Fat-Modified Diets Influence Serum Concentrations of Cholesterol Precursors and Plant Sterols in Hypercholesterolemic Subjects

Essi S. Sarkkinen, Matti I.J. Uusitupa, Helena Gylling, and Tatu A. Miettinen

Serum noncholesterol sterols, cholesterol precursors and plant sterols, are indicators of cholesterol absorption and synthesis. Serum plant sterol concentrations correlate positively with cholesterol absorption, but have also been found to correlate with dietary unsaturated to saturated fatty acid ratios. We studied the concentration of serum noncholesterol sterols during four different fat-modified diets, (1) high-fat, saturated fat–enriched (control), (2) reduced-fat, sunflower oil–enriched (SO-enriched), (3) rapeseed oil–enriched (RO-enriched), and (4) reduced-fat, saturated fat–enriched (reduced-fat), followed for 6 months in hypercholesterolemic subjects in a parallel design. The proportion of lathosterol (micrograms per 100 mg cholesterol), a precursor of cholesterol synthesis, increased significantly ($P < .05$) in both SO-enriched (mean ± SD 147 ± 57 *v* 167 ± 76, 0 *v* 6 months) and RO-enriched (147 ± 54 *v* 157 ± 52) groups, where the reduction in low-density lipoprotein (LDL) cholesterol was also significant. The proportion of sitosterol, a plant sterol, decreased significantly in the control group (137 ± 48 *v* 122 ± 42), and the proportion of another plant sterol, campesterol, increased in the RO-enriched group (280 ± 141 *v* 333 ± 162), reflecting changes in the use of vegetable oils in these two groups rather than increased cholesterol absorption. In the whole study population, the proportion of linoleic and α-linolenic acid (a marker of the use of RO) in cholesterol esters (CEs) correlated ($P < .001$) with the proportion of sitosterol ($r = .43$) and campesterol ($r = .36$) in serum at the end of the study. In conclusion, serum cholesterol precursors were found to be useful indicators of cholesterol metabolism, but changes in serum plant sterols reflected dietary changes rather than cholesterol metabolism during long-term dietary intervention with fat-modified diets.

THERE IS A CLEAR need to measure the efficiency of cholesterol absorption and the activity of cholesterol synthesis in outpatient dietary intervention studies,[1-3] since these factors appear to regulate the concentration of serum cholesterol.[4,5] The higher the fractional and absolute absorption of dietary cholesterol, the lower the rates of biliary secretion, fecal elimination, and endogenous synthesis of cholesterol.[5]

Recently, serum noncholesterol sterols have been used to study cholesterol absorption and synthesis.[6-9] Cholesterol precursor sterols in serum, like desmosterol, lathosterol, and squalene, reflect cholesterol synthesis, while serum plant sterols (campesterol and sitostanol) and, to some extent, cholestanol reflect absorption and biliary elimination of sterols.[6-10] Thus, measurement of serum noncholesterol sterols is valuable for monitoring cholesterol metabolism in large-scale outpatient studies.[6-10]

Plant sterols are present in varying amounts in vegetable oils, and the amounts present in the diet are related to the serum levels. Thus, serum concentrations of plant sterols exhibit positive correlations with the polyunsaturated to saturated fatty acid ratio of dietary fat and with the proportion of linoleic acid in serum and dietary lipids.[8]

A large dietary intervention study was performed previously in an outpatient setting with four different dietary regimens.[2] Enrichment of the diet with sunflower oil (SO) or rapeseed oil (RO) significantly decreased serum low-density lipoprotein (LDL) cholesterol concentrations, probably through altered fatty acid composition. However, the contribution of altered cholesterol metabolism also could have been involved in this, and attempts were made to reveal possible alterations by examining the proportion of serum cholesterol precursors and plant sterols during four different fat-modified diets: high-fat, saturated fat–enriched (control), reduced-fat, SO-enriched (SO-enriched), RO-enriched (RO-enriched), and reduced-fat, saturated fat–enriched (reduced-fat). Furthermore, the relations of plant sterols in plasma with the proportion of unsaturated fatty acids in dietary and serum lipids were examined.

SUBJECTS AND METHODS

Subjects

One hundred sixty free-living subjects with mild to moderate hypercholesterolemia aged 23 to 58 years consumed four (including control) different fat-modified diets for 6 months.[2] Subjects were recruited through the occupational health care system in the area of Kuopio in Eastern Finland. The original screening criterion was a serum total cholesterol level between 6.5 and 8.0 mmol/L before the study.

Exclusion criteria were known diseases affecting serum lipid levels, irregular eating patterns, or excess consumption of alcohol (>45 g ethanol/d). Only one subject withdrew from the study, due to a diagnosis of cancer, and for six subjects the samples for analysis of noncholesterol sterols were not taken. Nineteen subjects were taking β-blockers and/or diuretics for hypertension or coronary heart disease, and 16 women received postmenopausal estrogen therapy. Drug treatments were not changed during the study. None of the subjects had previous treatment with drugs for hyperlipidemia. Baseline characteristics of the subjects are shown in Table 1.

Study Design

During a 2-week run-in period, the subjects on their habitual diet visited the research unit twice. At the first visit, routine laboratory measurements were taken to ensure normal health status. In addition, medical history, drug use, smoking habits, alcohol consumption, and physical activity were reviewed by a questionnaire. The subjects were requested to keep their smoking habits and physical activity constant during the study. At the third visit (baseline), the subjects were

From the Department of Clinical Nutrition, University of Kuopio, Kuopio; and Department of Medicine, University of Helsinki, Helsinki, Finland.

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Address reprint requests to Essi S. Sarkkinen, PhD, Department of Clinical Nutrition, University of Kuopio, PO Box 1627, 70211 Kuopio, Finland.

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Table 1. Baseline Characteristics and Serum Lipids During the Study

Characteristic	Diet Group			
	Control (n = 36)	SO-Enriched (n = 37)	RO-Enriched (n = 41)	Reduced Fat (n = 39)
Men/women (n)	17/19	16/21	19/22	18/21
Age (yr)	43.1 ± 8.3	47.9 ± 7.7	46.4 ± 7.4	45.6 ± 7.5
Body mass index (kg/m²)	25.5 ± 4.2	26.1 ± 4.1	26.6 ± 3.8	26.5 ± 3.4
Total cholesterol (mmol/L)				
0 mo	6.40 ± 1.12	6.60 ± 1.03	6.54 ± 1.12	6.31 ± 1.08
6 mo	6.53 ± 1.08	6.28 ± 1.03*	6.30 ± 1.11	6.32 ± 1.18
LDL cholesterol (mmol/L)				
0 mo	4.33 ± 1.00	4.58 ± 0.88	4.55 ± 0.95	4.38 ± 1.00
6 mo	4.38 ± 0.97	4.25 ± 0.86†	4.25 ± 0.95†	4.24 ± 1.04
HDL cholesterol (mmol/L)				
0 mo	1.43 ± 0.38	1.38 ± 0.26	1.35 ± 0.28	1.36 ± 0.35
6 mo	1.54 ± 0.40‡	1.42 ± 0.28	1.38 ± 0.30	1.38 ± 0.34
Triglycerides (mmol/L)				
0 mo	1.41 ± 0.74	1.48 ± 1.06	1.55 ± 0.94	1.38 ± 0.68
6 mo	1.36 ± 0.84	1.27 ± 0.60§	1.40 ± 0.84	1.44 ± 0.80

NOTE. Values are the mean ± SD, and were analyzed by multivariate ANOVA for repeated measurements.
*$P < .05$, *v* control group.
†$P < .01$ to $.001$, *v* baseline (0 mo).
‡$P < .001$, *v* baseline (0 mo).
§$P < .05$, *v* baseline (0 mo).

randomized into one of the four diet groups for the next 6 months. They visited the research unit five times (at 2 weeks, 6 weeks, 3 months, and 6 months) during this period. Height was measured at the baseline visit only. Weight with light clothing was measured at every visit. Blood samples for laboratory measurements were taken at every visit, after a 12-hour overnight fast. Serum total cholesterol, triglycerides, and high-density lipoprotein (HDL) cholesterol were determined from the blood samples at every visit, but the LDL cholesterol concentration and fatty acid composition of serum cholesterol esters (CEs) were analyzed at baseline and at 3 and 6 months. The concentrations of squalene and noncholesterol sterols were determined at baseline and at 6 months.

Both the subjects and the nutritionists participating in nutritional counseling were unaware of the serum lipid values during the study. Patients provided informed consent for the study, and the study plan was approved by the Ethics Committee of the University of Kuopio.

Experimental Diets

The goals for the experimental diets and their actual composition are presented in Table 2. Subjects received detailed written instructions on the diets at their own energy level (7.6, 8.4, 10.1, or 11.8 MJ/d).

The diets were composed of normal food items, substituting low-fat items for high-fat foods typically consumed in the Finnish diet. The fatty acid composition was adjusted by changing the quality and quantity of fat spreads and oils. The following fats were provided on a single-blind basis: butter (Valio, Helsinki, Finland) and a small amount

Table 2. Dietary Goals and the Actual Composition of the Diets During the Study

Nutrient	Diet Group							
	Control (n = 36)		SO-Enriched (n = 37)		RO-Enriched (n = 41)		Reduced Fat (n = 39)	
	Goal	Actual	Goal	Actual	Goal	Actual	Goal	Actual
Energy (MJ/d)		8.0 ± 1.4		7.2 ± 1.5		7.6 ± 1.6		6.8 ± 1.8
Fat (E%)*	38	35.0 ± 3.8	30	31.9 ± 3.5	38	33.9 ± 3.6	28	29.8 ± 4.1
Saturated fat†	18	14.5 ± 1.8	10	10.2 ± 1.3	14	10.6 ± 1.8	14	11.8 ± 2.1
Monounsaturated fat†	15	10.0 ± 1.4	10	7.9 ± 1.0	16	11.3 ± 1.4	10	8.2 ± 1.2
Polyunsaturated fat†	5	3.6 ± 0.8	10	7.9 ± 1.6	8	5.4 ± 0.8	4	3.2 ± 0.5
Linoleic acid								
g/d		5.1 ± 1.4		13.3 ± 3.5		7.5 ± 2.0		3.9 ± 1.4
E%		2.5 ± 0.6		7.0 ± 1.7		3.7 ± 0.6		2.1 ± 0.5
Oleic acid								
g/d		9.4 ± 3.3		8.6 ± 2.4		15.2 ± 4.6		4.9 ± 2.1
E%		4.4 ± 1.3		4.4 ± 0.8		7.5 ± 1.5		2.7 ± 0.8
Protein (E%)	15	16.2 ± 1.7	15	17.7 ± 1.5	15	17.2 ± 1.6	15	18.1 ± 2.1
Carbohydrate (E%)	47	47.9 ± 5.7	55	49.7 ± 4.8	47	48.4 ± 4.6	57	52.8 ± 5.2
Alcohol (E%)		2.4 ± 4.1		2.4 ± 2.6		1.9 ± 2.7		1.0 ± 1.7
Fiber (g/d)	15-20	20.1 ± 4.2	15-20	20.8 ± 6.1	15-20	20.9 ± 6.0	15-20	20.4 ± 6.3
Cholesterol (mg/d)		301 ± 83		230 ± 82		230 ± 50		228 ± 71

NOTE. Values are the mean ± SD.
*Percent of calories of total energy intake.
†Fatty acids as energy percent.

of low–erucic acid RO ([LEAR] Van den Bergh Foods, Helsinki, Finland) for the control group; SO margarine and SO (Van den Bergh Foods) for the SO-enriched group; LEAR oil margarine and LEAR oil (Van den Bergh Foods) for the RO-enriched group; and a mixture of butter and LEAR oil (80% milk fat) (Valio) for the reduced-fat group.

The degree of adherence to the diets was evaluated during the study with repeated dietary records. The subjects kept food consumption records using household measures for 3 consecutive days 2 weeks before every visit to the research unit, altogether for 15 days during the study. At the study visits, all amounts and qualities of foods in the records were checked for completion, filling in data that were lacking. Photos of portion sizes and food models were used in checking the food records.

The analyses of nutrients were made using the software system developed at the National Public Health Institute, Helsinki, Finland. Composition data are a mixture of values obtained from the Finnish food analyses and the international food composition tables. Data on fiber and available carbohydrates and fatty acids are based on recent analyses of Finnish foods.[11]

Laboratory Measurements

Lipoproteins were separated by ultracentrifugation for 12 hours at density 1.006 to remove very–low-density lipoprotein (VLDL). HDL in the infranatant was separated from LDL by precipitation of LDL using dextran sulfate and magnesium chloride.[12] LDL cholesterol was calculated as the difference between the mass of cholesterol in the infranatant and in HDL, and VLDL cholesterol was calculated as difference between the whole serum and the infranatant.

Enzymatic colorimetric methods were used for determination of cholesterol and triglycerides from whole serum and separated lipoproteins using commercial kits (no. 237574 and 701904; Boehringer, Mannheim, Germany) with a Kone Specific Clinical Analyzer (Kone, Espoo, Finland). The interassay coefficient variation of control serum samples for total cholesterol was 2.3%, HDL cholesterol 3.1%, and triglycerides 2.2%.

Serum concentrations of the plant sterols campesterol, sitosterol, and cholestanol and the cholesterol precursor sterols squalene, Δ^8-cholestenol, lathosterol, and desmosterol were measured with gas-liquid chromatography[13] from samples obtained at the same time in the morning to avoid diurnal variation.[14] In brief, 0.2 mL serum was removed, nonsaponifiable serum lipids were derivatized with trimethylsilyl ether after addition of an internal standard (5α-cholestane), and the sterols were quantified by gas-liquid chromatography with a 50-m SE-30 capillary column. Respective retention times calculated in relation to the time for 5α-cholestane were as follows for these seven compounds: 1.867, 2.118, 1.640, 0.908, 1.649, 1.680, and 1.733. The accuracy of the method, eg, for campesterol is ±2.5%, and the reproducibility is ±4.8%. For the small peaks like Δ^8-cholestenol, the values were slightly higher. To eliminate the effect of altered lipoprotein concentration, squalene and noncholesterol sterols are expressed as micrograms per 100 mg cholesterol. Thus, the values presented are proportions or ratios to cholesterol, not serum concentrations.

For determination of the fatty acid composition of serum cholesterol esters, the lipids were extracted with dichloromethane-methanol.[15] The lipid fractions were separated with thin-layer chromatography, and the esters were transesterified with acidic methanol.[16] The percentage composition of the fatty acids was determined by an HRGC 412 Micromat gas chromatograph (HNU-Nordion Instruments, Espoo, Finland) with an NB-351 column, split injection, and helium as carrier gas.

The apolipoprotein E (apo E) phenotype was determined from the plasma after delipidation using isoelectric focusing and immunoblotting techniques.[17,18]

Statistical Analyses

Analyses of the data were performed using the Statistical Package for Social Sciences.[19] Values are expressed as the mean ± SD. Serum lipid variables and other variables normally distributed and homogenous in variance were tested with multivariate ANOVA for repeated measurements. Data for all time points of measurements are not shown in Table 1, but were used in the statistical analysis. Noncholesterol sterol variables were measured only twice, once before randomization and once at the end of the 6-month dietary period, and were not normally distributed or homogenous in variance. Nonparametric tests were used for these variables: the Wilcoxon test for within-group changes and the Mann-Whitney U test for between-group differences. In addition, Spearman correlation coefficients were calculated for the concentration of plant sterols and some dietary fatty acid variables of interest.

RESULTS

Baseline Characteristics, Diet, and Serum Lipids

Table 1 lists the baseline characteristics of the study population by diet group. There were no significant differences in the age, sex distribution, medication, body mass index, or serum lipid values between diet groups. Table 2 shows the goals for the diets and the composition of the actual diets during the study. The fatty acid composition adhered well to the goals, but the amount of fat exceeded the goal in the SO-enriched group remained below the goal in the control and RO-enriched diet groups.[2] The fatty acid composition of serum CEs verified the good compliance to the fatty acid modification of the diets.[20] The intake of campesterol increased by 72 mg in the RO-enriched diet group and intake of sitosterol increased 46 mg due to the test fats, while the respective figures in the SO-enriched diet group were about 9 and 54 mg during the dietary intervention.

The decrease in LDL cholesterol was significant and equal in the SO-enriched and RO-enriched diet groups (Table 1). Total triglycerides decreased significantly ($P < .001$) only in the SO-enriched diet group. Serum total and HDL cholesterol increased slightly in the control group, while HDL cholesterol remained unchanged with the other diets. No significant improvement in the serum lipid profile was found in the reduced-fat diet group (Table 1).

Relationship of Precursor Sterols and Plant Sterols to Apo E Phenotype

At baseline, the precursor sterol proportions tended to relate negatively to the subscript of the apo E phenotype, with the respective relation tending to be positive for plant sterols (Table 3). In fact, comparison of campesterol and sitosterol proportions between subjects with apo E 3/2 + 4/2 and subjects with apo E 3/4 + 4/4 showed significantly ($P < .05$) higher ratios in the latter group. The finding may indicate that subjects with the E2 allele had a lower absorption and higher synthesis of cholesterol than those with the E4 allele and without the E2 allele. However, due to the small number of subjects of each apo E phenotype within each diet group, no consistent differences in dietary responses were observed among the phenotypes.

Table 3. Noncholesterol Sterols (μg/100 mg cholesterol) at Baseline (0 months) by Apo E Phenotype

Sterols	Apo E Phenotype		
	3/2 + 4/2 (n = 9)	3/3 (n = 84)	4/3 + 4/4 (n = 59)
Precursor sterols			
Squalene	27.4 ± 11.2	25.5 ± 12.2	28.2 ± 10.6
Cholesterol	26.4 ± 16.8	18.3 ± 10.8	19.3 ± 14.5
Lathosterol	159.6 ± 49.3	142.6 ± 50.0	144.5 ± 52.1
Desmosterol	79.7 ± 12.7	75.2 ± 19.3	73.5 ± 18.4
Cholestanol	124.6 ± 15.4	124.7 ± 32.0	124.5 ± 29.1
Plant sterols			
Campesterol	206.6 ± 54.0	249.2 ± 115.4	261.0 ± 129.1*
Sitosterol	116.4 ± 38.4	140.0 ± 53.6	149.1 ± 59.2*

NOTE. Values are the mean ± SD.

*$P < .05$, apo E 3/2 + 4/2 *v* 4/3 + 4/4, analyzed with the Mann-Whitney nonparametric *U* test for independent variables.

Changes in Precursor Sterols and Plant Sterols During the Dietary Intervention

Table 4 shows the serum proportion of squalene and noncholesterol sterols during the study in the different diet groups. There were no significant differences in the baseline proportion of serum squalene or noncholesterol sterols between the diet groups.

The proportion of lathosterol increased significantly in both the SO-enriched and RO-enriched diet groups, while desmosterol only tended to increase in the SO-enriched group during the study. The proportion of cholesterol decreased significantly during the control and RO-enriched diets, but sitosterol decreased significantly only in the control group. At the end of the study, sitosterol ratios were higher in the SO-enriched and RO-enriched diet groups compared with the control group ($P < .05$). The proportion of campesterol increased markedly with the RO-enriched diet, even significantly above the respective proportion in the control group ($P < .001$). The results were also analyzed as the absolute concentration of noncholesterol sterols per serum, but it did not change the main findings or conclusions. The changes were slightly smaller and were affected by the overall changes in serum lipid levels in these groups (data not shown).

Correlations Between Dietary Variables and Plant Sterol Ratios in Serum

The amount of oleic acid (grams per day; parallel to the amount of LEAR oil) in the RO-enriched diet (n = 41) during the study (average of all food records) correlated significantly ($r = .32$, $P = .040$) with the campesterol level at the end of the study. When all groups (N = 153) were combined, there was also a significant correlation between oleic acid in the diet during the whole study period and the serum concentration of campesterol at the end of the study ($r = .23$, $P = .004$).

As previously reported, the proportion of α-linolenic acid in

Table 4. Noncholesterol Sterols (μg/100 mg cholesterol) During the Study by Diet Group

Sterols	Diet Group			
	Control (n = 36)	SO-Enriched (n = 37)	RO-Enriched (n = 41)	Reduced Fat (n = 39)
Precursor sterols				
Squalene				
0 mo	25.3 ± 12.7	28.7 ± 9.4	27.4 ± 12.0	25.2 ± 11.9
6 mo	25.6 ± 11.5	29.8 ± 16.0	28.0 ± 10.4	27.1 ± 24.2
Cholestenol				
0 mo	19.5 ± 13.5	20.0 ± 15.3	18.9 ± 13.3	18.3 ± 8.7
6 mo	22.4 ± 17.1	19.5 ± 10.8	18.2 ± 11.0	18.3 ± 8.8
Lathosterol				
0 mo	137.2 ± 40.9	146.7 ± 58.6	147.3 ± 54.5	145.5 ± 47.5
6 mo	139.8 ± 43.2	166.8 ± 75.7	156.7 ± 51.6	147.9 ± 52.2
		$P < .05$*	$P < .05$*	
Desmosterol				
0 mo	76.0 ± 18.5	72.1 ± 17.3	74.6 ± 18.2	76.4 ± 10.4
6 mo	75.9 ± 19.6	75.8 ± 16.0	73.9 ± 14.7	77.9 ± 28.3
Cholestanol				
0 mo	123.8 ± 23.3	126.0 ± 26.6	125.9 ± 38.4	122.8 ± 29.5
6 mo	115.6 ± 19.2	121.5 ± 25.1	121.8 ± 44.8	123.8 ± 23.6
		$P \leq .05$*		$P \leq .05$*
Plant sterols				
Sitosterol				
0 mo	136.9 ± 48.5	150.2 ± 61.4	146.8 ± 60.7	134.3 ± 49.7
6 mo	121.5 ± 42.3	149.6 ± 55.2†	152.6 ± 63.4†	138.8 ± 51.2
	$P < .01$*			
Campesterol				
0 mo	238.5 ± 98.2	254.1 ± 125.2	280.4 ± 141.5	229.6 ± 98.9
6 mo	231.3 ± 85.7	240.9 ± 102.5	333.2 ± 162.8‡	232.6 ± 96.8
			$P < .001$*	

NOTE. Values are the mean ± SD.

*Significance of change from baseline to 6 mo within group analyzed with nonparametric test for dependent variables (Wilcoxon).

†$P < .05$, ‡$P < .001$, significance of difference between groups *v* control group analyzed with Mann-Whitney nonparametric *U* test for independent variables.

CEs increased significantly in the control and RO-enriched groups where α-linolenic acid–rich LEAR oil was used, and linoleic acid increased in both oil groups (SO-enriched and RO-enriched).[21] In the whole study population (N = 153), the proportion of both linoleic acid and α-linolenic acid in CEs correlated positively with the proportion of campesterol in serum at the end of the study (Table 5). The respective correlation coefficient between linoleic acid and the proportion of sitosterol was even higher (Table 5). These correlations were significant but weaker within each diet group (Table 5).

DISCUSSION

Methods available to study cholesterol metabolism include radioactive stable isotopes and the sterol balance technique.[3] The balance studies are laborious, even though they can be performed also for outpatient diet studies with certain methodological limitations. In the present study, the proportions of serum squalene and noncholesterol sterols were examined as a part of a large dietary intervention study with four different fat-modified diets in free-living subjects.[2]

Precursor Sterols

In both groups on diets enriched with either SO or RO, the proportion of lathosterol increased significantly. Lathosterol is related negatively to both the fractional and absolute absorption of cholesterol but positively to overall cholesterol synthesis.[8-10] Thus, the increased lathosterol proportion could reflect an enhanced endogenous cholesterol synthesis during these two diets. This plausible increased synthesis of cholesterol could be a feedback reaction to reduced cholesterol absorption,[3] resulting in a diminished hepatic cholesterol concentration. Secondly, in turn, this could have activated the LDL apo B receptor activity, ultimately causing the decrease in total and LDL cholesterol.[21] In line with this view is our previous finding that the lathosterol proportion increased along with the reduced LDL cholesterol concentration obtained in lovastatin-treated subjects by adding soluble dietary fiber, which apparently further reduced the hepatic cholesterol content.[9] The amount of dietary cholesterol was slightly lower during SO-enriched and RO-enriched diets compared with the habitual diet of the subjects. This could also contribute to an enhanced cholesterol synthesis. The reason a respective increase was not observed in the ratio of desmosterol clearly could be due to the fact that the unsaturated pathway of cholesterol synthesis via lathosterol is usually dominant (Fig 1).

In a previous study by Miettinen and Vanhanen,[22] the relatively low cholesterol absorption efficiency of men during increased intake of RO suggested that absorption of cholesterol could be reduced during consumption of a RO-enriched diet.[22] This could be due to the increased intake of plant sterols from

Table 5. Correlation Coefficients Between the Proportion of Certain Fatty Acids in CEs and Serum Plant Sterols

Plant Sterol (μg/100 mg cholesterol)	Proportion (%) of Fatty Acid in CEs	
	Linoleic Acid	α-Linolenic Acid
Campesterol		
All (N = 153)	.36*	.24*
Control (n = 36)	.34†	.34†
SO-enriched (n = 37)	.33†	.10
RO-enriched (n = 41)	.58*	.26
Reduced fat (n = 39)	.46‡	−.01
Sitosterol		
All (N = 153)	.43*	.10
Control (n = 36)	.49‡	.22
SO-enriched (n = 37)	.39†	.03
RO-enriched (n = 41)	.52*	.21
Reduced fat (n = 39)	.39†	.03

*$P < .001$.
†$P < .05$.
‡$P < .01$.

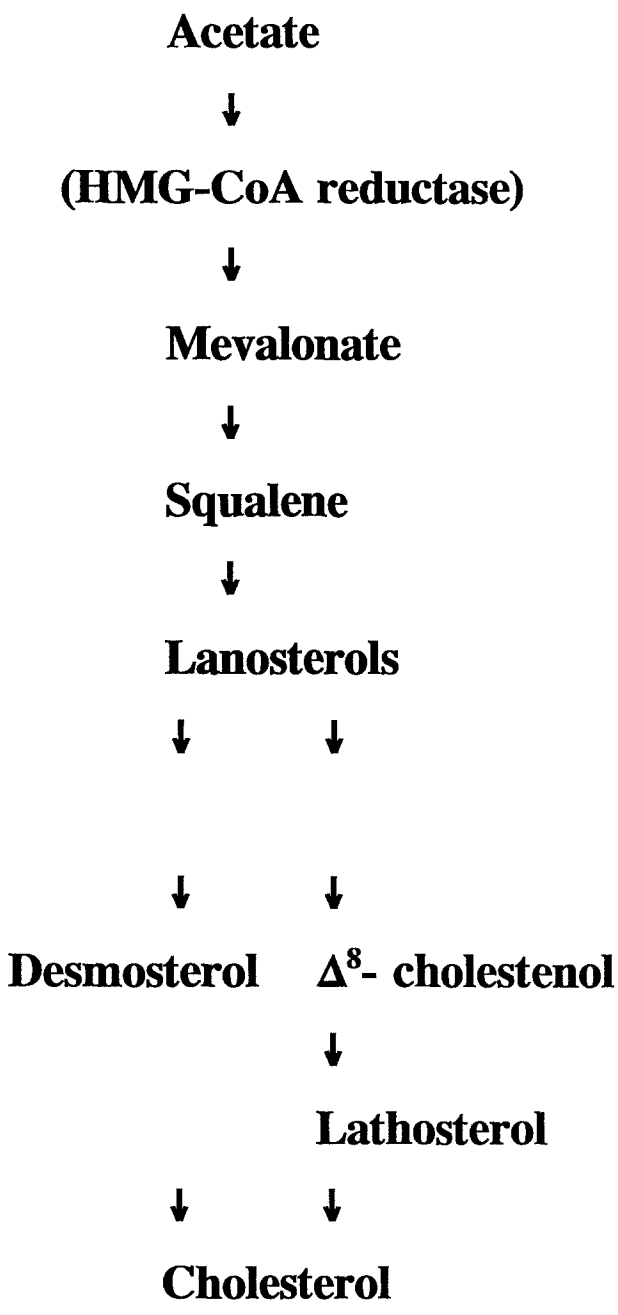

Fig 1. Schematic of cholesterol synthesis via certain main precursor sterols in human serum. HMG-CoA, hepatic hydroxymethyl glutaryl coenzyme A.

RO. Dietary sterols appear to compete for absorption during digestion.[23] This was probably the case in the present study: ingestion of test fats increased the intake of plant sterols and the proportion in serum also in both vegetable oil groups. Recently, the effect of plant sterols on cholesterol absorption has been verified by adding sitostanol-ester margarine to the diet.[24] The role of a reduced proportion of saturated fat in decreasing cholesterol absorption has been more controversial,[25] but in the present study, the proportion of saturated fatty acids was markedly reduced during both vegetable oil–enriched diets, which might have affected cholesterol absorption at least to some extent.[2]

Altogether, the significant reductions in the concentration of LDL cholesterol in both of these oil diet groups seem mainly due to the changes in dietary fatty acid composition, possibly contributed by vegetable oil–induced cholesterol malabsorption, which in turn resulted in a compensatory increase of cholesterol synthesis (Fig 1).

Plant Sterols

During the present study, the only change in the serum concentration of plant sterols was a marked increase in the campesterol concentration of the RO-enriched group and a reduction of sitosterol in the control group. Plant sterols correlate positively with cholesterol absorption, but they correlate with the dietary unsaturated to saturated fatty acid ratio, as well, probably due to a high plant sterol content in vegetable oils.[8] LEAR oil was used as the main source of unsaturated fatty acids in the RO-enriched group. The enhanced proportion of campesterol in the serum is likely a consequence of the use of test fats that, according to our analysis, increased the intake of campesterol about 72 mg. The amount of campesterol in RO is markedly higher than in other vegetable oils (30 to 40 mg/100 g).[26] Thus, the increased concentration of campesterol cannot be considered a marker of increased cholesterol absorption, but rather an indicator of the use of this particular campesterol-rich vegetable oil in our study. The significant correlation between the proportion of campesterol in the serum and dietary oleic and monounsaturated fatty acid in the RO-enriched diet group strengthens this view: the higher the biochemical indicators of vegetable oil use (linoleic and α-linolenic acid in CEs), the higher the concentration of plant sterols in serum. Furthermore, in the entire study population, the proportions of campesterol and sitosterol correlated positively with the proportions of linoleic acid and α-linoleic acid in CEs, biomarkers of the use of vegetable oils. This result supports the idea that in this case plant sterols reflect both the use of vegetable oils and absorption of plant sterols rather than cholesterol absorption. In line with this was the finding that the sitosterol ratio decreased significantly during the control diet, which was intentionally low in vegetable oil and thus low in unsaturated fatty acids. The altered plant sterol intake most likely also reduced the proportion of cholesterol in the control and RO-enriched diet groups significantly and in the SO-enriched group nonsignificantly. As mentioned earlier, dietary sterols tend to compete for absorption during digestion.[23] For instance, in previous studies, addition of dietary sitosterol decreased the proportion of cholestanol and campesterol but increased sitosterol, while dietary sitostanol reduced the proportion of campesterol, sitosterol, and cholestanol in serum.[23] In this particular study, the competition could have occurred between dietary plant sterols: campesterol or sitosterol and cholestanol and cholesterol.

In conclusion, an increase in the concentration of lathosterol was found in both RO- and SO-enriched diets, indicating that the significant reduction in the LDL cholesterol concentration in both of these oil diet groups could be due to cholesterol malabsorption with a compensatory increase in cholesterol synthesis, in addition to the changes induced by dietary fatty acid composition. No changes in cholesterol precursors were found in the diet groups (control and reduced-fat) in which serum cholesterol concentrations remained unchanged. The increases in the concentration of plant sterols (campesterol and sitosterol) indicate the use of vegetable oils and an increased plant sterol intake, rather than increased cholesterol absorption, in hypercholesterolemic subjects of this study.

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