addition, the small sample size may affect the results, because there was a similar trend for campesterol and sitosterol ratios compared to avenasterol both in serum and chylomicrons. Structurally, avenasterol resembles sitosterol, but it has a double bond in the side chain. Its amount in diet is much smaller than that of campesterol and especially that of sitosterol, but its distribution in food items is similar to that of sitosterol and campesterol. A question arises in which lipoprotein fractions these postprandial serum changes are explainable.

Analysis of chylomicron sterols showed that the postprandial behavior of cholesterol could not cover the respective serum cholesterol change. Chylomicrons might contribute to the development of atheromatous arterial disease [14, 15]. Diurnal rhythm of cholesterol metabolism shows a marked reduction in cholesterol synthesis early in the morning lasting to afternoon hours. Normal breakfast with no sterol addition increases, however, markedly squalene and free precursor sterol concentrations in chylomicrons and VLDL, but owing to a marked increase in the respective cholesterol level their ratios of cholesterol are decreased during the first four postprandial hours [16]. The reduced synthesis and absorption marker ratios especially on staest indicate that chylomicron particles released in circulation on additional staest are low in non-cholesterol sterols. This difference was significant only for cholestenol. Since the other synthesis marker ratios also tended to be reduced more frequently on staest than in controls, a general morning reduction in cholesterol synthesis [16] could explain the observation. Thus, the trend of increased synthesis sterols and decreased absorption sterols may be antiatherogenic on chronic absorption inhibition with staest consumption. The additional stanol ester dose at breakfast may have reduced both synthesis and absorption markers of chylomicron particles. Chylomicron squalene and non-cholesterol sterols, especially in the staest group, could have contributed to their serum changes.

It is interesting that no baseline increase in sitostanol was seen on long-term staest consumption in chylomicrons in spite of the doubled sitostanol values in other lipoproteins. Rapid turnover of chylomicron particles may explain why the over night-produced new fasting particles next morning were poor in sitostanol. Sitostanol levels increased in the VLDL particles. Transfer of sitostanol from circulation to tissues, including liver and endothelial cells, is interesting since it is under speculations whether plant stanols and sterols accumulate to arterial endothelium

during long-term consumption. It has been documented, however, that staest consumption increased plant stanols in serum but not in endarterectomized carotid arteries [17]. The present findings appear to eliminate chylomicrons as harmful transporters of plant stanols to arterial tissue.

The difference between plant sterol and plant stanol ester consumption shown in several earlier studies is that during plant sterol consumption, serum plant sterols increase, and during plant stanol consumption, serum plant sterols decrease [18]. Furthermore, serum plant sterol levels are related to their respective levels in arterial and aortic wall [19–21]. In children with familial hypercholesterolemia, consumption of plant sterol esters increased serum and tissue (red blood cells) plant sterol levels, but during consumption of plant stanol esters, serum and tissue plant sterols were decreased [22]. However, the clinical relevance of increased endothelial plant sterol levels is not known.

The limitations of this study are the relatively small sample size and only one postprandial sample at 4 h. However, the 4-h postprandial time is usually the shortest period to see constant increases in triglycerides and changes in plant sterols or stanols. Because of the inconvenience to the patients to stay longer in the laboratory, we had to choose the shortest possible time. That the postprandial changes in non-cholesterol sterols could not be demonstrated in every individual sterol is not a surprise, because their own metabolic pathways determine their behavior in different populations and under different circumstances; this is the reason why all of them were analyzed and presented.

In conclusion, long-term inhibition of cholesterol absorption with consuming a large staest dose decreased plant sterols in serum, chylomicrons, and VLDL (including intermediate-density lipoprotein, IDL). Acute additional breakfast-included staest decreased cholestenol (cholesterol synthesis marker) in chylomicrons and avenasterol (plant sterol) in serum. Because of the previous consumption of 8.8 g of plant stanols for 10 weeks, the 0-h sitostanol was doubled in serum but not in chylomicrons. The acute additional dose of staest increased sitostanol contents in VLDL only. These results suggest that acute absorption inhibition with plant stanol esters increases the sitostanol content in triglyceride-rich lipoproteins but suggests to decrease the risk of plant sterol and plant stanol accumulation into vascular wall by chylomicrons.

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Conflict of interest The authors claim no conflicts of interest associated with the manuscript.

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