Cholesterol Reduction by Different Plant Stanol Mixtures and With Variable Fat Intake

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Our aim was to investigate (1) whether different campestanol/sitostanol mixtures in margarine differ in reducing serum cholesterol, and (2) whether sitostanol ester in butter decreases serum cholesterol and alters cholesterol absorption and metabolism. Twenty-three postmenopausal women replaced 25 g dietary fat with (1) sitostanol ester–rich (campestanol to sitostanol ratio 1:11) and (2) campestanol ester–rich (campestanol to sitostanol ratio 1:2) rapeseed oil margarine, (3) butter, and (4) sitostanol ester–rich (campestanol to sitostanol ratio 1:13) butter. The respective scheduled stanol intake was 3.18, 3.16, and 2.43 g/d. The 6-week margarine periods and, after an 8-week washout, 5-week butter periods were double-blind and in random order. Serum cholesterol precursor sterols (indicators of cholesterol synthesis) and plant sterols (indicators of cholesterol absorption) were quantified with gas-liquid chromatography (GLC). Low-density lipoprotein (LDL) cholesterol was reduced by 8% and 10% with the sitostanol and campestanol ester–rich margarines versus baseline ($P < .05$ for both) and high-density lipoprotein (HDL) cholesterol was increased by 6% and 5% ($P < .05$), so the LDL/HDL cholesterol ratio was reduced by 15% ($P < .05$ for both). Sitostanol ester–rich butter decreased LDL cholesterol 12% and the LDL/HDL cholesterol ratio 11% ($P < .05$ for both) versus the butter period. The serum proportions of plant sterols and cholestanol were similarly reduced and those of cholesterol precursor sterols were similarly increased during all periods ($P < .05$ for all). Serum proportions of sitostanol and campestanol were slightly increased, indicating that their absorption related to their dietary intake. During all stanol interventions, serum vitamin D and retinol concentrations and α-tocopherol to cholesterol ratios were unchanged, whereas those of α- and β-carotenes were significantly reduced. We conclude that varying the campestanol to sitostanol ratio from 1:13 to 1:2 in margarine and in butter similarly decreased cholesterol absorption, LDL cholesterol, and the LDL/HDL cholesterol ratio such that the serum lipids became less atherogenic.
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SERUM CHOLESTEROL is regulated by the interplay of cholesterol absorption, cholesterol synthesis, and low-density lipoprotein (LDL) receptor activity. However, dietary factors also affect cholesterol homeostasis such that dietary cholesterol and saturated fatty acids independently elevate[1,2] and dietary plant sterols decrease[3-10] serum cholesterol in humans. Plant sterols have been studied since the 1950s as potential hypocholesterolemic agents.[3-10] These studies have been performed mainly with tall oil sterols containing a relatively large amount of sitosterol and less campesterol and saturated stanols. However, sitosterol and especially campesterol are absorbed about 5% and 16%,[11-15] but the saturated derivative of sitosterol, sitostanol, is virtually unabsorbable,[16,17] whereas campestanol may be absorbed to some extent.[14]

We have previously shown that sitostanol (with small amounts of campestanol), when made fat-soluble by transesterification with rapeseed oil fatty acids and dissolved in mayonnaise or margarine, decreases serum total and LDL cholesterol by at least 10% and 14% in mildly hypercholesterolemic populations,[18-20] women with coronary artery disease,[21] children with familial hypercholesterolemia,[22] and type 2 diabetics.[23,24] Sitostanol and its esters decrease serum cholesterol by inhibiting the absorption and increasing the synthesis of cholesterol.[15,18-25] These changes are also reflected in decreased serum plant sterols and increased precursor sterols of cholesterol.[21,22] It has been demonstrated that plant sterols reduce serum cholesterol effectively in subjects consuming a saturated fatty acid–enriched diet.[26] Dietary saturated fatty acids elevate serum cholesterol mainly by enhancing LDL production and downregulating LDL receptor activity,[27,28] with no[29,30] or some[31] effect on cholesterol absorption.

Now, several questions arise to be answered in the present stanol ester mixture feeding study: (1) Is a reduction in serum cholesterol dependent on the campestanol to sitostanol ratio in margarine?; (2) Is absorption of campestanol and sitostanol detectable by their serum values?; (3) Are the serum levels of campestanol and sitostanol and their parent plant sterols dependent on their dietary intake?; and finally, (4) How effective is sitostanol ester in butter to reduce serum cholesterol and alter the absorption and synthesis of cholesterol?

SUBJECTS AND METHODS

Patients

The study population consisted of 24 moderately hypercholesterolemic postmenopausal women aged 50 to 55 years, with a mean of 52.7 ± 1.2 (mean ± SE) years. They were recruited from a random age cohort based on the population register of the Helsinki area. The inclusion criteria for this study were as follows: serum cholesterol between 5.5 and 8.0 mmol/L, serum triglycerides less than 2.5 mmol/L, and body mass index less than 28 kg/m^2. Postmenopausal status was determined by the absence of menstruation and serum follicle-stimulating hormone greater than 30 μmg/L. Eight women had postmenopausal hormone replacement therapy, four with tablets and four with transdermal estrogen, and they had no change in the therapy during the intervention. The study subjects had no prior hypolipidemic treatment or thyroid, gastrointestinal, or hepatic disease or diabetes mellitus. All volunteered for the study and provided informed consent. The study was approved by the Ethics Committee of our hospital.

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Study Design

After two baseline blood samples 1 week apart on the ad libitum home diet, the subjects were advised to replace 25 g of their normal dietary fat with (1) sitostanol ester–rich (campestanol to sitostanol ratio 1:11) and (2) campestanol ester–rich (campestanol to sitostanol ratio 1:2) rapeseed oil margarine and (3) butter without and (4) with sitostanol (campestanol to sitostanol ratio 1:13) ester. We did not have a margarine-only period because we were not investigating the efficacy of plant stanols per se, but only in combination with margarine or butter. The margarines contained 3.18 and 3.16 g stanols/25 g margarine, and the butter 2.43 g stanols/25 g butter. The margarines contained no *trans*-fatty acids. However, the daily intake of plant sterols was different (Table 1). Sitostanol ester–rich margarine contained 82.8% sitostanol and 7.5% campestanol of the tall oil–based plant sterols, whereas the campestanol-rich margarine of the vegetable oil–based sterols contained 65.2% sitostanol and 28.1% campestanol, respectively. In addition, the sitostanol ester–rich margarine contained two times more sitosterol than the other margarine, but the campesterol intake was similar. The sitostanol butter contained 92.0% sitostanol and 7.3% campestanol of the total plant sterols. The margarine interventions lasted 6 weeks and the butter periods 5 weeks. The margarine periods were double-blind in random order with a crossover design. After a washout of 8 weeks, the same women were randomly and double-blindly assigned the butter without or with sitostanol ester.

Neither vitamin A nor vitamin D were added to the margarine. β-Carotene was used as a coloring agent. The butter products contained normal levels of carotenoids and vitamins. The margarine stanols were transesterified with rapeseed oil fatty acids, and butter stanols with butter fatty acids (Raisio Group, Raisio, Finland).

Two blood samples for lipid, vitamin, cholesterol precursor sterol (indicators of cholesterol synthesis),[32] and plant sterol and cholestanol (indicators of cholesterol absorption efficiency)[32] measurements were taken from the fasting subjects at baseline during the home diet and 1 week apart at the end of each intervention period. The mean value for the two specimens is presented. During the home diet and the margarine and butter periods, nutrients were analyzed from a 7-day food record according to a computer program.[33] Serum for the vitamin analyses was taken in dark test tubes, and all analyses were performed in subdued light. All samples were immediately frozen to −70°C.

Methods

Serum total cholesterol and triglycerides were determined enzymatically with commercial kits (Boehringer Diagnostica, Mannheim, Germany). High-density lipoprotein (HDL) cholesterol was determined enzymatically after apolipoprotein B (apo B) containing lipoproteins were precipitated. LDL cholesterol was calculated.[34] Serum noncholesterol sterol concentrations were analyzed with gas-liquid chromatography (GLC) on a 50-m SE-30 capillary column[32,35] (Ultra 1; Hewlett Packard). However, sitostanol and campestanol were quantified by GLC on an Ultra 2 capillary column. Identification of campestanol and sitostanol was based on their retention time relative to their parent compounds campesterol and sitosterol, respectively. The fact that the peaks with these retention times contained respective stanols was evidenced by mass spectrometry for larger respective peaks from a patient with phytosterolemia. Owing to low concentrations of campestanol and sitostanol in normal serum, no mass spectrometric evidence was obtained for the presence of these two stanols. Vitamin D was analyzed by quantifying 25(OH)cholecalciferol in serum.[36] Retinol, α-tocopherol, and α- and β-carotenes were analyzed with reverse-phase high-performance liquid chromatography[37] using α-tocopherol acetate as an internal standard.

Table 1. Scheduled Daily Intake (mg/d) of Plant Sterols in the Margarine and Butter Diets

Variable	Sitostanol Ester–Rich Margarine	Campestanol Ester–Rich Margarine	Sitostanol Ester Butter
Campesterol	79	84	8
Campestanol	264	952	202
Sitosterol	262	143	11
Sitostanol	2,914	2,206	2,232
Total stanols	3,178	3,158	2,434
Total sterols	3,519	3,385	2,453

Table 2. Weight, Body Mass Index, and Dietary Cholesterol and Fat Intake During the Different Diets

Variable	Home Diet (n = 23)	Margarines (n = 23)	Butter (n = 21)
Weight (kg)	66.7 ± 1.5	66.9 ± 1.6	66.7 ± 1.6
Body mass index (kg/m²)	25.7 ± 0.7	25.7 ± 0.7	25.6 ± 0.7
Dietary cholesterol (mg/d)	269 ± 19	262 ± 19	323 ± 19*
Dietary fat (g/d)	80 ± 7	93 ± 6*	97 ± 6*
Polyunsaturated/saturated fatty acid ratio	0.40 ± 0.03	0.58 ± 0.03*	0.26 ± 0.02*

NOTE. Results are the mean ± SE.
*Significantly different *v* home diet.

Statistical Analysis

Statistical significance was tested with ANOVA and covariance with repeated measures (BMDP Statistical Software, Los Angeles, CA) and a paired *t* test. Logarithmic transformations were used with skewed distributions. Serum values for noncholesterol sterols, α-tocopherol, and α- and β-carotene were standardized and also expressed in proportion to serum cholesterol, because the noncholesterol sterols, α-tocopherol, and carotenes are transported by lipoproteins, mainly LDL, in serum. A *P* value less than .05 was considered significant.

RESULTS

Twenty-four subjects participated in the two margarine interventions, and 21 subjects completed the whole study. One subject had to be excluded because of violation of the protocol, and two subjects withdrew for reasons not connected with the study.

The weight and body mass index were unchanged throughout the study (Table 2). Daily cholesterol intake was low during the margarine period but increased during butter consumption, and fat intake was increased by 13 ± 1 g/d during the margarine period and 17 ± 1 g/d during the butter period. Scheduled plant sterol intake was variable (Table 1). However, total stanol intake was similar in the sitostanol ester–rich and campestanol ester–rich margarine periods, but lower during the butter period.

Sitostanol and Campestanol Ester–Rich Margarines

Serum total and LDL cholesterol were significantly reduced by 4% ± 2% and 8% ± 3% with sitostanol ester–rich margarine and by 6% ± 2% and 10% ± 2% with campestanol ester–rich margarine versus the baseline home values (Table 3). HDL cholesterol levels were significantly increased by 6% ± 2% and 5% ± 2% and the LDL/HDL cholesterol ratio was reduced by 15% with both stanol ester margarines. The baseline data and the changes in lipids were not related to estrogen treatment.

The proportion of serum campesterol, sitosterol, and choles-

Table 3. Serum and Lipoprotein Lipids (mmol/L) During the Different Diets

Variable	Home Diet (n = 23)	Sitostanol Ester–Rich Margarine (n = 23)	Campestanol Ester–Rich Margarine (n = 23)	Butter (n = 21)	Sitostanol Ester Butter (n = 21)
Serum cholesterol	6.06 ± 0.16	5.79 ± 0.17*	5.71 ± 0.18*	6.34 ± 0.21*	5.88 ± 0.18†
LDL cholesterol	3.98 ± 0.14	3 62 ± 0.14*	3.58 ± 0.17*	4.15 ± 0.18	3.70 ± 0.16†
HDL cholesterol	1.54 ± 0.09	1.63 ± 0.10*	1.62 ± 0.09*	1.63 ± 0.10*	1.64 ± 0.10
Serum triglycerides	1.21 ± 0.14	1.18 ± 0.13	1.15 ± 0.12	1.26 ± 0.17	1.18 ± 0.13
LDL/HDL cholesterol	2.80 ± 0.20	2.44 ± 0.19*	2.42 ± 0.20*	2.77 ± 0.23	2.46 ± 0.19†

NOTE. Results are the mean ± SE and were analyzed by ANOVA and analysis of covariance for repeated measures.
*Significantly different *v* home diet.
†Significantly different *v* butter

tanol was reduced with the stanol ester margarines by 6% to 21% versus the baseline values (Table 4). The sitosterol proportion was even more effectively reduced by campestanol versus sitostanol ester–rich margarine, probably due to the higher sitosterol intake with the latter (Table 1). Compared with the home diet, the serum campestanol proportion (Table 4) was slightly increased by both stanol ester margarines, significantly more so by the campestanol ester–rich period, most likely due to a higher dietary intake of campestanol (264 *v* 952 mg/d). The serum sitostanol proportion was also slightly but significantly increased, more so by the sitostanol ester–rich margarine with a higher sitostanol intake (2,914 *v* 2,206 mg/d). The increase seems smaller for campesterol versus sitosterol in each stanol mixture. The serum cholesterol precursor sterols Δ^8-cholestenol, desmosterol, and lathosterol were compensatorily similarly increased by +12% to +19%, respectively.

Serum concentrations of vitamin D and retinol were unchanged from baseline values during both periods (Table 5). The serum concentration of α-tocopherol was significantly reduced during both margarine periods, but the α-tocopherol to cholesterol ratio was unchanged. The serum concentration and proportion of α- and β-carotenes were significantly reduced by both stanol ester margarines.

Butter Versus Sitostanol Ester Butter

Butter alone increased serum total and LDL cholesterol by 4% and HDL cholesterol by 6% (Table 3) without any constant changes in the serum noncholesterol sterol proportions (Table 4). The addition of sitostanol ester to butter significantly decreased serum total and LDL cholesterol by 8% ± 2% and 12% ± 2% versus butter alone and decreased the LDL/HDL ratio. The serum plant sterol and cholestanol proportions were decreased by 12% to 29% ($P < .05$ for both) compared with butter alone and the proportion of serum precursor sterols was compensatorily increased. The proportion of campestanol was slightly increased and sitostanol was doubled, yet the final values were only about one fourth of the respective sitosterol value.

The vitamin D concentration was increased versus the home values similarly by the two butter preparations, but the α-tocopherol proportion and retinol concentration were unchanged by the butters (Table 5). The α-carotene concentration and proportion were decreased by butter versus the home diet, but the sitostanol ester decreased the β-carotene concentration and proportion versus the butter-alone period.

DISCUSSION

This study shows for the first time that campestanol ester–rich margarine with 28% campestanol decreases serum total and LDL cholesterol as effectively as sitostanol ester–rich margarine with a low campestanol content (7.5%). We were not interested in the pure plant stanol effect per se, because we have described it previously[21] and it is not used alone; for this reason, we did not have a margarine-only study period. Sitostanol esterified with butter fat fatty acids and dissolved in butter decreased serum cholesterol as effectively as the respective stanol mixture in margarine, despite a slightly smaller stanol dose. These results suggest that the stanol and fatty acid composition of stanol ester can be varied and dissolved either in monoene-enriched margarine or in butter without diminishing the cholesterol-lowering effect. In addition, all stanol ester–containing products increased HDL cholesterol followed by a 15% decrease of the LDL/HDL cholesterol ratio such that the serum lipids became less atherogenic during both margarine-

Table 4. Serum Noncholesterol Sterol to Cholesterol Proportion (10^2 μmol/mmol cholesterol) During the Different Diets

Variable	Home Diet (n = 23)	Sitostanol Ester–Rich Margarine (n = 23)	Campestanol Ester–Rich Margarine (n = 23)	Butter (n = 21)	Sitostanol Ester Butter (n = 21)
Δ^8-Cholestenol	19.2 ± 1.5	22.1 ± 1.2*	22.0 ± 1.3*	17.6 ± 1.4	20.1 ± 1.4†
Desmosterol	73.7 ± 5.2	80.6 ± 3.9*	80.3 ± 4.0*	73.8 ± 4.6	84.8 ± 5.1*†
Lathosterol	182.8 ± 10.1	204.9 ± 10.9*	207.1 ± 11.4*	175.1 ± 14.8	202.3 ± 12.9*†
Campesterol	210.9 ± 17.0	164.9 ± 13.0*	164.7 ± 12.7*	209.4 ± 17.8	151.2 ± 15.3*†
Campestanol	1.84 ± 0.18	3.44 ± 0.30*	8.52 ± 0.66*‡	2.35 ± 0.27*	2.52 ± 0.14*
Sitosterol	125.0 ± 7.8	107.8 ± 6 1*	97.0 ± 5.9*‡	125.8 ± 9 6	90.2 ± 6.2*†
Sitostanol	11.1 ± 0.6	23.2 ± 1.1*	20.9 ± 0.9*‡	11.2 ± 0.5	23.1 ± 1.2*†
Cholestanol	127.9 ± 6.6	114.2 ± 5.6*	119.6 ± 5.5*	122.8 ± 6.9	111.9 ± 6.1*†

NOTE. Results are the mean ± SE and were analyzed by ANOVA and analysis of covariance for repeated measures.
*Significantly different *v* home diet.
†Significantly different *v* butter.
‡Significantly different *v* sitostanol ester–rich margarine.

Table 5. Serum Levels of Vitamin D, Retinol, α-Tocopherol, and α- and β-Carotene During the Different Diets

Variable	Home Diet (n = 23)	Sitostanol Ester–Rich Margarine (n = 23)	Campestanol Ester–Rich Margarine (n = 23)	Butter (n = 21)	Sitostanol Ester Butter (n = 21)
Vitamin D (nmol/L)‡	52.7 ± 5.1	58.0 ± 6.2	56.2 ± 5.9	65.4 ± 5.6*	65.5 ± 6.0*
Retinol (μmol/L)	2.25 ± 0.08	2.27 ± 0.07	2.33 ± 0.09	2.23 ± 0.08	2.33 ± 0.08
α-Tocopherol (μmol/L)	41.7 ± 1.9	38.1 ± 1.7*	38.3 ± 1.6*	42.7 ± 2.0	39.8 ± 1.8*†
α-Tocopherol/cholesterol	6.94 ± 0.33	6.62 ± 0.25	6.79 ± 0.30	6.78 ± 0.28	6.84 ± 0.32
α-Carotene (μmol/L)	0.46 ± 0.06	0.32 ± 0.04*	0.31 ± 0.04*	0.35 ± 0.04*	0.32 ± 0.05*
α-Carotene/cholesterol	0.07 ± 0.01	0.06 ± 0.01*	0.05 ± 0.01*	0.06 ± 0.01*	0.05 ± 0.01*
β-Carotene (μmol/L)	1.63 ± 0.17	1.10 ± 0.13*	1.10 ± 0.15*	1.57 ± 0.19	1.19 ± 0.13*†
β-Carotene/cholesterol	0.27 ± 0.03	0.19 ± 0.02*	0.19 ± 0.02*	0.25 ± 0.03	0.21 ± 0.02*†

NOTE. Results are the mean ± SE and were analyzed by ANOVA and analysis of covariance for repeated measures.
*Significantly different *v* home diet.
†Significantly different *v* butter.
‡Normal range, 22-72 nmol/L.

and butter-based stanol ester diets. The 8% and 10% LDL cholesterol decreases represent the combined effect of margarine and stanol, a smaller effect versus some earlier studies,[18-24] especially in coronary women,[21] but is now associated with increased HDL cholesterol, found only infrequently in our prior experiments.[22,23] Serum sitostanol and campestanol, being less than one tenth of the sitosterol and campesterol concentrations at baseline, were significantly increased during the margarine- and butter-based stanol ester diets even though the final serum concentrations remained one fifth to one seventh of the respective parent-compound values. The stanol esters, despite inhibiting cholesterol absorption, had no effect on the serum concentrations of vitamin D and retinol and the α-tocopherol to cholesterol proportion, whereas serum carotene levels and proportions were reduced.

The stanol esters were obtained from different sources. The tall oil–based stanol product contained 83% sitostanol and 7.5% campestanol of the total sterol mixture, with the respective values being 65% and 28% for the campestanol ester–rich sterol mixture obtained from vegetable oil sterols. The two stanol mixtures, the dietary intake of which was similar in the two groups, seemed equally effective in inhibiting cholesterol absorption efficiency and compensatorily upregulating cholesterol synthesis and reducing serum cholesterol. Thus, the reduction in serum cholestanol and plant sterols and the increase in cholesterol precursors were similar during the two sterol group feedings. The dietary intake of plant sterols seems to be associated with their serum level.[38] Thus, the changes in serum stanols and sterols depended on their scheduled dietary intake. Accordingly, campestanol seemed to be absorbed especially from the campestanol-rich sterol mixture, and sitostanol especially from the sitostanol-rich margarine. The present study actually evidences for the first time that these stanols are also absorbed slightly in normal subjects. It is known from previous studies that sitostanol is only slightly absorbed from the intestine, such that 2% of orally fed sitostanol is recovered from rat lymph in 24 hours[39] and it has been used in human studies as a nonabsorbable marker.[40] The absorption of campestanol is less well known, but it can be presumed to be less well absorbed than campesterol because, in general, stanols seem to be less absorbable than the respective parent sterols. In an intestinal perfusion study, 12.5% of infused campestanol was absorbed in humans,[14] but the overall quantities were small. In the present study, the relative increase of the campestanol proportion was about 36%, whereas for sitostanol it was about 110%. The smaller increase of campestanol versus sitostanol, even on the campestanol-rich diet, may be due to an effective inhibitory action of large amounts of sitostanol on smaller amounts of campestanol in the intestinal sterol mixture. Previous studies have shown that an alteration in dietary plant sterol composition sensitively changes their serum levels.[38] The serum campestanol concentration increased by 11 μg/100 mL during the campestanol ester–rich margarine treatment but the amount remained small, suggesting that these minor serum concentrations are probably meaningless, especially since other serum plant sterol levels were markedly decreased. However, there is one group of patients in whom even small amounts of stanols may accumulate in the body: homozygous patients with phytosterolemia. This extremely rare disease is characterized by increased absorption[41,42] and decreased biliary secretion[41] of plant sterols and accelerated atherosclerosis.[42]

The serum concentrations of vitamin D and retinol and the α-tocopherol proportion were unaffected by the stanol ester margarine consumption. β-Carotene, having a nonpolar chemical structure with two β-ionone rings connected by an isoprene chain, was the only vitamin derivative for which the serum level was significantly reduced by sitostanol yet its metabolite retinol exhibited an unchanged serum concentration. This is in agreement with previous findings that β-carotene feeding does not increase serum retinol levels.[43-44] Retinol is chemically more polar than β-carotene, so the latter is oxidized to two molecules of retinol. α-Carotene produces only one molecule of retinol because it has only one β-ionone and one non–β-ionone ring. Serum levels of β-carotene reflect dietary supplementation,[43-45] but the present findings suggest that its absorption was related to the cholesterol absorption efficiency. β-Carotene is absorbed from 9% to 22%,[46-48] with the absorbed fraction mostly converted to retinol in the enterocyte.[46,47] In a recent study, 57% of absorbed β-carotene was converted to retinoids in enterocytes and 43% in the liver.[48] All serum β-carotene is transported in lipoproteins, mainly LDL.[49] The clinical importance of the carotene decrease remains obscure, since β-carotene treatment has resulted in harmful clinical effects.[50,51]

Diets high in saturated fatty acids increase serum cholesterol

by increasing cholesterol absorption, diminishing cholesterol synthesis and LDL apo B receptor activity, and increasing LDL apo B production.[35] However, the present study revealed that stanol esters are able to decrease serum total and LDL cholesterol and the LDL/HDL cholesterol ratio even in a saturated environment, ie, when stanol is esterified with butter fatty acids and then dissolved in butter. By inhibiting cholesterol absorption, a probable mechanism, despite upregulation of cholesterol synthesis, is an activation of LDL apo B transport due to reduced intestinal flow of cholesterol to the liver without altered LDL apo B removal.[23,24] A lack of hepatic cholesterol decreases apo B synthesis, resulting in reduced transport of VLDL apo B to LDL.[52] Accordingly, the stanol esters are effective cholesterol-lowering agents despite the type of dietary fat intake.

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