Serum sterols during stanol ester feeding in a mildly hypercholesterolemic population

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Abstract **We investigated the changes of cholesterol and non-cholesterol sterol metabolism during plant stanol ester margarine feeding in 153 hypercholesterolemic subjects. Rapeseed oil (canola oil) margarine without (n = 51) and with (n = 102) stanol (2 or 3 g/day) ester was used for 1 year. Serum sterols were analyzed with gas–liquid chromatography. The latter showed a small increase in sitostanol peak during stanol ester margarine eating. Cholestanol, campesterol, and sitosterol proportions to cholesterol were significantly reduced by 5–39% ($P < 0.05$ or less for all) by stanol esters; the higher their baseline proportions the higher were their reductions. The precursor sterol proportions were significantly increased by 10–46%, and their high baseline levels predicted low reduction of serum cholesterol. The decrease of the scheduled stanol dose from 3 to 2 g/day after 6-month feeding increased serum cholesterol by 5% ($P < 0.001$) and serum plant sterol proportions by 8–13% ($P < 0.001$), but had no consistent effect on precursor sterols. In twelve subjects, the 12-month level of LDL cholesterol exceeded that of baseline; the non-cholesterol sterol proportions suggested that stimulated synthesis with relatively weak absorption inhibition contributed to the non-responsiveness of these subjects. In conclusion, plant stanol ester feeding lowers serum cholesterol in about 88% of subjects, decreases the non-cholesterol sterols that reflect cholesterol absorption, increases the sterols that reflect cholesterol synthesis, but also slightly increases serum plant stanols. Low synthesis and high absorption efficiency of cholesterol results in the greatest benefit from stanol ester consumption.**—Gylling, H., P. Puska, E. Vartiainen, and T. A. Miettinen. **Serum sterols during stanol ester feeding in a mildly hypercholesterolemic population.** ***J. Lipid Res.*** **1999.** 40: **593–600.**

Supplementary key words cholesterol absorption • cholesterol malabsorption • cholesterol synthesis • non-cholesterol sterols • sitostanol • campestanol

Plant sterols, mainly as a mixture of sitosterol and sitostanol and small amounts of campesterol and campestanol, have been shown to reduce serum cholesterol since the 1950s when administered orally to human subjects in crystalline, microcrystalline, or homogenized forms (1–9). Sugano, Morioka, and Ikeda (10) and Ikeda and Sugano (11) pointed out that in experimental animals the corresponding stanols, which are saturated 5α-plant sterols, were more effective in preventing cholesterol absorption and lowering serum cholesterol than the unsaturated parent compounds, and that stanols were unabsorbable. Similar observations were found in human experiments showing that in familial hypercholesterolemia serum cholesterol was dramatically reduced by sitostanol, and that this reduction was associated with enhanced fecal elimination of cholesterol due to absorption inhibition (12, 13). However, a similar study with a homogenized sitostanol fed in capsules resulted in no changes of serum cholesterol (14). We considered that in order to get an optimal effect, the insoluble sitostanol should be given in a fat-soluble form. This was successfully obtained by preparing sitostanol esters through transesterification with rapeseed oil fatty acids (15, 16). This procedure increases fat solubility of sitostanol improving also its consumption, because stanol ester could be solubilized in nutritional fats, for instance, in mayonnaise and margarines, general nutritients in many western countries. In clinical studies, these fat preparations significantly reduced serum total and LDL cholesterol through cholesterol malabsorption (15–24). Finally, in an 1-year study in a mildly hypercholesterolemic population, sitostanol ester margarine feeding significantly reduced serum total (−10%) and LDL (−15%) cholesterol with no changes in HDL cholesterol or triglyceride levels (25). In the present study, we investigated the changes in serum non-cholesterol sterols of this mildly hypercholesterolemic population, cholesterol changes reported previously (25), to determine *1*) what are the cholesterol lowering mechanisms; *2*) what are the mechanisms in those subjects in whom serum cholesterol is not reduced; *3*) how non-cholesterol sterols are related to each other and to serum cholesterol; and *4*) are stanols unabsorbable.

Abbreviations: BMI, body mass index; GLC, gas–liquid chromatography.

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MATERIALS AND METHODS

Study population

A random mildly hypercholesterolemic (total cholesterol ≥5.7 mmol/l) population of 153 subjects was defined from about 1500 subjects of a study (Finnrisk) performed half a year earlier in a countryside county, as shown briefly in our previous paper (25). Serum triglycerides had to be less than 2.5 mmol/l and body mass index (BMI) <30 kg/m^2. Subjects with renal, liver, or thyroid disease and diabetes mellitus were excluded. The subjects were informed of the study and they volunteered for the investigation, protocol of which had been accepted by our hospital Ethics Committee.

Design of the study

Initially after a blood sample was obtained for lipid and other variables for eligibility, all volunteers were advised to replace 24 g of their home dietary fat by rapeseed oil margarine without stanol esters for 6 weeks. At the end of this period, two blood samples were obtained a week apart, and 51 of the participants were randomized to continue on the rapeseed oil margarine for the subsequent 12 months. The remaining 102 subjects were similarly advised to continue with rapeseed oil margarine, now con-

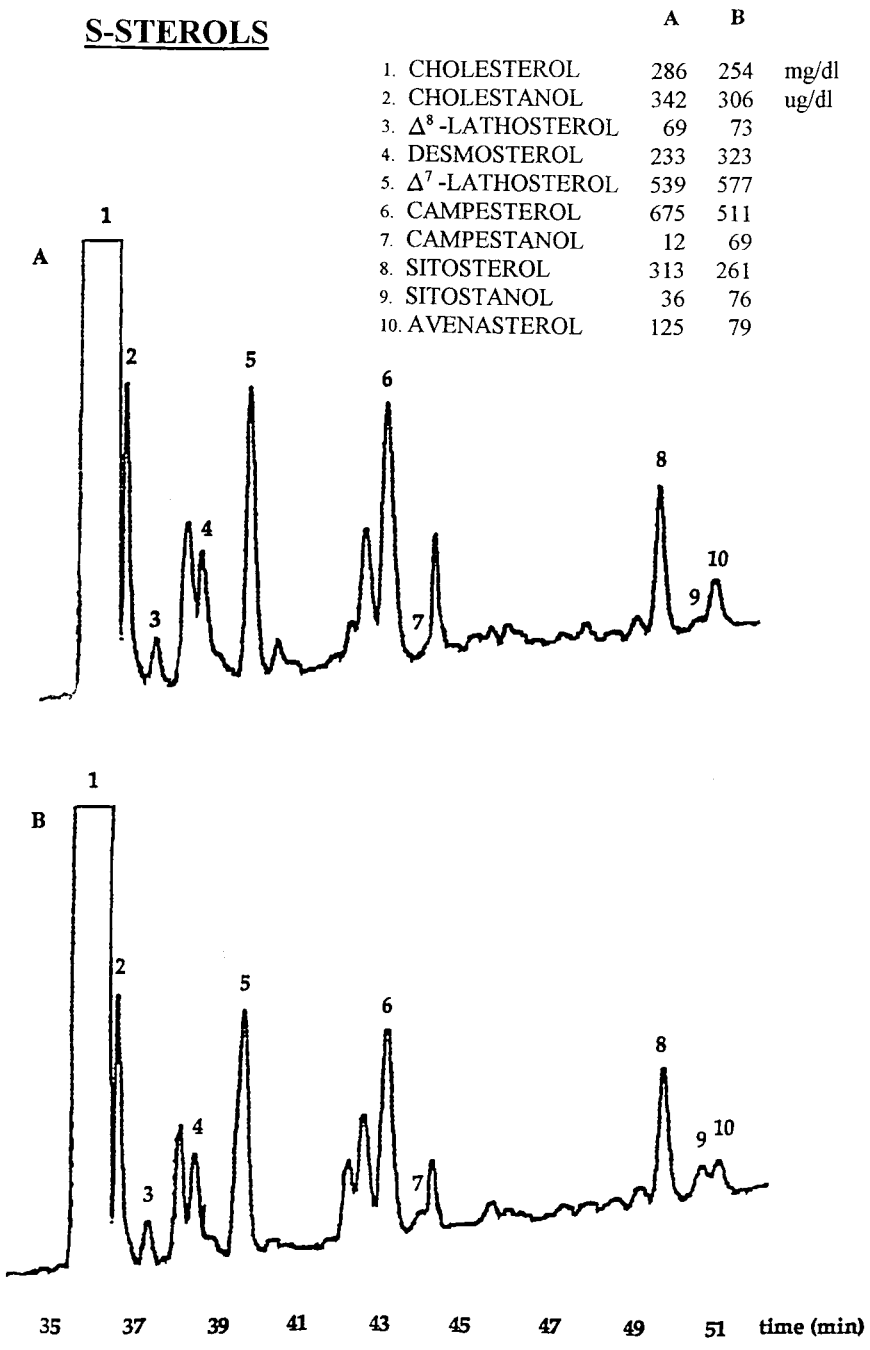

Fig. 1. Gas–liquid chromatogram of serum sterols of a subject at baseline (upper panel, A) and on stanol ester feeding (lower panel, B) for 1 year using Ultra 2 column; 1, cholesterol; 2, cholestanol; 3, Δ^8-lathosterol; 4, desmosterol; 5, Δ^7-lathosterol; 6, campesterol; 7, campestanol; 8, sitosterol; 9, sitostanol; 10, avenasterol.

taining stanol esters corresponding to a scheduled amount of 3 g/day of stanols (sitostanol/campestanol = 9:1). A closer analysis showed that the 3-g dose contained 0.27 g of campestanol. As the margarine packages were similar, and especially because the 153 participants could not distinguish who consumed stanol ester-added and -non-added margarine, the study was a randomized and double-blind study (25). After 6 months on stanol ester margarine, 50 subjects were randomized to use rapeseed oil margarine scheduled to contain 2 g/day of stanol for the subsequent 6 months, whereas the remaining 52 subjects continued on the 3-g dose for the 6 additional months. The subjects did not taste the change in composition of the margarines. After 12 months, all subjects discontinued the use of margarine and were advised to return back to their original home diet.

Consumption of margarine was checked once by measuring the weight of three returned daily-consumed 8 g margarine packages, and during each blood sampling, the subjects reported to nurses the remaining number of packages at home. The margarine was advised to be used in three daily doses on a piece of bread during three major daily meals. The measurements indicated that the actual stanol intakes were 1.8 and 2.6 g/day in the two stanol groups, respectively.

Analytical methods

The subjects were weighed during each blood sampling, and the mean body weight did not change during the study period. Lipid measurements for this study were made solely by gas–liquid chromatography (GLC) (26, 27), because all sterol analyses could be done by a single GLC run for each serum sample. Thus, total cholesterol, cholestanol, Δ^8-lathosterol, desmosterol, lathosterol, campesterol, and sitosterol were analyzed by GLC on a 50 m long SE-30 column (Ultra 1, Hewlett-Packard) from saponified serum extract as their trimethylsilyl derivatives. All these sterols were quantitated from a single run using 5α-cholestane as internal standard. Because the campestanol peak was slightly contaminated and sitostanol had the same retention time as avenasterol in Ultra 1 runs, the quantitation of the stanols was performed on a slightly more polar column (Ultra 2, Hewlett Packard) in a small subgroup of subjects, viz. ten controls and fifteen subjects on stanol esters in pre-stanol phase and again after 1 year (**Fig. 1**). Campestanol and sitostanol peaks were located by running serum sterols of a patient with phytosterolemia on both columns. The non-cholesterol values are given as concentrations and also in terms of mmol/mol of cholesterol, the latter expressed as ratios or proportions to cholesterol of the same GLC run in order to standardize the variability of serum cholesterol level. Single serum analyses were made from samples at −6 weeks (first home value) and 3, 6, and 9 months, whereas two serum analyses a week apart were performed before the start of the stanol ester margarine, at 12 months and at 14 months, i.e., at the end of the second home period. Non-responsiveness to the stanol ester treatment was considered when serum LDL cholesterol at 12 months (measured from the data of previous paper; ref. 25) was above the level of the zero time at the end of the 6-week margarine period. This group included twelve subjects, five of them being on 2 g/day of stanols after 6 months. As dietary consumption of stanol ester margarine would be chronic, the 12-month treatment point was considered to reveal the actual non-responders most reliably.

Statistical treatment was performed by analysis of variance, Student's two-sided *t*-test, paired *t*-test, and analysis of variance and covariance for repeated measures using BMDP Statistical Software package (28). Correlation coefficients were calculated with Pearson's test.

RESULTS

Serum cholesterol

Total cholesterol values, measured by GLC, were significantly higher during the second home diet period compared to the first (+3.5 ± 0.9 (SE)%; $P < 0.001$ for n = 153; **Table 1**, **Fig. 2**). Starting the margarine diet for the first 6 weeks decreased cholesterol similarly in the two groups by only −1.5 ± 0.1% ($P < 0.05$). The mean cholesterol concentration was 9.5% ($P < 0.001$) lower in the stanol than control group during the stanol ester feeding period. The higher the pre-stanol cholesterol level, the higher was the absolute reduction of cholesterol ($r = 0.567$, $P < 0.001$).

Non-responders (shown by broken-ring line in Fig. 2) reduced serum cholesterol more effectively than the responders (−7.0% vs. −0.5%; $P < 0.05$) during the margarine diet for the first 6 weeks, followed, in contrast to the responders, by a significant gradual increase during the stanol feeding period.

Non-cholesterol sterols

Control group. The proportions and concentrations of Δ^8-lathosterol and desmosterol, but not those of lathosterol, increased during the study period in the control group (Table 1, **Fig. 3**). Starting the control margarine diet increased the proportions and concentrations of

TABLE 1. Serum sterol concentrations of control (C) and stanol ester treatment (T) groups during different dietary periods of 14 months

Period, Weeks	Diet	Group	Cholesterol	Cholestanol	Δ^8Lathosterol	Desmosterol	Lathosterol	Campesterol	Sitosterol
			mg/dl	*μg/dL*	*μg/dL*	*μg/dL*	*μg/dL*	*μg/dL*	*μg/dL*
−6	Home diet	C	6.01 ± 0.11	287 ± 11	36.8 ± 2.7	163 ± 6[a]	413 ± 20	658 ± 41[a]	343 ± 19
		T	5.95 ± 0.07	299 ± 8[a]	30.3 ± 1.4	166 ± 4[a]	386 ± 11	639 ± 29[a]	344 ± 14[a]
0	RS	C	5.92 ± 0.10	280 ± 10	38.4 ± 2.5	159 ± 6	419 ± 19	715 ± 42	351 ± 18
	RS	T	5.85 ± 0.07	291 ± 7	31.9 ± 1.2	164 ± 4	378 ± 10	712 ± 31	358 ± 15
26	RS	C	6.07 ± 0.10[a]	283 ± 10	43.2 ± 2.7[a]	166 ± 6[a]	415 ± 20	739 ± 45	357 ± 19
	RS + stanol	T	5.51 ± 0.06[ab]	251 ± 6[ab]	43.0 ± 1.7[ab]	175 ± 4[a]	414 ± 11[ab]	419 ± 19[ab]	274 ± 10[ab]
52	RS	C	6.17 ± 0.10[a]	284 ± 11	48.4 ± 2.6[a]	175 ± 5[a]	434 ± 18	761 ± 46	359 ± 21
	RS + stanol	T	5.54 ± 0.07[ab]	255 ± 6[ab]	48.7 ± 1.8[ab]	180 ± 4[a]	425 ± 12[ab]	430 ± 19[ab]	272 ± 9[ab]
90	Home diet	C	6.34 ± 0.12[a]	301 ± 12[a]	50.5 ± 3.2[a]	185 ± 5[a]	442 ± 19	706 ± 38	362 ± 20
		T	6.12 ± 0.08[a]	309 ± 8[a]	40.8 ± 1.5[a]	177 ± 4[ab]	385 ± 11	684 ± 32	362 ± 15

Values are given as mean ± SE; RS, rapeseed oil margarine; RS + stanol, rapeseed oil margarine + stanol ester.
[a] $P < 0.05$ vs. respective RS value; [b] $P < 0.05$ for changes T vs. C from respective RS value; analysis of variance and covariance for repeated methods.

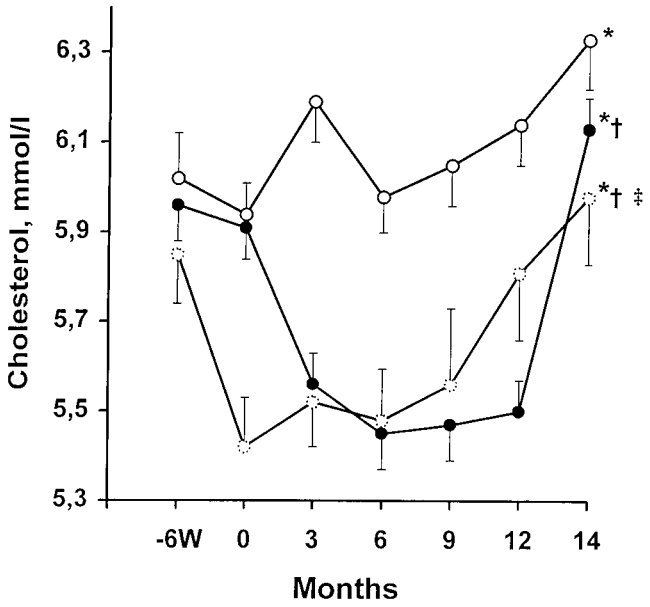

Fig. 2. Serum cholesterol at home diet (−6 week and 14 months) and during margarine treatment without (controls, n = 51, open circles) or with stanol ester in responders (n = 90, closed circles), and in non-responders (n = 12, open broken circles). Mean ± SE (indicated by vertical lines). *$P < 0.001$ for change; †$P < 0.001$ from control curve; ‡$P < 0.001$ from responders. Analysis of variance and covariance for repeated measures.

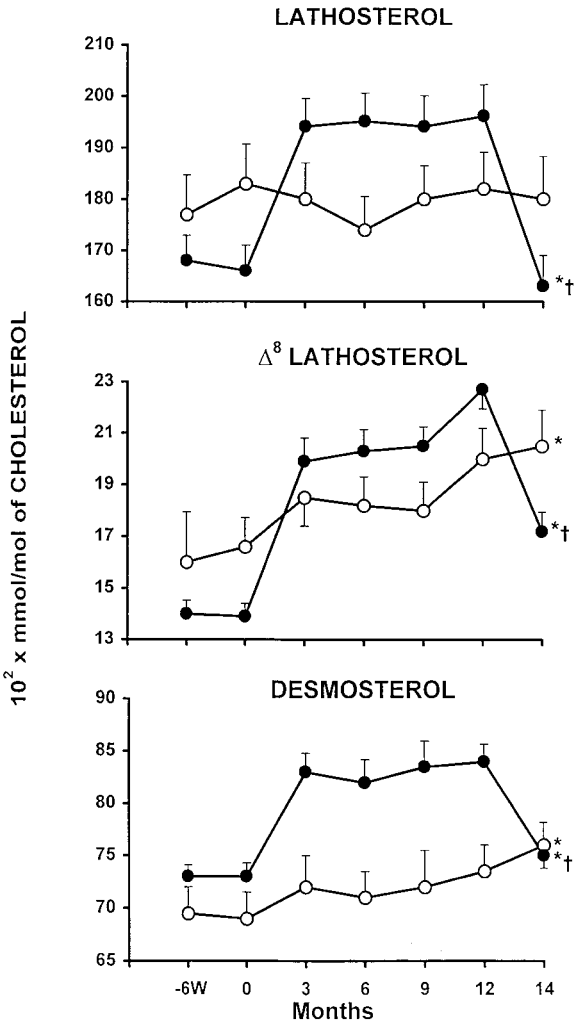

Fig. 3. Serum lathosterol, Δ^8-lathosterol, and desmosterol to cholesterol proportions at home diet (−6 week and 14 months) and during margarine treatment without (controls, n = 51, open circles) or with stanol ester margarine (n = 102, closed circles). Mean ± SE (indicated by vertical lines). *$P < 0.001$ for change; †$P < 0.001$ from control curve. Analysis of variance and covariance for repeated measures.

campesterol, but not that of sitosterol (Table 1, **Fig. 4**). Opposite changes were recorded at the end of the study by discontinuation of the margarine feeding.

Stanol ester group. The proportions and concentrations of the cholesterol precursor sterols were significantly increased from the preceding margarine period or the second home values during the stanol ester feeding ($P < 0.001$, Table 1, Fig. 3), while those of cholestanol, campesterol and sitosterol recorded opposite changes ($P < 0.001$, Fig. 4). The increments of the proportions ranged from 10% to 46% for the precursors at 6 and 12 months, respectively, as compared with the control group, the respective decrements being up to 8% for cholestanol, 42% for campesterol, and 16% for sitosterol ($P < 0.05$ or less for all).

Correlations. The higher the basal home (pre- or post study) or pre-stanol proportions of cholestanol and plant sterols and the lower those of the precursor sterols, the higher were their changes ($r = -0.601$ to -0.795, $P < 0.0001$; n = 102, and $r = -0.286$ to -0.285; $P < 0.01$, respectively) in the stanol ester group. For the whole study population (n = 153), the respective *r*-values ranged from −0.491 to −0.577 ($P < 0.001$) and from −0.307 to −0.483 ($P < 0.001$).

The proportions of the precursor sterols were related to each other at each time point (*r*-values ranged from 0.285 to 0.731), to cholestanol (*r*-values ranged from −0.409 to −0.512) and to the plant sterols (*r*-values ranged from −0.224 to −0.384) in the stanol-fed group (n = 102).

Serum stanols. Sitostanol and campestanol concentrations showed no changes in the controls, but roughly doubled the values at 12 months in the stanol-fed group (**Table 2**).

Correlations of non-cholesterol sterols to cholesterol

Pre- and post-study home or pre-stanol proportions of the precursor sterols were only weakly related to the respective cholesterol concentrations, e.g., for Δ^8-lathosterol $r = -0.213$ ($P < 0.05$). From among the non-cholesterol sterols, only the high precursor sterol proportions of the whole study population predicted low response (n = 153; $r = 0.165$–0.180, $P < 0.05$) of the serum total cholesterol levels. The decrements of the latter were insignificantly related to those of the cholestanol and plant sterol proportions ($r = 0.110$ to 0.188), but the higher the decrement of serum cholesterol the higher were the increments of the precursor sterol proportions ($r = -0.226$ to -0.272, $P < 0.05$–0.01) in the stanol-treated subjects. The respec-

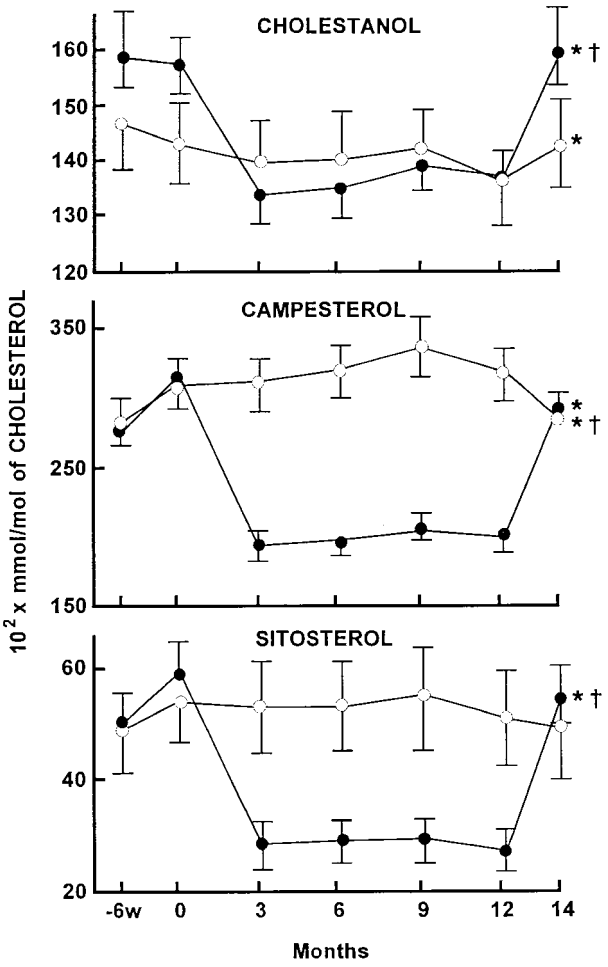

Fig. 4. Serum cholestanol, campesterol, and sitosterol to cholesterol proportions at home diet (−6 week and 14 months) and during margarine treatment without (controls, n = 51, open circles), or with stanol ester margarine (n = 102, closed circles). Mean ± SE (indicated by vertical lines). *$P < 0.001$ for change; †$P < 0.001$ from control curve. Analysis of variance and covariance for repeated measures.

TABLE 2. Serum campestanol and sitostanol concentrations in subgroups of control and stanol-fed subjects

	Campestanol, μg/dL		Sitostanol, μg/dL	
	Home Diet	12 Months	Home Diet	12 Months
Control, n = 10	6.7 ± 1.4	7.2 ± 1.6	25 ± 3	27 ± 1
Stanol ester, n = 15	6.5 ± 0.8	11.1 ± 3.5	27 ± 1	53 ± 5[a]

Values given as mean ± SE.
[a]$P < 0.001$ from home diet and from controls.

tive correlations were more significant for the total study population (n = 153, $r = 0.240$ to 0.320, $P < 0.01$–0.001; $r = -0.301$ to -0.362, $P < 0.001$).

Stanol ester dose

The decrease of the scheduled stanol dose from the scheduled 3 to 2 g/day doses after 6 months feeding increased serum total cholesterol on 2 g/day by 0.21 ± 0.08 mmol/l ($P < 0.001$) and depressed by −0.05 ± 0.06 mmol/l on 3 g/day ($P < 0.001$ vs. the two changes) during the next 6 months. The respective changes in the proportions of the precursor sterols were not significant, but they increased on 2 g/day by 13% for campesterol and 8% for sitosterol ($P < 0.001$ vs. respective 3 g dose for both).

Reasons for non-responding

As already noted, stanol ester feeding increased serum cholesterol level at 12 months in 12 subjects (Fig. 2 and **Fig. 5**). To determine more closely reasons for non-responding, the changes brought by 12-month stanol ester treatment in the non-cholesterol sterols and cholesterol were categorized into four subgroups as shown in Fig. 5; Group 1: cholesterol increased and lathosterol proportion decreased; group 2: both values increased; group 3: both values decreased; and group 4: the lathosterol proportion increased and cholesterol decreased. The respective number of subjects were 3, 9, 17, and 72. Cholesterol and lathosterol values were changed significantly from the pre-stanol ester period in all but the smallest subgroup. The increments of cholesterol in the subgroups 1 and 2 were similar in the 2 or 3 g/day subgroups. The decreased cholesterol level in subgroup 4 was associated with an increased proportion of lathosterol and in subgroup 3 with a decreased lathosterol proportion. Similar results were obtained for Δ^8-lathosterol and desmosterol (data not shown) even though in group 3, in contrast to lathosterol, the Δ^8-lathosterol proportions were increased (+2.9 ± 1.1; $P < 0.05$).

The proportions of cholestanol and plant sterols were significantly decreased (**Table 3**) in subgroups 3 and 4, less consistently in subgroups 1 and 2, and the changes in subgroup 4 were higher than in subgroups 1 and 2. The respective combined non-responder reductions of subgroups 1 and 2 were significant ($P < 0.001$), but significantly less negative than in the responders (Table 3). The increase of sitostanol in the non-responders was +24 ± 3 μg/dL, the change being similar to that of the 13 re-

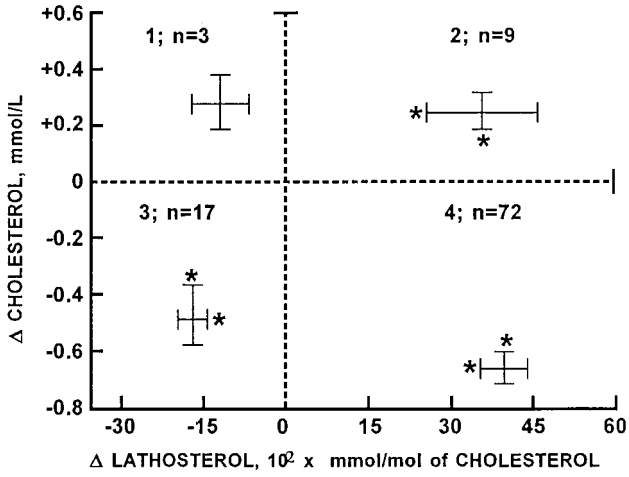

Fig. 5. Changes in serum cholesterol and lathosterol to cholesterol proportion by stanol ester margarine at the end of 1 year treatment from pre-stanol stage. *$P < 0.05$ for changes.

TABLE 3. Reductions of plant sterol and cholestanol proportions at 12 months from pre-stanol stage in non-responders (groups 1 and 2) and responders (groups 3 and 4) to stanol ester

Group	n	Campesterol	Sitosterol	Cholestanol
1	3	-41.7 ± 7.5^{ab}	-5.7 ± 13.8^{b}	-0.5 ± 4.1^{b}
2	9	-62.8 ± 35.4^{b}	-17.4 ± 12.4	-5.9 ± 2.1^{ab}
3	17	-96.8 ± 27.1^{a}	-21.7 ± 9.4^{a}	-6.4 ± 3.1^{a}
4	72	-138.1 ± 9.0^{a}	-35.3 ± 3.6^{a}	-13.2 ± 1.5^{a}

Values given as mean ± SE. Groups 1–4 as in Fig. 5.
$^{a}P < 0.05$ or less; $^{b}P < 0.05$ or less from group 4.

sponders, +27 ± 5 μg/dL, in Table 2. During the whole stanol ester period, the curves of sitosterol and campesterol proportions tended to locate on a lower level in the non-responders than responders.

BMI was positively related to the precursor sterol proportions both during stanol ester margarine feeding and home periods ($r = 0.276$ to 0.470), the respective r-values, from −0.218 to −0.290, being negative for cholestanol and plant sterols in the stanol-fed group. However, the higher the BMI the lower were the stanol-induced decreases of the cholestanol and plant sterol proportions ($r = 0.305$ to 0.377), yet the responses of the precursor sterol proportions or the cholesterol concentration were not related to BMI.

DISCUSSION

The present paper shows that: *1*) the stanol ester feeding-induced decrease of serum cholesterol for a year is associated with a constant decrease in the proportions of the non-cholesterol sterols reflecting cholesterol absorption, and with a constant increase in those reflecting cholesterol synthesis; *2*) basal non-cholesterol sterol proportions predict serum cholesterol response to stanol esters; *3*) the higher the basal proportions of cholestanol and the plant sterols and the lower those of the precursor sterols, the higher are their responses to stanol esters; *4*) campestanol and sitostanol are slightly absorbed; *5*) non-responders to stanol esters effectively decrease their cholesterol concentrations to those observed after consumption of rapeseed oil margarine, followed during subsequent stanol treatment by increased cholesterol synthesis (increased precursor sterols) and a tendency to reduced cholesterol absorption inhibition.

The proportions of cholestanol and plant sterols to cholesterol have been shown to correlate positively with cholesterol absorption efficiency (29, 30). Also, stanol ester feeding reduces cholesterol absorption efficiency by up to 65% in short-term studies (20, 24). Thus, the marked reductions in the proportions of cholestanol and plant sterols to the low level for the whole year of stanol ester feeding indicated that cholesterol absorption was significantly and permanently affected and was most likely the major reason for the decrease of serum total and LDL cholesterol. In addition, the findings between 6 and 12 months of the study indicated that a decrease of stanol dose from 3 to 2 grams per day only slightly increased serum cholesterol by increasing cholesterol absorption efficiency as indicated by an increase of the plant sterol proportions. The marked reduction of serum plant sterols is highly recommendable because phytosterolemia is strongly atherosclerotic (31) and high plant sterol levels may provoke atheromatosis (32).

A long-term lowering of cholesterol absorption efficiency should finally result in depletion of body cholesterol, unless synthesis were compensatorily increased or biliary cholesterol secretion were reduced. In the latter case, absolute absorption of cholesterol should be markedly decreased because of lowered absorption efficiency and reduced intestinal cholesterol pool size, but fecal output of cholesterol as neutral sterols should be increased or remain unchanged. However, sterol balance studies have indicated that biliary cholesterol secretion was not detectably decreased during stanol ester consumption and that fecal elimination of cholesterol was, in fact, increased (20, 24). In addition, constantly decreased plant sterol proportions for the whole year (Fig. 4) indicate reduced absorption. A consistently observed increase of plant sterol proportions during statin treatments (33–37) has been interpreted to be due to decreased biliary sterol secretion during reduced cholesterol synthesis. It does seem most probable that stanol ester treatment increases cholesterol synthesis, a finding reported already in earlier sitosterol feeding sterol balance studies (8) and shown also in several later studies (13, 18–20, 22–24). Increased cholesterol synthesis apparently enhances cholesterol turnover and may increase biliary secretion of plant sterols to bile, a factor contributing to reduced proportions of serum plant sterols.

The weak negative correlation of the basal precursor sterol proportions with the serum cholesterol concentrations suggests that the lower the cholesterol value in this hypercholesterolemic population the higher was cholesterol synthesis. Correspondingly, absorption efficiency should be low, suggesting that stanol ester might not be very effective in these subjects. In fact, the response of cholesterol was positively related to basal cholesterol values and the higher the basal precursor sterol proportions the lower the decrease of serum cholesterol appeared to be. This type of finding has been observed earlier in our short-term studies (16, 24).

Proportions of cholesterol precursor sterols are positively related to cholesterol synthesis and negatively to those of cholestanol and plant sterols and cholesterol absorption efficiency (18, 29, 30, 38–40). Thus, the marked increase of their proportions to cholesterol in the present population indicates that cholesterol synthesis was increased to balance the decreased absorption-induced enhancement of cholesterol elimination from the body by stanol ester feeding. The increase in cholesterol synthesis is a reason why the serum cholesterol level decreased only by about 10%. Plant sterol concentrations in serum were reduced over 50%, and their proportions to cholesterol were reduced ca. 40%. The actual difference between cholesterol and plant sterol metabolism is that the lowering of plant sterols by stanol ester-induced sterol malab-

sorption is not balanced by enhanced synthesis of plant sterols in the body, while the lowering of cholesterol results in the stimulation of cholesterol synthesis. This may contribute to plant sterol reduction, but it may also be the reason why sitostanol ester feeding occasionally does not lower serum cholesterol or may even increase it.

In the present population, 12 subjects did not lower their LDL cholesterol level at 12 months, raising questions about impaired absorption inhibition or extensively increased synthesis of cholesterol. These subjects lowered serum cholesterol by addition of margarine significantly more than the responders for unknown reasons (Fig. 2). Most of the non-responders increased lathosterol proportions (Fig. 5) and had a lower decrease of absorption-related sterols (Table 3), indicating that stimulated synthesis with relatively weak absorption inhibition of cholesterol contributed to a slight increase of serum cholesterol in the non-responders. Earlier studies, even in 1950s, have shown that some patients with familial hypercholesterolemia increased their serum cholesterol during plant sterol feeding (4). Impaired stanol ester intake could have contributed to the relatively low decrease of plant sterols in the non-responders. However, the increase of sitostanol indicates similar compliance with the responders. In group 4 of Fig. 5 or Table 3, the increase of cholesterol synthesis (shown by lathosterol) is similar to that in group 2, but absorption (shown by campesterol and cholestanol in Table 3) is reduced more effectively. In group 3, absorption markers are clearly reduced, perhaps explaining the decrease of cholesterol, but the synthesis shown by lathosterol was inconsistently lowered, even though the Δ^8-lathosterol proportion was increased, indicating a proportionally high increase in activity of enzymes converting lathosterol to 7-dehydrocholesterol and cholesterol, the conversion of Δ^8-lathosterol to lathosterol being a rate-limiting step. A marked reduction of different degree in cholesterol precursor sterols was detected earlier in subjects on statins despite unchanged cholesterol synthesis as judged by the sterol balance technique (23, 34, 41). Increased enzyme activities have been assumed to convert precursor sterols to cholesterol as effectively as possible.

Campestanol and sitostanol concentrations were slightly increased during the year consumption of stanol esters, yet the final values were 15 and 19% of the respective parent sterol concentrations. Longer use of this stanol mixture (up to 8 years in occasional cases) has not further increased the concentrations. In addition, consumption of a stanol mixture with a higher campestanol content (sitostanol/campestanol = 77/23) did not increase further campestanol concentration (42) despite its high absorption rate (43). Stanol feeding to phytosterolemic patients reduced serum plant sterols, the decrease of campestanol and sitostanol being inconsistent (44). Metabolism of stanols, especially their biliary elimination, is not completely understood at the moment. Possible side effects of absorbed stanols, especially their atherogenicity, is not known either. In relation to the serum plant sterol levels, the concentrations of plant stanols remained very low and the levels of the two sterol series were not related with each other. The plant stanol ester margarine has been free of any side effects, even in relatively long-term Finnish experience since 1995 (25), yet factors regulating serum levels and metabolic aspects of stanols require further studies.

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