Serum cholesterol, cholesterol precursors, and plant sterols in hypercholesterolemic subjects with different apoE phenotypes during dietary sitostanol ester treatment

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Abstract A randomized double-blind study was made in 67 modestly hypercholesterolemic subjects by replacing 50 g of daily dietary fat by the same amount of a rapeseed oil preparation without and with fat-soluble sitostanol esters. The diet became relatively rich in dietary fat (37%) especially in subjects with a low basal calorie intake. The esters were prepared by transesterification of sitostanol with rapeseed oil fatty acids. The effects of sitostanol esters were studied on serum cholesterol and cholesterol synthesis (measuring cholesterol precursors in serum) and absorption (measuring serum plant sterols). The results were related to different apoE phenotypes. A 6-week regimen of about 3.4 g/day of sitostanol lowered total and low density lipoprotein (LDL) cholesterol levels by 7.5% and 10%, respectively, over that due to rapeseed oil alone. High density lipoprotein (HDL) cholesterol and triglyceride concentrations were unchanged. Thus, the HDL/LDL cholesterol ratio was significantly increased. The decrease in LDL cholesterol level was more consistent in subjects with the $\epsilon 4$ allele than in those with homozygous $\epsilon 3$ alleles. Sitostanol markedly decreased serum campesterol (−46%) and sitosterol (−30%), especially in subjects with the $\epsilon 4$ alleles known to have high cholesterol absorption. The decreases of LDL cholesterol and plant sterols were interrelated, suggesting that reduced cholesterol absorption contributed to the lowering of LDL cholesterol. Serum sitostanol was unchanged, while the serum cholesterol precursors, Δ^8-cholestenol, desmosterol, and lathosterol, were compensatorily increased by 10% ($P < 0.05$), most consistently in the subjects with $\epsilon 4$ alleles, indicating an increase in cholesterol synthesis. The study demonstrates that sitostanol esters dissolved in dietary fat can be recommended for treatment of modest primary hypercholesterolemia and are apparently practical and suitable for cholesterol lowering in a general population. **—Vanhanen, H. T., S. Blomqvist, C. Ehnholm, M. Hyvönen, M. Jauhiainen, I. Torstila, and T. A. Miettinen.** Serum cholesterol, cholesterol precursors, and plant sterols in hypercholesterolemic subjects with different apoE phenotypes during dietary sitostanol ester treatment. *J. Lipid Res.* 1993. **34:** 1535–1544.

Supplementary key words rapeseed oil • sitostanol • serum cholesterol lowering • lathosterol • sitosterol • campesterol • cholestanol

Plant sterols, campesterol and sitosterol, are not synthesized endogenously in man. Despite their structural similarity to cholesterol, the respective presence of methyl or ethyl groups in the side chains of campesterol and sitosterol results in their poor absorption (1–4). Thus, only small quantities, 0.3–1.7 mg/dl (2, 5, 6) are found in human serum under normal conditions compared with daily dietary intakes of 160–360 mg of plant sterols (5). Within the intestinal lumen the plant sterols apparently compete with cholesterol for incorporation into mixed micelles and other mucosal absorption steps (7–12). Accordingly, intestinal cholesterol absorption is reduced (13–17) to the extent that large doses of plant sterols, usually administered in crystalline form, are used for treatment of hypercholesterolemia (3, 4, 18, 19). Only modest reductions, however, have been observed in serum cholesterol levels. Thus, the interest in using unsaturated plant sterols as hypocholesterolemic agents has recently decreased particularly because other agents, including modern statins (20), have turned out to be effective cholesterol-lowering agents. On the other hand, saturated forms of plants sterols, the 5α-derivatives, especially sitostanol, appear to be almost totally unabsorbable (21–23). They reduce cholesterol absorption more effectively than sitosterol, and even at a relatively small doses, especially when administered in soluble micellar-type form (7, 24, 25), they reduce serum cholesterol level effec-

Abbreviations: campesterol, cholest-5-en-24-methyl-3β-ol; cholestanol, 5α-cholestan-3β-ol; cholesterol, cholest-5-en-3β-ol; desmosterol, cholest-5,24-dien-3β-ol; GLC, gas–liquid chromatography; Δ^8-cholestanol, 5α-cholest-8-en-3β-ol; HMG-CoA, hydroxymethylglutaryl-CoA; lathosterol, 5α-cholest-7-en-3β-ol; SaE, sitostanol ester; sitosterol, cholest-5-en-24-ethyl-3β-ol.

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tively (25, 26). We have prepared a soluble sitostanol preparation by dissolving sitostanol esters in dietary fat. This preparation may introduce sitostanol to the micellar phase during fat digestion and appears to interfere with sterol absorption. Preliminary findings suggest that serum cholesterol is distinctly reduced by relatively small sitostanol ester doses (26).

The purpose of the present randomized, double-blind study was to investigate serum cholesterol changes caused by dietary sitostanol, transesterified with rapeseed oil fatty acids and dissolved in rapeseed oil, in modestly hypercholesterolemic subjects. Additional questions were: is the response to sitostanol ester related to *a)* apoE phenotype, *b)* cholesterol synthesis as measured by cholesterol precursors in serum, and *c)* cholesterol absorption as measured by serum plant sterols and cholestanol; in addition, *d)* are the apoE phenotypes related to changes in cholesterol synthesis and absorption.

SUBJECTS AND METHODS

Study population

Hypercholesterolemic individuals (serum cholesterol >6.0 mmol/l) were screened in occupational health care systems at two different companies (Pohjola Insurance Company and Helsinki Energy Board) at the beginning of 1991. Those having triglycerides above 2.5 mmol/l, on drug medication, or with liver or kidney disease were excluded. Altogether 96 subjects were interested in participating in the study. After screening measurements, 72 subjects (48 men and 24 women) were selected for the study. They were 25 to 60 years of age (**Table 1**).

Preparation of sitostanol ester mayonnaise

Our purpose was to develop a sitostanol preparation that could be used as a component of daily meals for cholesterol-lowering during normal dietary habits. Sitostanol in moderate doses such as 1.5 g/day in soluble preparations significantly lowers absorption and serum levels of cholesterol (25). To get sitostanol soluble in sufficient concentrations in dietary fats, it had to be esterified because oil-solubility of unesterified sitostanol is so low that, for instance, 1 g of free sitostanol intake would require about 100 g of fat to be ingested. Administration of sitostanol in soluble form can be considered to be important to get a high enough content in micellar phase for inhibition of cholesterol absorption. Esterified sterols are known to be hydrolyzed during lipolysis of dietary fats (12). Accordingly, as monoene-rich triglycerides are hypocholesterolemic, sitostanol was transesterified with rapeseed oil (Raisio Inc., Turku, Finland) and the esters were dissolved in rapeseed oil. Thus, the final sitostanol-rapeseed oil "mayonnaise" contained only native rapeseed oil fatty acids. The solubility was such that 1 g of sitostanol could be easily consumed in 10 g of oil or fat preparation.

For the purposes of the present study two different types of rapeseed oil mayonnaise (including 40% water and 60% oil) were prepared (Raisio Inc., Turku, Finland), one containing only rapeseed oil plus water and the other containing additional 15% sitostanol esters (7 g of unesterified sitostanol/100 g of mayonnaise fat). The mayonnaise was delivered in tubes containing 100 g fat. Sitostanol ester (SaE) did not change the taste or smell of the rapeseed oil mayonnaise. Compliance was improved by offering four different mayonnaise flavors with taco, lemon, garlic or natural.

TABLE 1. Study population and demographic data according to group, sex, apoE polymorphism, and body weight

Variable	Control	Sitostanol
Number of subjects	33	34
Male/female	26/7	21/13
Age (y)	43 ± 2	48 ± 2
ApoE phenotypes		
2/3	0	1
3/3	22	16
4/3	10	12
4/4	1	5
Height (cm)	175 ± 1.4	171 ± 1.6
Basal BMI[a]	25.8 ± 0.7	25.2 ± 0.7
Weight by period		
Basal	77.3 ± 2.2	75.3 ± 1.7
Rapeseed oil	77.7 ± 2.2	76.0 ± 1.7
Rapeseed oil ± sitostanol ester	77.7 ± 2.2	76.0 ± 1.7

[a]BMI, body mass index.

Experimental design

The subjects first consumed 50 g of rapeseed oil mayonnaise to replace 50 g of daily fat intake. The mayonnaise was used on bread and as sauce in meals and salads both at work and at home. Mayonnaise tubes could be easily taken to work, or even on vacation trips. Oral and written instructions were given to the participants to reduce their basal diet of 50 g of visible dietary fat as butter, margarine, milk fat, sausages, and cheeses. The rapeseed oil mayonnaise tubes, containing 100 g fat, corresponded to a 2-day use. The participants received the tubes at 2-week intervals at the time of blood sampling. The consumption of the mayonnaise was controlled at 2 weeks during the whole study period by counting the returned tubes and recording their weights. All drop-outs occurred during this period. Four women withdrew during the baseline diet period because they felt the rapeseed oil mayonnaise dose was too much for them. One man withdrew because of vacation. Thus, 67 subjects (Table 1) completed the study.

No baseline dietary recording was made but a questionnaire indicated that the consumption of saturated fat and dietary cholesterol had been reduced. A 7-day diet record indicated that during the rapeseed oil mayonnaise period

energy percent from fat was 37%, saturated fat 12%, monoene fat 19%, and polyene fat 10%; cholesterol intake was 270 mg/day.

After 4 weeks the subjects were randomized into two subgroups: one continued on the rapeseed oil mayonnaise (33 subjects) for an additional 6 weeks and the other used rapeseed oil plus sitostanol ester mayonnaise (34 subjects). In the latter group the subjects consumed, on average, 3400 mg sitostanol daily. The tubes of the rapeseed oil and rapeseed oil plus sitostanol ester mayonnaise could be used blindly because the sterol ester addition did not change the taste. Thus, the study was blinded for both the participants and investigators. A second 7-day diet record performed during the second period indicated that diet composition was similar to that during the first period.

Body weight was recorded at each blood sample drawing. Fasting blood samples were taken before and twice at the end of the rapeseed oil period at weeks 3 and 4, and three times during the second period at weeks 7, 9, and 10. The study protocols were accepted by the Ethics Committee of the hospital.

Laboratory methods

Serum total cholesterol, triglycerides, and HDL-cholesterol were analyzed with commercial enzymatic kits (Boehringer Mannheim, Germany). LDL-cholesterol was calculated according to Friedewald, Levy, and Fredrickson (27). ApoE phenotypes were determined from serum by isoelectric focusing (28). Subjects with the apoE 3/3 phenotype are designated the apoE3 group and those with apoE3/4 and apoE4/4, the apoE4 group.

Cholesterol precursors (squalene, Δ^8-cholestenol, lathosterol, and desmosterol), cholestanol and plant sterols (campesterol, sitosterol, and their saturated forms campestanol and sitostanol) were analyzed in nonsaponifiable material of serum, the sitostanol preparations and the rapeseed oil mayonnaises by gas–liquid chromatography (GLC) using a 50-meter-long SE-30 capillary column (29, 30). Noncholesterol sterols in serum are transported in a mixture with cholesterol in lipoproteins, up to 90%, by low density lipoprotein (31). Accordingly, concentrations of noncholesterol sterols usually have positive correlation coefficients with that of cholesterol. Thus, simple changes in the serum concentration of cholesterol would also change proportionately those of noncholesterol sterols with no change of the noncholesterol sterol concentration in the serum sterol mixture. Thus, noncholesterol sterols in serum are expressed as mmol/mol of cholesterol (the ratio or proportion of noncholesterol sterols to cholesterol) to eliminate the effect of altered lipoprotein level on noncholesterol sterols and to show their changes in the serum sterol mixture. The plant sterol/cholesterol ratio has been shown to be proportional to cholesterol absorption efficiency (32–34).

The rapeseed oil contained mainly campesterol and sitosterol, but small peaks corresponding to campestanol and sitostanol were also found in GLC runs (23). Mass spectrometric analysis on a Hewlett-Packard instrument equipped with a nonpolar SE-30 capillary column showed that the sitostanol peak actually contained only traces of sitostanol, and contained mainly avenasterol. Sitostanol, used for the sitostanol ester preparation, was synthesized from sitosterol by hydration (Kaukas Inc., Lappeenranta, Finland), and GLC revealed the presence of saturated sterols, the amounts of nonhydrated parent unsaturated sterols being negligible. It should be noted that the campestanol peak was not found in any serum samples and mass spectrometric analysis of the "sitostanol" peak in the serum sterol pattern indicated the presence of $\Delta^{8,24}$ dimethylsterol precursor of cholesterol synthesis and avenasterol, while traces of sitostanol were found only during the sitostanol feeding. Therefore, the "sitostanol" values represent the mean of the mixture of these three sterols. Cholestanol and other plant sterol peaks obtained by GLC from sterols in serum appeared to include only the respective sterol. Repeated determinations of a serum pool agreed within 6% (SD). Plant sterol standards were obtained from Kaukas Inc. Finland.

Statistical analyses

Paired *t*-test was used to compare the average changes in lipid levels and in the contents of noncholesterol and plant sterols at different sample drawings; the changes between the groups were compared by Student's *t*-test. Correlation coefficients were calculated with the least-square method.

RESULTS

Table 1 shows that the body weights of the individuals were unchanged during the study. The two mayonnaise preparations were reported to be tasteful and they could not be distinguished from each other. Calculations based on returned mayonnaise tubes indicated that the compliance was good. In the control group the consumption percentages measured at three time points were 93.5%, 88.0%, and 89.5% at 4, 7, and 10 weeks, respectively. The respective figures in the sitostanol group were 93.2%, 91.1%, and 90.1%.

Effects of sitostanol ester on serum lipids

Fig. 1 demonstrates concentrations of serum sterols as a function of time and **Table 2** shows the changes in serum lipids caused by dietary sitostanol. Total and LDL-cholesterol are seen to be reduced by the dietary addition of rapeseed oil.

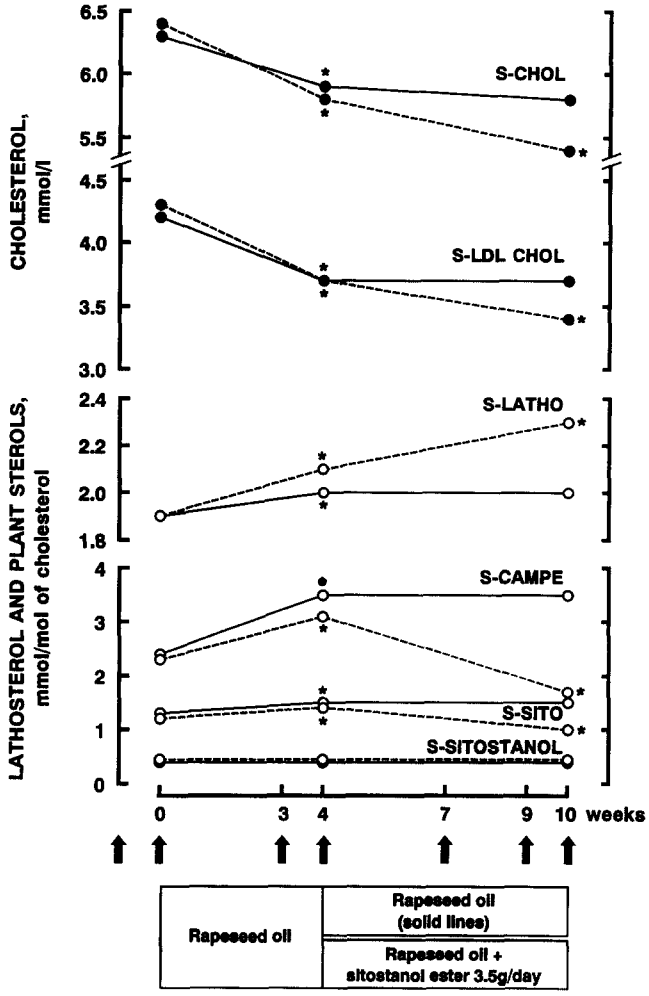

Fig. 1. Changes in serum cholesterol, lathosterol, and plant sterols (campesterol, sitosterol, sitostanol) during rapeseed oil and sitostanol (3.4 g/day) periods (broken lines) (n = 34) in comparison with the rapeseed oil control group (solid line) (n = 33). Serum cholesterol mmol/l, lathosterol and plant sterols mmol/mol of cholesterol. *$P < 0.05$ or less for change from previous period; ↑ = laboratory measurements.

The addition of sitostanol ester to rapeseed oil further reduced serum total and LDL cholesterol levels from the rapeseed oil-period value of 5.84 mmol/l by 0.44 mmol/l (7.5%; $P < 0.05$) and from 3.71 mmol/l by 0.37 mmol/l (10%; $P < 0.05$). The decreases in serum cholesterol were similar in males and females so that there was no gender difference. In comparison with the control group, which used rapeseed oil spread alone (respective reduction 1.2% and 1.1% for total and LDL cholesterol), the net sitostanol ester-induced reductions of the serum total and LDL cholesterol levels were 6.5% and 9%. A reduction of the LDL cholesterol level by 5% or more occurred in 68% of the participants, the reduction occurring insignificantly more frequently in women than men. The decrease in total or LDL cholesterol by sitostanol ester was not quite significantly related to the respective basal, presitostanol levels, even though the correlation was significant when both groups were included ($r = -0.342$; $P = 0.005$).

Serum HDL-cholesterol and triglyceride levels were unchanged, but because of the significant fall in the LDL-cholesterol fraction, the LDL/HDL ratio fell 8.5% ($P < 0.05$) in the sitostanol ester (SaE) group (Table 2). The change of the latter ratio was significantly different from a small increase (1%) in the respective ratio of the rapeseed oil control group. The total LDL-cholesterol reductions from the basal home diet were 14.5% for the control and 21.9% for the SaE group (Fig. 1).

Apolipoprotein E phenotypes and cholesterol change

During the basal home diet and after the rapeseed oil period, the total and LDL-cholesterol levels were not significantly higher in the apoE4 group than in the apoE3 group. However, **Table 3** shows that the SaE-induced LDL-cholesterol reductions, as compared with those in the respective rapeseed oil control groups, were significant only for the E4 group but not for the E3 group. However,

TABLE 2. Effects of rapeseed oil without and with sitostanol ester on serum lipids and the changes

Variable	Group	Rapeseed Oil	Change[a]
		mmol/l	*mmol/l*
Serum total cholesterol	Control	5.88 ± 0.14	−0.07 ± 0.06
Serum total cholesterol	Sitostanol	5.84 ± 0.13	−0.44 ± 0.06[b]
HDL-cholesterol	Control	1.39 ± 0.06	−0.03 ± 0.02
HDL-cholesterol	Sitostanol	1.37 ± 0.06	−0.03 ± 0.02
LDL-cholesterol	Control	3.71 ± 0.11	−0.04 ± 0.07
LDL-cholesterol	Sitostanol	3.74 ± 0.14	−0.37 ± 0.06[b]
Serum triglycerides	Control	1.74 ± 0.26	−0.02 ± 0.13
Serum triglycerides	Sitostanol	1.63 ± 0.15	−0.09 ± 0.07
LDL/HDL ratio	Control	3.71 ± 0.11	+0.04 ± 0.07
LDL/HDL ratio	Sitostanol	3.74 ± 0.14	−0.25 ± 0.07[b]

Values given as mean ± SE; n = 33 for control group and n = 34 for sitostanol group.
[a]Caused by continuous rapeseed oil feeding in the control group and by rapeseed oil plus sitostanol ester in sitostanol group.
[b]$P < 0.05$ or less for change and difference from control.

TABLE 3. Effects of sitostanol ester on serum total and LDL-cholesterol (mmol/l), cholesterol precursors and plant sterols (10^2 × mmol/mol cholesterol) according to apoE phenotype (E3/3 vs. E3/4 + E4/4)

Variable	Group	Rapeseed Oil Diet	Rapeseed Oil + Sitostanol Ester	Change, %
Total cholesterol	E3 C	5.84 ± 0.16	5.77 ± 0.15	−1.0
	E3 SaE	5.89 ± 0.18	5.50 ± 0.19[a]	−6.6
	E4 C	5.83 ± 0.26	5.87 ± 0.23[b]	−1.7
	E4 SaE	5.74 ± 0.19	5.28 ± 0.24[a]	−8.0
LDL-cholesterol	E3 C	3.63 ± 0.10	3.57 ± 0.09	−1.7
	E3 SaE	3.68 ± 0.10	3.46 ± 0.20[a]	−6.0
	E4 C	3.86 ± 0.25	3.86 ± 0.18[b]	±0.0
	E4 SaE	3.82 ± 0.22	3.37 ± 0.21[a]	−11.8
Squalene	E3 C	37 ± 5	38 ± 4	+2.7
	E3 SaE	37 ± 6	41 ± 8	+11.4
	E4 C	26 ± 3	24 ± 3	−5.4
	E4 SaE	26 ± 2	30 ± 2	+1.8
Desmosterol	E3 C	104 ± 22	91 ± 12	−12.1
	E3 SaE	76 ± 3	82 ± 4[a]	+8.6
	E4 C	125 ± 55	104 ± 38	−16.9
	E4 SaE	70 ± 3	78 ± 4[a]	+11.1
Δ^8-Cholestenol	E3 C	18 ± 2	20 ± 2	+10.0
	E3 SaE	19 ± 2	24 ± 2[a]	+26.3
	E4 C	20 ± 3	18 ± 2[b]	−6.5
	E4 SaE	16 ± 2	20 ± 2[a]	+24.4
Lathosterol	E3 C	203 ± 15	205 ± 15[b]	+1.1
	E3 SaE	220 ± 18	247 ± 19[a]	+12.5
	E4 C	204 ± 19	199 ± 17[b]	−2.7
	E4 SaE	192 ± 17	210 ± 15[a]	+8.9
Cholestanol	E3 C	122 ± 7	121 ± 6	−0.4
	E3 SaE	105 ± 6	99 ± 5[a]	−5.7
	E4 C	123 ± 5	120 ± 5	−2.5
	E4 SaE	114 ± 4	110 ± 4	−3.6
Campesterol	E3 C	330 ± 27	331 ± 31[b]	+0.4
	E3 SaE	273 ± 21	154 ± 13[a]	−43.6
	E4 C	377 ± 42	385 ± 41[b]	+2.3
	E4 SaE	353 ± 28	178 ± 14[a]	−49.5
Sitosterol	E3 C	143 ± 12	141 ± 13[b]	−1.5
	E3 SaE	123 ± 8	90 ± 6[a]	−26.8
	E4 C	166 ± 16	160 ± 16[b]	−3.7
	E4 SaE	155 ± 12	102 ± 8[a]	−34.0

Values given as mean ± SE. Abbreviations: C, control; SaE, sitostanol ester.
[a] $P < 0.05$ or less for change from previous period.
[b] $P < 0.05$ for changes between C and SaE.

the reductions were not statistically significant between the apoE3 (−6.0%) and apoE4 (−11.8%) groups. The overall reductions in the total and LDL cholesterol levels from the home diet values by SaE differed significantly from those in the respective rapeseed oil controls in the apoE4 group only (data not shown), even though the SaE-induced changes between the apoE3 and E4 groups (−0.74 vs. −1.05 mmol/l for LDL and −0.86 vs. 1.03 mmol/l for total cholesterol) were not significant.

Squalene and noncholesterol sterols

The replacement of 50 g of daily dietary fat by 50 g of rapeseed oil (contains 360 mg of plant sterols) increased serum lathosterol, campesterol, and sitosterol (Fig. 1). Desmosterol and Δ^8-cholestenol were also increased (data not shown), while "sitostanol" was unchanged. The addition of SaE to the rapeseed oil did not change serum squalene (**Table 4**). On the contrary, the serum levels of Δ^8-cholestenol, lathosterol, and desmosterol increased significantly, and the increases were higher than the respective changes in the rapeseed oil control group. The respective net increases were 21%, 10%, and 27%. The cholesterol precursor/plant sterol ratios were significantly increased by SaE but not by the control mayonnaise. For instance, the lathosterol/campesterol ratio increased from 0.772 ± 0.084 to 1.585 ± 0.164 ($P < 0.001$).

In contrast to the preceeding rapeseed oil period, the levels of serum campesterol and sitosterol were significantly reduced in the SaE group by 47.2% and 31.4%, respectively, while the continuation of rapeseed oil alone

TABLE 4. Effects of sitostanol ester on serum squalene and non-cholesterol sterols (10^2 × mmol/mol of cholesterol)

Variable	Group	Rapeseed Oil	Change[a]
Cholesterol	Control	5.86 ± 0.14	−0.07 ± 0.06[b]
Cholesterol	Sitostanol	5.84 ± 0.13	−0.44 ± 0.06[c]
Squalene	Control	33.4 ± 3.6	+0.2 ± 2.2
Squalene	Sitostanol	30.9 ± 3.2	+4.3 ± 2.2
Desmosterol	Control	111.0 ± 23.1	−15.4 ± 10.6[b]
Desmosterol	Sitostanol	72.8 ± 2.0	+6.8 ± 1.2[c]
Δ^8-Cholestenol	Control	18.5 ± 1.4	+0.8 ± 0.9[b]
Δ^8-Cholestenol	Sitostanol	17.5 ± 1.2	+4.3 ± 0.8[c]
Lathosterol	Control	203.4 ± 11.8	+0.4 ± 5.2[b]
Lathosterol	Sitostanol	205.0 ± 12.0	+21.3 ± 3.7[c]
Cholestanol	Control	122.2 ± 5.2	−1.4 ± 2.0
Cholestanol	Sitostanol	110.0 ± 3.4	−4.8 ± 1.7[c]
Campesterol	Control	345.5 ± 22.7	+3.8 ± 7.3[b]
Campesterol	Sitostanol	314.2 ± 18.3	−148.3 ± 11.5[c]
Sitosterol	Control	150.9 ± 9.8	−3.5 ± 2.6[b]
Sitosterol	Sitostanol	139.9 ± 7.3	−44.0 ± 4.0[c]

[a]Caused by continuous rapeseed oil feeding in control group and by rapeseed oil plus sitostanol ester in sitostanol group.
[b]Different ($P < 0.05$ or less) from sitostanol group.
[c]Significant ($P < 0.05$ or less) change.

made no additional change in the control group. The campesterol/sitosterol ratio was significantly decreased from 2.30 ± 0.04 to 1.72 ± 0.04 by SaE but not by the rapeseed oil control mayonnaise. The proportion of serum "sitostanol" was unchanged, while that of cholestanol was significantly decreased, albeit insignificantly in relation to the rapeseed oil control group.

The changes caused by SaE in the cholesterol precursor/cholesterol proportions were weakly negatively associated with those in the serum cholesterol levels, although in the whole population a significant correlation was found for Δ^8-cholestenol ($r = -0.244$; $P < 0.05$). Also, the changes in the precursors exhibited negative correlation coefficients with those in cholestanol and plant sterols (e.g., $r = -0.258$; $P < 0.05$ for lathosterol vs. campesterol). The changes found during the SaE and control periods of the whole population in serum campesterol and sitosterol were negatively associated with their respective pretreatment values ($r = -0.901$ and $r = -0.812$; both $P < 0.001$) and positively with those in the serum LDL-cholesterol levels ($r = 0.458$ and 0.406; $P < 0.001$). These findings indicated that the higher the plant sterol values were during the standard rapeseed oil period the more their values were reduced during the SaE period. In addition, the high reductions in the plant sterol/cholesterol ratios were associated with the high decrease in the LDL cholesterol levels. The increases of the cholesterol precursor sterol/plant sterol ratios were negatively related to the respective decreases in LDL-cholesterol (P values 0.05–0.01) of the whole population during the SaE period.

ApoE phenotypes, squalene, and noncholesterol sterols

The changes caused by SaE in cholestanol and cholesterol precursors were similar in the E3 and E4 groups, while the respective reductions in campesterol and sitosterol were significantly higher in the apoE4 than in the apoE3 group (Table 3). On the other hand, analogous to the reduction of LDL-cholesterol, the increases of cholesterol precursors caused by SaE in relation to those in the respective rapeseed oil control groups tended to be higher in the apoE4 group than in the apoE3 group; the increase of Δ^8-cholestenol was actually significant only in the E4 group, not in the apoE3 group (**Fig. 2**).

DISCUSSION

The present study shows that the addition of sitostanol ester to dietary fat lowers serum total and LDL-cholesterol levels by about 10% and decreases the LDL/HDL cholesterol ratio. The study also demonstrates that the decrease in LDL cholesterol occurs significantly in the subjects with the $\epsilon 4$ alleles but less so in the subjects homozygous for the $\epsilon 3$ alleles. In addition, the findings on cholesterol precursors and plant sterols in serum suggest that the decrease in LDL-cholesterol is associated with reduced cholesterol absorption and compensatorily enhanced cholesterol synthesis (**Fig. 3**). Furthermore, the changes in synthesis and absorption are related to the apoE genotypes.

Recent studies have shown that sitostanol lowers cholesterol absorption more effectively than sitosterol (7, 24,

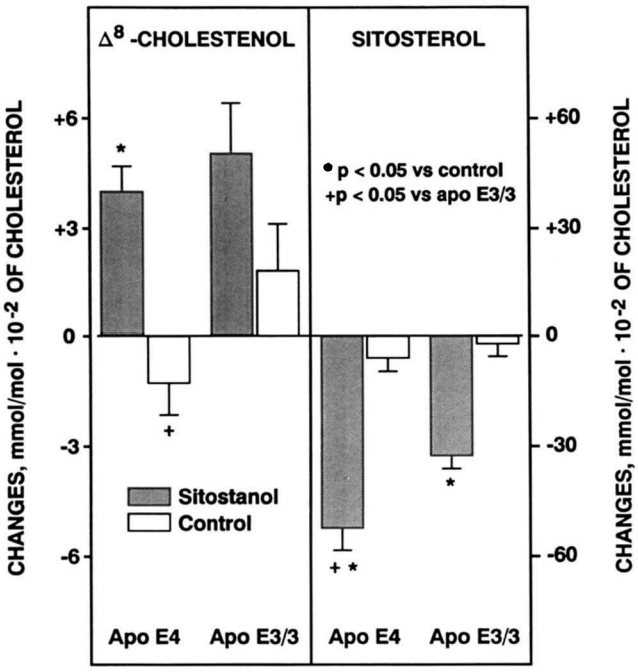

Fig. 2. Changes in Δ^8-cholestenol and sitosterol during rapeseed oil (control) and sitostanol (3.4 g/day) periods in different apoE phenotypes. Mean ± SE (mmol/mol of cholesterol). The lower value of Δ^8-cholestenol on rapeseed oil only for apoE4 subjects may reflect low synthesis due to greater absorption rates.

25). Accordingly, serum cholesterol reduction is also more prominent during oral sitostanol administration at the comparable doses of the two plant sterols (25, 26). An advantage of sitostanol administration has apparently been that the stanol has been given in micellar form, which offers the sterol in soluble form into the intestine where the stanol might replace and precipitate cholesterol from the absorbable micelles more effectively than crystalline sitostanol might do (7, 24). Administration of sitostanol in this type of soluble form has been reported to lower serum cholesterol level detectably in doses of 1.5 g/day (25), even though larger doses may be more effective, and then even more beneficial than sitosterol (25, 26). The increase of sitostanol dose over 3 g/day may not further increase serum cholesterol reduction. This is a reason that in our study the sitostanol intake was 3.4 g/day, though even this figure could have been unnecessarily high. Considering its taste, good compliance of subjects, and effectiveness to lower serum cholesterol, our sitostanol ester–rapeseed oil mayonnaise preparation seems to be a good example for administering sitostanol on a large scale to humans during the normal food consumption for LDL-lowering. Sitostanol-monoene ester could also be similarly mixed with other fats, such as margarine, vegetable oils, fat spreads, and even butter or butter mixes. In the present study dietary cholesterol and saturated fat intakes were relatively low. Thus, administration of sitostanol ester to a diet with higher cholesterol and saturated fat intakes could have resulted in a greater reduction in LDL cholesterol levels. Even under the present study conditions the clearcut LDL cholesterol reduction and the decrease in the LDL/HDL cholesterol ratio could have been beneficial in prevention of coronary heart disease. An additional advantage of using high sitostanol–fat mixtures is that, especially in obese subjects, the energy content of the fat mixtures is reduced by sitostanol.

Apolipoprotein E phenotypes and cholesterol reduction

Despite the finding that the apoE phenotypes were not related to the baseline serum total or LDL cholesterol levels, the decrease in the LDL cholesterol level was significant only in the apoE4 group but not in the apoE3 group. A reason for this may be related to the findings that high cholesterol absorption efficiency in the subjects with the $\epsilon 4$ alleles (28, 35) could be effectively inhibited by sitostanol ester. Cholesterol absorption efficiency is positively related to serum level of LDL cholesterol and apoE phenotypes under normal conditions (35–37). Also, the decrease in dietary fat and cholesterol reduces LDL

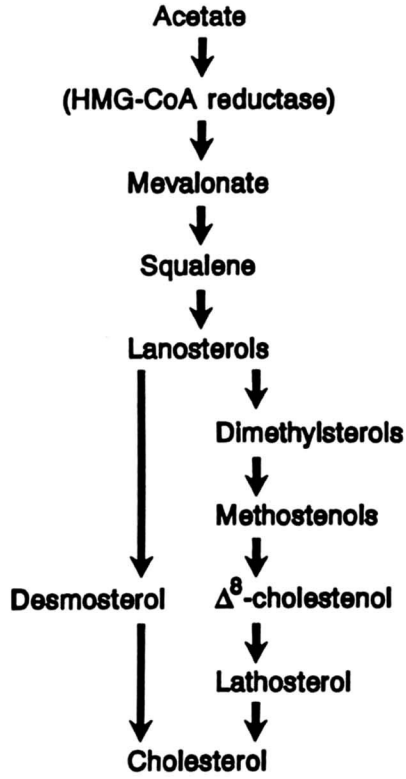

Fig. 3. Schedule of cholesterol synthesis via major precursor sterols in human serum. Cholesterol malabsorption (or bile acid malabsorption) causes activation of cholesterol synthesis by a feed-back mechanism, resulting in enhanced release of precursor sterols and in increased precursor/cholesterol ratios in serum.

cholesterol in proportion to apoE phenotypes and cholesterol absorption (36). The high responsiveness of the apoE4 group to sitostanol ester is in accordance with dietary studies in which dietary manipulations, such as fat and cholesterol reductions or cholesterol feeding, result in highest responses in the apoE4 phenotype (36–39). However, this phenomenon of high serum cholesterol response to dietary manipulations has not been recorded in all studies (40–43).

Cholesterol precursors and plant sterols

The present findings demonstrated also, for the first time, that the reductions in the serum total and LDL-cholesterol levels, obtained during the SaE periods, were related to the increases in serum cholesterol precursors and decreases in plant sterols. As an increase in cholesterol precursors is known to reflect enhanced cholesterol synthesis (30, 44–47) and a decrease in serum plant sterols is known to be related to reduced cholesterol absorption (48), the findings suggest that SaE reduced cholesterol absorption which subsequently increased compensatory cholesterol synthesis. Sitostanol also has been shown to inhibit intestinal cholesterol absorption (7, 24, 25). Examples of our findings are summarized in **Table 5,** which shows a higher LDL cholesterol reduction by SaE associated with a higher decrease in an absorption marker sitosterol and a higher increase in a synthesis marker Δ^8-cholestenol in the apoE4 subjects than in apoE3 subjects. Our preliminary findings by fecal analysis (48) in diabetic patients showed that the dietary SaE was hydrolyzed during intestinal passage, inhibited cholesterol absorption over 60%, and increased cholesterol synthesis; the change in absorption was positively related to those in serum plant sterols. Thus, the decrease in the serum plant sterols caused by SaE in the present study apparently reflects a proportionate reduction in cholesterol absorption efficiency. Accordingly, the positive correlation of the change in plant sterols caused by SaE with their pretreatment values might also indicate that the highest LDL-cholesterol reductions are obtained by SaE in subjects with the highest cholesterol absorption. The finding that the most consistent lowering of LDL-cholesterol was obtained in the subjects with the $\epsilon 4$ alleles, known to have a high cholesterol absorption (28, 35, 36, 38), is in good agreement with this assumption. Negative correlation coefficients between cholesterol precursors and plant sterols suggest that the highest plant sterol and LDL-cholesterol reductions are also associated with the highest compensatory increase in cholesterol synthesis. The positive correlation of the change in the cholesterol precursor sterols/plant sterol ratios with that in LDL-cholesterol also suggests that the highest reduction in LDL-cholesterol was associated with the highest increase in overall cholesterol metabolism.

Some evidence was obtained that apoE4 genotypes were related not only to the high SaE-induced lowering of LDL-cholesterol but also to a high decrease in cholesterol absorption and a large increase in cholesterol synthesis. Thus, the different decreases of the plant sterol values and the increases of the cholesterol precursor values in serum of the different apoE genotypes suggested that the decrease of LDL-cholesterol was related to greater reduction in absorption and a greater increase in synthesis in the subjects with $\epsilon 4$ alleles than in the subjects with the homozygous $\epsilon 3$ allele. Earlier findings in a random male population have shown that cholesterol absorption and plant sterols are higher and the indicators of cholesterol synthesis are lower in subjects with the $\epsilon 4$ alleles than in those with the $\epsilon 2$ alleles (28, 35, 49). Analogous to the present findings, dietary manipulations change cholesterol synthesis and absorption according to apoE genotypes (36).

In agreement with our earlier results (23) the addition of SaE decreased serum levels of campesterol more (−47%) than that of sitosterol (−17%) from the rapeseed oil period so that the campesterol/sitosterol ratio was significantly decreased. The "sitostanol" value was unchanged, indicating that sitostanol is apparently unabsorbable and that sitostanol may not be absorbed even in subjects with high sterol absorption efficiency such as the apoE 4/4 phenotype or even in sitosterolemia. Sitostanol has been suggested to be unabsorbable in the rat (21–23). Unabsorbability of sitostanol means that the stanol might not be taken up by the intestinal mucosa so that it hardly interferes with mucosal esterification of cholesterol or plant sterols, a procedure known to contribute to sterol absorption (10, 50). Thus, its inhibitory effect on sterol absorption may actually include an inhibition of micellar solubilization of other sterols by mass action. Unabsorbability of sitostanol and its action to reduce serum levels of other plant sterols may be important because hyperphytosterolemia has been considered to increase coronary risk in hypercholesterolemic subjects (51).

In conclusion, sitostanol ester dissolved in dietary fats produces a tasty preparation that significantly decreases serum total and LDL cholesterol levels. The sterol is

TABLE 5. Rapeseed oil–sitostanol ester-induced relative changes[a] (percent) in LDL-cholesterol, Δ^8-cholestenol (reflects cholesterol synthesis), and sitosterol (reflects cholesterol absorption) in patients with different apoE genotypes

Sterol	ApoE 3/3	ApoE 3/4/ApoE 4/4
LDL-cholesterol	− 4.4	− 11.8[b,c]
Δ^8-Cholestenol	+ 13.7	+ 38.9[b,c]
Sitosterol	− 25.8[b]	− 31.7[b,c]

[a]Changes corrected by changes in respective rapeseed oil control period in Table 3.
[b]Significant changes ($P < 0.05$ or less).
[c]Change different from apoE 3/3 ($P < 0.05$ or less).

apparently not absorbed so that consumption of the sitostanol ester–fat preparation appears to be safe from the nutritional point of view even in large-scale population use at sufficiently high doses for lowering of serum cholesterol. As the use of esterified sitostanol markedly increases solubilization of sitostanol in dietary fats, only a modest daily intake (<30 g) of sitostanol ester–fat preparation is needed for effective serum cholesterol reduction.

The expert technical assistance of Mrs. E. Gustafsson, P. Hoffström, and L. Kaipiainen as well as secretarial help of Mrs. Marja Aarnio and Soile Aarnio is gratefully acknowledged. The study was supported by a grant to Tatu Miettinen from Helsingin Yliopisto and Juho Vainio Foundation which is gratefully acknowledged.

Manuscript received 15 December 1992 and in revised form 26 March 1993.

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