Effect of 3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase Inhibitor on Sterol Absorption in Hypercholesterolemic Subjects

Fady Y. Ntanios, Peter J.H. Jones, and Jiri J. Frohlich

To investigate the potential effects of high-dose 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitor on plasma phytosterol, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG), hypercholesterolemic subjects received 40 or 80 mg/d simvastatin in a 24-week prospective clinical trial. Plasma lipid levels were analyzed enzymatically, and plasma phytosterol concentrations were determined using gas-liquid chromatography. The change in the plasma phytosterol-campesterol level was used as an indicator of cholesterol absorption in humans. Simvastatin treatment reduced plasma campesterol (−24%, $P = .017$) but did not affect circulating stigmasterol and sitosterol levels. A dose of 80 mg/d simvastatin produced a larger decrease ($P = .050$) in plasma campesterol (0.1680 mmol/L) than 40 mg/d (0.0237 mmol/L) versus baseline. There was a positive correlation between plasma campesterol and TC both before ($r = .54$, $P = .027$) and after ($r = .63$, $P = .009$) treatment. Plasma TC and TG levels did not differ between groups receiving 40 or 80 mg/d simvastatin. Simvastatin treatment reduced circulating TC, LDL-C, and TG by 40%, 50%, and 33% ($P < .007$), respectively. There was no significant effect of simvastatin on plasma HDL-C, but the HDL-C/LDL-C ratio increased 1.3-fold ($P < .0001$). In conclusion, this HMG-CoA reductase inhibitor reduces the plasma campesterol level, a marker of cholesterol absorption, which may contribute to the mechanism by which simvastatin decreases circulating cholesterol levels.

SIMVASTATIN is among the most effective agents for the treatment of hypercholesterolemia in humans. The Scandinavian Simvastatin Survival Study[1] demonstrated a significant reduction in overall morbidity and mortality with this lipid-lowering agent. Simvastatin is a potent inhibitor of 3-hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase. It decreases cholesterol biosynthesis by a competitive and reversible inhibition of HMG-CoA reductase activity, which leads to an increased expression of low-density lipoprotein (LDL) receptors.[2] These receptors bind LDL-cholesterol (LDL-C) particles and remove them from the circulation, thus decreasing the circulating total cholesterol (TC) level.

Other mechanisms by which HMG-CoA inhibitors may decrease plasma TC have been proposed. Since the observed benefits were manifest before plaque stabilization or regression may have occurred, it has been suggested that statins in general[3-5] and simvastatin in particular[6-10] exert effects other than inhibition of HMG-CoA reductase activity. Simvastatin has been shown to decrease the synthesis of apolipoprotein B (apo B) lipoproteins,[11-13] to reverse endothelial dysfunction in hypercholesterolemia[14] to improve arterial topographic morphology after 4 years in the Multicenter Anti-Atheroma Study,[15] and to reduce ex vivo platelet aggregation in patients with type IIa hypercholesterolemia.[16] Moreover, HMG-CoA reductase inhibitors have been suggested to decrease cholesterol absorption in animals.[17,18] For instance, Ishida et al[19,20] reported that the hypolipidemic effect of simvastatin in cholesterol-fed rabbits is related to a marked reduction of exogenous cholesterol absorption from the intestinal wall due to inhibition of acylcoenzyme A:cholesterol acyltransferase (ACAT) activity. However, this effect has not been clearly demonstrated in humans.[21-24] Whether simvastatin affects cholesterol absorption in hypercholesterolemics is the subject of the current study.

One approach for determining cholesterol absorption is to measure the content of phytosterol-campesterol in plasma.[25-27] Campesterol, derived from the diet and found in small quantities in human plasma, is mainly transported in the high-density lipoprotein cholesterol (HDL-C) fraction.[28] Like other phytosterols, campesterol is not catabolized in vivo, as shown in rat liver perfusate.[29] In addition, phytosterols are found in small concentrations in human bile,[30,31] suggesting that they are absorbed from the intestine, transported in plasma, and secreted in part as neutral sterols in bile. Plasma campesterol levels have been shown to positively correlate with dietary cholesterol absorption efficiency in normal,[25,26,32] hypercholesterolemic,[27] and celiac disease subjects.[33] Variation in campesterol levels in plasma is used as an index of cholesterol absorption.

The aim of the current study was to further explore the effect of 40 and 80 mg/d simvastatin on plasma campesterol and lipoprotein levels in hypercholesterolemic subjects fed a National Cholesterol Education Program (NCEP) step 1 diet over a 24-week treatment period.

SUBJECTS AND METHODS

Subjects

Eighteen subjects (eight women and 10 men) were recruited from patients participating in a simvastatin parallel clinical trial at the Atherosclerosis Speciality Laboratory of the Lipid Clinic at St. Paul's Hospital (Vancouver, British Columbia, Canada). The subjects (mean age, 49 ± 11 years) underwent measurement of plasma TC, LDL-C, HDL-C, and triglyceride (TG) levels 4 weeks before the trial (during the washout period), at the baseline, every 6 weeks, and at the end of the 24-week trial. Subjects were admitted to the study based on the following selection criteria: plasma LDL-C greater than 4.16 mmol/L, TG 3.95 mmol/L or less, and age between 21 and 70 years. Subjects with a body mass index ([BMI] weight in kilograms divided by the square of the height in meters) of 31 or less were accepted into the study.

From the School of Dietetics and Human Nutrition, McGill University, Ste-Anne-de-Bellevue, Quebec; and Department of Pathology, University of British Columbia, Vancouver, British Columbia, Canada.

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Address reprint requests to Peter J.H. Jones, PhD, School of Dietetics and Human Nutrition, McGill University, Macdonald Campus, 21,111 Lakeshore Rd, Ste-Anne-de-Bellevue, Quebec H9X 3V9, Canada.

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Any previous lipid-lowering medications were suspended during the washout period. The subjects were evaluated for hypertension, diabetes, and coronary heart problems. In addition, the plasma chemical profile, hematology, and urinalysis were performed. Patients with renal failure, hypertension, myocardial infarction, diabetes type I or II, hypersensitivity to HMG-CoA, type I, III, IV, or V hyperlipidemia, homozygous familial hypercholesterolemia (FH), or hypothyroidism were excluded from the study. The experimental protocol was approved by the Ethics Committee of the Lipid Clinic at St. Paul's Hospital and the Department of Pathology at the University of British Columbia.

Study Design

The study was performed for a 24-week period during which seven (two women and five men) and 11 (six women and five men) subjects received either 40 or 80 mg/d simvastatin (Zocor; Merck, Sharp & Dohme, Rahway, NJ). Subjects in whom the plasma TC level did not decrease with 40 mg/d simvastatin to a desirable concentration set by the Ethics Committee were changed to a dose of 80 mg/d simvastatin. Blood samples were taken 4 weeks before the start of the study after the subjects had fasted for 12 hours. During a 1-month washout period, the subjects ate a low-cholesterol diet (step 1 of the American Heart Association diet: 30% total fat, 7% to 10% saturated fat, 200 to 300 mg/d cholesterol, 15% to 16% protein, and 54% to 55% total carbohydrate; National Institutes of Health NCEP step 1 diet),[34] and all prior lipid-lowering drug treatment was discontinued. Four weeks later, subjects who fulfilled the selection criteria were admitted to the study, and the plasma lipid concentrations, height, and weight were measured at baseline. After 24 weeks of daily oral administration of 40 or 80 mg simvastatin, blood samples were collected and body weight was remeasured. Dietary instructions were given to the subjects, with follow-up evaluation by dietitians to monitor adherence to the diet. Food frequency records were obtained from the subjects during each visit for blood sampling. Subjects were encouraged to maintain food intake within the step 1 guidelines.

Plasma TC, HDL-C, and TG concentrations were measured using commercial enzymatic kits (Abbott, Montreal, Quebec, Canada). HDL-C concentrations were measured in plasma after precipitation of apo B lipoproteins with dextran sulfate/magnesium chloride.[35] The LDL-C concentration was calculated according to the method of Friedewald et al.[36] Plasma phytosterol concentrations were determined by gas-liquid chromatography from the nonsaponifiable material of plasma lipid.[37] Briefly, 0.5-mL plasma samples were saponified with 0.5 mol/L KOH for 2 hours, and nonsaponifiable material was extracted with petroleum ether. 5α-Cholestane was used as an internal standard. Samples were injected into a chromatograph equipped with a flame ionization detector (HP 5890 Series II; Hewlett-Packard, Palo Alto, CA) using a 30-m capillary column (SAC-5; Supelco, Bellefonte, PA). Detector and injector temperatures were set at 320° and 300°C, respectively. Samples were analyzed isothermically at 285°C. Phytosterol peaks were identified by comparison to standards (Supelco).

Statistical Analysis

The results are presented as the mean ± SD. The effects of simvastatin on the measured variables of plasma lipid and phytosterol, were tested using a paired Student's *t* test. Student's *t* test was applied to measure any difference in the variables between men and women. The relationship between the variables of phytosterol and plasma TC was determined using Pearson product-moment correlation coefficients.

RESULTS

The anthropometric measure for the men and women are shown in Table 1. The body weight and BMI did not change over the 24 weeks of the trial. The reported dietary cholesterol and fractions of energy derived from saturated, monounsaturated, and polyunsaturated fat did not differ between men and women during the study period (data not shown). Since the subjects consumed the same diet before and during treatment, the macronutrient composition did not vary. No adverse clinical side effects from simvastatin were reported among subjects. The only differences observed between men and women were for plasma HDL-C levels. Women had consistently higher ($P = .016$ and $P = .049$, pretreatment and during treatment, respectively) circulating HDL-C than men (Table 2). Male and female subjects were pooled into one group because there were no gender differences in plasma TC, LDL-C, TG, and phytosterol profiles at baseline.

Table 1. Anthropometric Measures for the Men and Women at Baseline (mean ± SD)

Parameter	Men (n = 10)	Women (n = 8)
Height (cm)	174.5 ± 7.7	161.6 ± 5.6
Weight (kg)	82.0 ± 11.7	66.7 ± 9.5
Age (yr)	42.7 ± 11.2	55.0 ± 8.8
BMI (kg/m²)	26.9 ± 2.6	25.5 ± 2.9

The plasma lipid profile during 24 weeks of treatment is shown in Fig 1. At week 6 of simvastatin treatment, plasma TC, LDL-C, and TG were significantly decreased ($P < .02$), but from weeks 6 to 24, there were no further changes in plasma lipid levels. At baseline, subjects who were administered 40 or 80 mg/d simvastatin exhibited similar lipid levels (Table 3). Similarly, the percent change in plasma lipid levels pretreatment and posttreatment did not differ between groups given 40 or 80 mg/d simvastatin (Table 4). Although the percent decrease in TC, LDL-C, and TG was larger in subjects administered 80 mg

Table 2. Comparison of Plasma Lipid and Phytosterol Levels (mean ± SD, mmol/L) in Hypercholesterolemic Women Versus Men at Baseline and 24 Weeks Posttreatment

Parameter	Women (n = 8)	Men (n = 10)	*P*	95% CI
TC				
Pre	10.34 ± 2.05	9.82 ± 1.87	.60	−1.52-2.56
Post	5.74 ± 1.09	6.36 ± 1.32	.33	−1.91-0.68
LDL				
Pre	8.61 ± 1.98	8.33 ± 1.88	.77	−1.74-2.29
Post	3.70 ± 0.84	4.62 ± 1.31	.13	−2.12-0.29
HDL				
Pre	1.39 ± 0.37	1.03 ± 0.17	.02	0.0767-0.6360
Post	1.44 ± 0.41	1.14 ± 0.15	.05	0.0045-0.6020
TG				
Pre	1.73 ± 0.77	2.31 ± 1.13	.26	−1.63-0.48
Post	1.28 ± 0.52	1.50 ± 0.67	.47	−0.87-0.42
Campesterol				
Pre	0.0205 ± 0.0096	0.0304 ± 0.0141	.11	−0.0223-0.0025
Post	0.0181 ± 0.0076	0.0214 ± 0.0073	.39	−0.0111-0.0046
Stigmasterol				
Pre	0.0121 ± 0.0066	0.0106 ± 0.0058	.61	−0.0047-0.0078
Post	0.0097 ± 0.0032	0.0108 ± 0.0042	.59	−0.0050-0.0030
Sitosterol				
Pre	0.0322 ± 0.0160	0.0466 ± 0.0206	.12	−0.0332-0.0044
Post	0.0305 ± 0.0109	0.0369 ± 0.0111	.26	−0.0140-0.0053

NOTE. Subjects were treated with 40 or 80 mg/d simvastatin. The 95% CI is for the difference of means.

Abbreviations: Pre, baseline; Post, posttreatment.

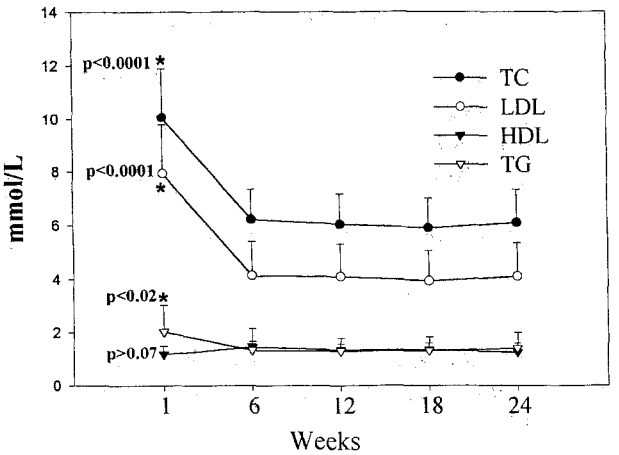

Fig 1. Plasma lipid profile in hypercholesterolemic subjects (N = 18) treated with 40 or 80 mg simvastatin for 24 weeks. The time effect within the same group is in contrast to week 1.

simvastatin, with 18 subjects (40 mg, n = 7; 80 mg, n = 11) participating in this study, the difference in the lipid profile between the doses did not reach statistical significance. On the other hand, 80 mg/d simvastatin produced a larger decrease ($P = .050$) in the mean plasma campesterol level (0.1680 mmol/L) than 40 mg/d (0.0237 mmol/L) versus baseline. Conversely, there were no differences in circulating stigmasterol and sitosterol concentrations between the 40- and 80-mg/d simvastatin doses (Table 4).

Table 5 shows the combined results of both subgroups of hypercholesterolemics. Simvastatin at both 40 and 80 mg/d decreased the mean plasma TC level by an average of 40%. The mean plasma TC level was decreased ($P < .0001$) from 10.04 ± 1.90 mmol/L to 6.11 ± 1.24 mmol/L (95% confidence interval [CI], 3.18 to 4.68) by simvastatin. The mean circulating LDL-C level was decreased ($P < .0001$) by 47% (7.9 ± 2.0 mmol/L to 4.2 ± 1.2 mmol/L; 95% CI, 2.94 to 4.46). Similarly, simvastatin decreased ($P = .007$) the mean plasma TG concentration by 32% (2.07 ± 1.02 mmol/L to 1.41 ± 0.60 mmol/L; 95% CI, 0.21 to 1.11). There was no effect of simvastatin on plasma HDL-C concentrations. On the other hand, the HDL-C/LDL-C ratio increased ($P < .0001$) 1.3-fold from 0.22 ± 0.04 to 0.30 ± 0.13 with simvastatin treatment.

The combined data from subjects with 40 and 80 mg/d simvastatin showed a lower (−24%, $P = .0172$) circulating phytosterol-campesterol concentration versus baseline

Table 3. Comparison of Plasma Lipid and Phytosterol Levels (mean ± SD, mmol/L) in Hypercholesterolemic Subjects with 40 or 80 mg/d Simvastatin at Baseline

Parameter	40 mg/d (n = 7)	80 mg/d (n = 11)	P	95% CI
TC	9.91 ± 1.31	10.17 ± 2.17	.78	−1.69-2.20
LDL	7.69 ± 1.51	8.12 ± 2.17	.65	−1.53-2.43
HDL	1.25 ± 0.36	1.13 ± 0.28	.44	−0.44-0.20
TG	2.12 ± 1.45	1.99 ± 0.64	.80	−1.18-0.92
Campesterol	0.026 ± 0.010	0.026 ± 0.017	.93	−0.0128-0.0140
Stigmasterol	0.010 ± 0.007	0.012 ± 0.008	.57	−0.0045-0.0079
Sitosterol	0.037 ± 0.011	0.043 ± 0.024	.48	−0.0133-0.0267

NOTE. The 95% CI is for the difference of means.

Table 4. Mean Percent Change (decrease) in Plasma Lipid and Phytosterol Profiles in Subjects With 40 or 80 mg/d Simvastatin During 24-Week Treatment (mean ± SD)

Parameter	40 mg/d (n = 7)	80 mg/d (n = 11)	P	95% CI
TC	63.2 ± 23.0	71.2 ± 32.0	.56	−20.5-36.4
LDL	92.8 ± 42.6	98.4 ± 43.7	.80	−41.4-52.6
HDL	−7.8 ± 15.9	−1.5 ± 20.5	.49	−12.5-25.0
TG	40.9 ± 73.1	58.0 ± 47.8	.56	−43.5-77.6
Campesterol	33.8 ± 72.5	64.1 ± 83.3	.44	−51.0-111.6
Stigmasterol	−34.0 ± 22.0	−29.4 ± 35.2	.75	−26.2-35.5
Sitosterol	−28.8 ± 43.3	−13.7 ± 51.3	.52	−34.3-64.5

NOTE. The 95% CI is for the difference of means.

(0.0256 ± 0.0083 mmol/L) after treatment (0.0195 ± 0.0037 mmol/L). The levels of stigmasterol and sitosterol were not significantly affected by simvastatin treatment. Moreover, the ratio of campesterol to cholesterol (2.65 ± 1.06 and 3.21 ± 0.98 mmol/mol) and of campesterol to sitosterol (0.66 ± 0.23 and 0.57 ± 0.15, $P = .08$) did not change significantly (Table 6). However, plasma TC concentrations were significantly correlated with campesterol levels ($r = .54$, $P = .027$ and $r = .63$, $P = .009$) in both pretreatment and posttreatment. Moreover, the percent change in circulating TC was positively correlated with the campesterol to sitosterol ratio in pretreatment ($r = .68$, $P = .003$) and posttreatment ($r = .66$, $P = .005$). The correlation of the percent change in plasma TC and LDL-C with the percent change in circulating campesterol, stigmasterol, and sitosterol was not significant. In addition, the ratio of campesterol to LDL-C did not vary between the start and end of the trial.

DISCUSSION

The main finding in this study is that plasma campesterol levels were reduced after 24 weeks on high-dose simvastatin treatment. On the other hand, circulating β-sitosterol and stigmasterol concentrations were unchanged by treatment. Plasma campesterol levels were comparable to those reported in healthy individuals,[38,39] hypercholesterolemics,[22,27,40] and subjects with non–insulin-dependent diabetes mellitus.[41]

Circulating phytosterol levels have been shown to be related to fractional cholesterol absorption in hypercholesterolemic subjects.[25-27,33,38] Tilvis and Miettinen[25] reported that changes in plasma campesterol concentrations, unlike β-sitosterol, indicate

Table 5. Plasma Lipid Levels (mean ± SD, mmol/L) in the Hypercholesterolemic Subjects (N = 18)

Parameter	Pretreatment	Posttreatment	P	95% CI
TC	10.04 ± 1.90	6.11 ± 1.24	<.0001	3.18-4.68
LDL	7.91 ± 1.96	4.22 ± 1.21	<.0001	2.94-4.46
HDL	1.77 ± 0.32	1.25 ± 0.35	.12	−0.16-0.02
TG	2.07 ± 1.02	1.41 ± 0.60	.007	0.21-1.11
HDL-C/LDL-C ratio	0.22 ± 0.04	0.30 ± 0.13	<.0001	−0.22-−0.12
Pearson product-moment correlation between plasma TC and campesterol	$r = .54$ $P = .003$	$r = .63$ $P = .009$		

NOTE. Subjects were administered a dosage of 40 or 80 mg/d simvastatin. The 95% CI is for the difference of means.

Table 6. Plasma Phytosterol Levels (mean ± SD, mmol/L) in the Hypercholesterolemic Subjects (N = 18)

Parameter	Pretreatment	Posttreatment	*P*	95% CI
Campesterol	0.026 ± 0.010	0.020 ± 0.009	.02	0.0013-0.0110
Stigmasterol	0.012 ± 0.010	0.010 ± 0.004	.39	−0.0017-0.0034
Sitosterol	0.041 ± 0.020	0.034 ± 0.011	.26	−0.0051-0.0175
Campesterol/sitosterol ratio	0.660 ± 0.229	0.569 ± 0.154	.08	−0.0160-0.2450

NOTE. Subjects were administered a dosage of 40 or 80 mg/d simvastatin. The 95% CI is for the difference of means.

an alteration in the cholesterol absorption rate in humans. Moreover, plasma phytosterol levels are unlikely to be modified by the NCEP step 1 diet,[34,42] which contains less cholesterol and fat but provides more vegetable and fruit servings than typical Western diets. In this study, subjects were placed on a 4-week washout period with the NCEP step 1 diet, which they also consumed during the treatment period. In addition, Salen et al[43] showed that a greater than eightfold increase in the dietary phytosterol load is needed to double plasma β-sitosterol levels in healthy subjects.

Recently, Robins and Fasulo[28] have clearly demonstrated that HDL-C, but not other lipoproteins, provides the vehicle for phytosterol transport to bile. Since there was no change in circulating HDL-C concentrations pretreatment and posttreatment, the lower plasma campesterol levels found after simvastatin treatment suggest reduced cholesterol absorption in these subjects. This finding is in agreement with studies in heterozygous FH subjects fed egg yolk and supplemented with lovastatin.[23] Moreover, the ratio of plasma campesterol to cholesterol significantly increased in both non-FH and FH patients on 40 mg/d simvastatin[22] or FH subjects on 80 mg/d pravastatin,[40] suggesting lower cholesterol absorption.

The mechanisms involved in reducing intestinal cholesterol absorption by HMG-CoA reductase inhibitors are not fully understood. Vanhanen et al[24] suggested that the entry of intestinal cholesterol into the liver as chylomicrons is reduced by pravastatin in FH subjects. In addition, they showed in the same subjects a reduction of the esterified methylsterol content of the chylomicrons, which may reflect a decrease in intestinal mucosal ACAT activity.[24] Unlike cholesterol, phytosterols are poorly esterified in the enterocytes and are consequently poorly absorbed.[44,45] The esterification rate of phytosterol is about 5% of the rate for cholesterol in vitro.[44] Hence, any reduction in ACAT activity due to simvastatin would reduce the esterification of phytosterol-campesterol and result in less campesterol and cholesterol absorption. For instance, in more invasive animal experiments, Ishida et al[19] demonstrated that a HMG-CoA reductase inhibitor reduced cholesterol absorption in cholesterol-fed rabbits, while Hajri et al showed similar effects in hamsters[17] and hypercholesterolemic rats.[18] Ishida et al[20] reported that this effect was due to a reduction in microsomal ACAT activity in the intestinal mucosa. Results from this study show a reduced campesterol concentration in the plasma and suggest that intestinal cholesterol absorption was reduced, but do not demonstrate a direct simvastatin effect on ACAT activity.

A simvastatin dose of 80 mg/d was more effective than 40 mg/d to decrease circulating campesterol levels. This superior effect of 80 mg/d suggests that simvastatin has a dose-dependent effect in decreasing plasma campesterol concentrations. On the other hand, Chisholm et al[21] failed to show any effect of approximately 30 mg/d simvastatin on plasma campesterol levels in FH subjects on low-fat and subsequent high-fat diets. The discrepancy between their results and this study may be due to the lower simvastatin dosage. The difference between the high-fat/cholesterol diets used by Chisholm et al[21] and the low-fat/cholesterol diets used in this study may also have confounded the results, due to the fact that dietary phytosterol absorption is affected by dietary fat intake.[46,47]

Another possible explanation for the simvastatin effect on plasma campesterol and cholesterol levels could be the marked decrease in cholesterol synthesis. Chang et al[48,49] showed that providing 20 mmol/L DL-mevalonate to Chinese hamster ovary cells grown in sterol-free medium increases the activity of ACAT enzyme sixfold. Such activation of ACAT by adding mevalonate was inhibited by adding squalene oxide cyclase inhibitor to the cells. These results suggest that active endogenous sterol synthesis is required to manifest the effect of mevalonate.[48,49] Simvastatin significantly inhibits endogenous cholesterol synthesis. Consequently, and as suggested by Ishida et al,[20] such reduction in endogenous cholesterol may inhibit ACAT activity and thus reduce campesterol and cholesterol absorption.

Finally, a number of investigators have demonstrated that simvastatin affects cellular membrane functions by decreasing the cholesterol content.[5,10] Such changes in the content of intestinal mucosal cells might alter the membrane fluidity and subsequently the function of membrane-associated proteins such as ACAT. Hence, cholesterol absorption in the intestinal tract might be impaired due to the effect of simvastatin on endothelial membranes.

In conclusion, our findings show that 24-week treatment with simvastatin decreases plasma TC, LDL-C, and TG levels, increases the HDL-C/LDL-C ratio, and reduces the circulating campesterol concentration. This suggests that simvastatin may have an inhibitory role in cholesterol absorption in hypercholesterolemic subjects.

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