

Plant Sterols in Serum and in Atherosclerotic Plaques of Patients Undergoing Carotid Endarterectomy

Tatu A. Miettinen, MD, PhD,* Mikael Railo, MD, PhD,† Mauri Lepäntalo, MD, PhD,†
Helena Gylling, MD, PhD‡§

Helsinki and Kuopio, Finland

OBJECTIVES	The purpose of this research was to determine whether serum plant sterol levels are associated with those in atheromatous plaque.
BACKGROUND	Cholesterol of low-density lipoprotein (LDL) particles contributes to atheromatous plaque formation; LDL also contains most serum non-cholesterol sterols, including plant sterols. The role of plant sterols in atheromatous plaque formation is open.
METHODS	Free, ester, and total cholesterol and the respective non-cholesterol sterols were measured by gas-liquid chromatography in serum and arterial tissue of 25 consecutive patients undergoing carotid endarterectomy. The population was ranked to triads according to tissue cholesterol concentration.
RESULTS	Cholesterol concentration increased markedly in tissues but not in serum with triads. The ester percentage was lower in the third than in the first triad (47% vs. 56%; $p < 0.01$) and lower than in serum triads (70%; $p < 0.001$). Ratios to cholesterol of non-cholesterol sterols decreased in increasing tissue triads, but were unchanged in serum. A major new observation was that the higher the ratio to cholesterol of the surrogate absorption sterols (cholestanol, campesterol, sitosterol, and avenasterol) in serum, the higher was their ratio also in the carotid artery wall (e.g., $r = 0.683$ for campesterol). Despite undetectable differences in serum and tissue cholesterol concentrations off and on statins, an additional important novel finding was that statin treatment was associated with increased ratios of the absorption sterols in serum and also in the arterial plaque.
CONCLUSIONS	The higher the absorption of cholesterol, the higher are the plant sterol contents in serum resulting also in their higher contents in atherosclerotic plaque. However, the role of dietary plant sterols in the development of atherosclerotic plaque is not known. (J Am Coll Cardiol 2005;45:1794–801) © 2005 by the American College of Cardiology Foundation

Atherothrombotic disease is the major source of mortality and morbidity in the Western world. Although it may affect any part of the arterial tree, its main clinical manifestations are coronary heart disease (CHD), cerebrovascular disease, and occlusive arterial disease of lower extremities. The threatened sequelae of cardiovascular diseases (CVDs) are myocardial infarction, stroke, threatening limb ischemia, and death. Increased serum total and low-density lipoprotein (LDL) cholesterol concentrations are among the major risk factors of arterial CVD. It is believed that cholesterol from blood is incorporated through arterial endothelium to subendothelial extracellular connective tissue mainly as LDL in proportion to its serum level (1). Thus, as the occurrence of clinical manifestations of CVD is higher, the higher is the serum level of LDL cholesterol. In fact, recent clinical statin trials have convincingly shown that effective LDL cholesterol lowering reduces the incidence of clinical manifestations of CVD (2). Even though LDL cholesterol

has been suggested to be below 2.5 mmol/l in coronary and diabetic patients, more current targets suggested that it should be even <1.6 mmol/l for a high-risk population (3).

Low-density lipoprotein particles contain not only cholesterol, however, but also small amounts of squalene and other sterols, called non-cholesterol sterols, including cholesterol precursors cholestanol, desmosterol, and lathosterol, and sterols reflecting cholesterol absorption (i.e., cholestanol and plant sterols campesterol, sitosterol, and avenasterol). In serum, cholesterol precursors, especially their ratios to cholesterol, reflect cholesterol synthesis, whereas the respective ratios of cholestanol and plant sterols are markers of cholesterol absorption (4). Because patients with high LDL cholesterol usually have high cholesterol absorption efficiency and high ratios of plant sterols to cholesterol in serum (5), mainly in LDL, we considered that plant sterols should also be rich in arterial atheromas. Postmortem plant sterol concentrations have previously been studied in arterial walls of a few infants, children, and adults (6), but comparison of serum non-cholesterol sterols and their ratios to cholesterol with those in atheromas have not been previously studied *in vivo*. In addition, long-term statin treatment, especially with large doses of effective drugs, is known to increase serum plant sterols reflecting cholesterol absorption (4,7–9), suggesting that increased plant sterol ratios to cholesterol in

From the *Departments of Medicine, Division of Internal Medicine, and †Vascular Surgery, University of Helsinki, Helsinki, Finland; ‡Department of Clinical Nutrition, University of Kuopio, Kuopio, Finland; and §Kuopio University Hospital, Kuopio, Finland. This study was supported by grants from the Finnish Heart Research Foundation, the Finnish Heart Foundation (February 19 Fund), the Yrjö Jahnsson Foundation, and the Helsinki University Hospital (EVO Research Grant).

Manuscript received December 14, 2004; revised manuscript received January 28, 2005, accepted February 8, 2005.

Abbreviations and Acronyms

BMI	=	body mass index
CHD	=	coronary heart disease
CVD	=	cardiovascular disease
GLC	=	gas-liquid chromatography
HDL	=	high-density lipoprotein
LDL	=	low-density lipoprotein
4S	=	Scandinavian Simvastatin Survival Study

serum might increase the ratios also in atheromatous tissues. So, to study this question more closely, non-cholesterol sterols, including plant sterols, were studied in serum and compared with those in carotid artery plaques obtained during endarterectomy of patients with signs of disturbed cerebral circulation. The samples were obtained from consecutive patients undergoing carotid surgery.

METHODS

Study population. A carotid endarterectomy specimen was obtained from 25 consecutive patients who underwent the procedure in order to prevent stroke or stroke-related death. Sixteen patients were men and nine were women, and their mean age was 67 years, range 52 to 84 years. All patients were symptomatic having transient ischemic attacks, amaurosis fugax, or recent stroke with favorable recovery as the clinical indication for operation. Duplex ultrasound evaluation and digital subtraction angiography were used for carotid artery assessment. All carotid arteries operated on showed a maximum diameter reduction of at least 70%.

Mean body mass index (BMI) of the study population was 25.0 ± 0.7 kg/m². Thus, the patients had mostly a normal body weight, and only three of them were obese with BMI >30 kg/m². The population included 5 patients with type 2 diabetes, 8 with chronic obstructive pulmonary disease as a consequence of smoking, 14 were current or ex-smokers, 14 had intermittent claudication, 6 had signs of CHD, and 7 had had an earlier stroke. Diagnosis of hypercholesterolemia had been made in 10 patients, yet serum cholesterol levels were relatively low (Table 1) in these patients, partly due to the fact that 12 of 25 were on statins (mainly simvastatin and atorvastatin). Unfortunately, the initial serum total or LDL cholesterol levels could not be explored.

All participants volunteered for the study and gave their

Table 1. Serum and Lipoprotein Lipids (mmol/l) Off and On Statin Treatment

Variable	Total (n = 25)	Statin – (n = 13)	Statin + (n = 12)
Cholesterol	3.56 ± 0.15	3.68 ± 0.17	3.43 ± 0.27
LDL cholesterol	2.23 ± 0.14	2.44 ± 0.17	1.99 ± 0.22
HDL cholesterol	0.82 ± 0.06	0.82 ± 0.08	0.82 ± 0.08
Triglycerides	1.13 ± 0.14	0.93 ± 0.10	1.35 ± 0.25

Mean \pm SE. For cholesterol, to obtain mg/dl, multiply by 38.6; for triglycerides, multiply by 88.2.

HDL = high-density lipoprotein; LDL = low-density lipoprotein.

informed consent. The study protocol was approved by the ethics committees of the Departments of Medicine and Surgery, Helsinki University Hospital.

Techniques. Arterial blood sample was obtained during the surgery at the time of removing the carotid arterial plaque. Serum was separated from the blood sample by mild centrifugation. Carotid artery plaque was washed in saline and sent to the laboratory with the serum sample. Attempts were made to separate from each arterial plaque macroscopically less atheromatotic endothelial parts in addition to more tight plaque areas by one researcher (T.A.M.) blinded to the history of the patients, clinical data, and laboratory results. Thus, one to five different-looking samples were prepared from each arterial sample. Macroscopic differentiation of the tissue specimen to clear endothelial parts without or with some atheroma plaques or with more severely atheromatotic areas was not possible. Thus, 68 vascular and 25 serum samples were collected.

Serum total and high-density lipoprotein (HDL) cholesterol and triglycerides were analyzed by the routine methods of our hospital (using kits from Boehringer Diagnostica, Mannheim, Germany, and Waco Chemicals, Neuss, Germany). Exact amounts of tissue samples, 50 to 200 mg each, were carefully weighted, 5- α cholestane and epicoprostanol added for internal markers, homogenized with chloroform/methanol, and the homogenate was extracted three times with the mixture. The extract was evaporated and applied in a small amount of ethyl ether on a thin-layer chromatography plate (on silica gel) for separation of free and ester sterols with hexane/ethyl ether 50:50. The fractions were extracted from the plate, and the ester fraction, containing also 5- α -cholestane and squalene, was saponified, and non-saponifiable lipids were extracted with ethyl ether. The sterol fractions were silylated, and the sterols and squalene were quantitated with gas-liquid chromatography (GLC) using epicoprostanol as the internal standard for free sterols and 5- α -cholestane for squalene and ester sterols (10). Serum squalene and free and ester sterols were analyzed in the same way; GLC was performed using a 50-m long ULTRA-1 SE-30 column (Hewlett-Packard, Wilmington, Delaware). The values are given as mg/100 g of tissue or mg/dl of serum for cholesterol. Squalene and non-cholesterol sterols are expressed as μ g/100 g of tissue or μ g/dl of serum, or as ratios to respective cholesterol value ($10^2 \times \mu$ g/mg of cholesterol). The sum of free and ester fractions reveals the total concentration of each compound in tissues and serum. The study population was also ranked to triads according to 66 (the weight of two tissues were missed) total cholesterol concentrations in tissues (upper limit for 22 cases in triad 1 was 838 mg/100 g and 2,020 mg/100 g for 22 cases in triad 2). So as to have comparable values, this case-ranking was also used for grouping of respective GLC values in serum. Large concentration differences of tissue cholesterol of the same individual case resulted in that several patients could be in all triads; the number of arterial samples was 22 in each triad. On the

other hand, one serum sample was available from each patient, and the patient occurred only once in each triad, but serum values of some patients could occur in more than one triad according to tissue cholesterol variation. Thus, the number of serum samples was 14, 13, and 12 in increasing number of triads.

The results are given as arithmetic mean and standard error of the values, and statistical differences between the groups were calculated by the Student *t* test, using logarithmic transformation for skewed distribution. The normality of the distribution was tested with the W statistic (BMDP, statistical software, Berkeley, California) first with arithmetic and, if skewed, with log values, which usually was more normally distributed. Comparison between the triad values was performed with analysis of variance. Correlation coefficients were calculated with the Spearman rank correlation. A *p* value <0.05 was considered to be statistically significant.

RESULTS

Characteristics of serum lipids. Operative arterial serum lipid concentrations are shown in Table 1; HDL cholesterol level was low, 0.82 ± 0.06 mmol/l, including 10 cases with HDL cholesterol <0.7 mmol/l (for conversion to mg/dl, multiply with 38.6). All total cholesterol values were <5 mmol/l, and only three LDL cholesterol values exceeded 2.5 mmol/l. Twelve patients had been treated with statins, but their total and LDL cholesterol levels were similar to those without statins (Table 1). Mean total lipid concentrations were similar in diabetic and non-diabetic subjects (data not shown).

Cholesterol and squalene concentrations. Total cholesterol concentrations of arterial tissue samples measured by GLC ranged from 125 to 5,177 mg/100 g of tissue, the respective range being 43 to 150 mg/dl (1.1 to 3.9 mmol/l) in serum. The mean free and esterified cholesterol concentrations (Table 2) were about 25 and 11 times higher in tissues than serum (*p* < 0.001 for all), respectively. Ester percentage of total cholesterol was lower in tissues ($54 \pm 2\%$) than serum ($70 \pm 2\%$, *p* < 0.001). A higher increase of

free than esterified tissue cholesterol concentration decreased the respective ester percentage from $56 \pm 3\%$ in triad 1 and $59 \pm 2\%$ in triad 2 to $47 \pm 2\%$ in the third trial. Serum cholesterol concentrations and ester percentages were similar in each triad. Squalene concentrations were similar in each triad, and the levels were about 17× higher in tissues than in serum.

Non-cholesterol sterol concentrations Squalene and non-cholesterol sterol concentrations of tissues were significantly correlated with those of cholesterol (*r* value ranged from 0.659 to 0.958, *p* < 0.01, respectively, highest for cholesterol). In serum, the respective correlation was highest for campesterol and desmosterol (*r* = 0.760 for both, *p* < 0.001). Means of tissue cholesterol (mg/100 g) and non-cholesterol sterols ($\mu\text{g}/100$ g of tissues) are shown in Figure 1.

Both free and esterified sterol concentrations were increased in tissues linearly with increasing triads (Fig. 1). The synthesis marker sterols of atheromata showed a roughly similar pattern as cholesterol in that the ester fraction tended to be lower in the third than in the two other triads. The highest ester percentages were found for cholesterol (up to 75%); the ester percentages in serum (dashed lines in Fig. 1) exceeded those of tissue cholesterol and desmosterol (*p* < 0.05), but for lathosterol it tended to be lower than in tissues.

The concentrations of vascular tissue ester and free absorption sterol markers exhibited similar patterns with respect to cholesterol, so that the esterified sterols formed only 36% to 43% of total in the third triad, the proportions being significantly higher, 49% to 55% (*p* < 0.05), in the other triads (Fig. 1). The respective ester percentages were markedly higher in serum than in atheromata, especially in the third triad. The ratios of campesterol/sitosterol concentrations in tissues increased from 1.0 to 1.5 and from 0.6 to 1.8 linearly (*p* < 0.01 for both) for the respective free and ester sterols from the first to the third triad.

Ratios of non-cholesterol sterols to cholesterol. Ratios of squalene and free, ester and total non-cholesterol sterols to respective cholesterol fractions in serum and tissues, and in

Table 2. Cholesterol and Squalene in Arterial Tissue (T) and Serum (S)*

Variable	Source	Total	Triad 1	Triad 2	Triad 3
		(n = 66 for T, n = 25 for S)	(n = 22 for T, n = 14 for S)	(n = 22 for T, n = 13 for S)	(n = 22 for T, n = 12 for S)
Cholesterol	T free	800 ± 85	198 ± 29	593 ± 46	1,609 ± 116†
	T ester	847 ± 70	261 ± 37	836 ± 45	1,443 ± 93†
	T total	1,647 ± 146	459 ± 57	1,429 ± 63	3,052 ± 166†
	T ester %	54 ± 2	56 ± 3	59 ± 2	47 ± 2†
	S free	34 ± 2	32 ± 2	36 ± 2	33 ± 2
	S ester	78 ± 4	76 ± 6	82 ± 3	75 ± 5
	S total	111 ± 5	108 ± 8	118 ± 5	108 ± 7
	S ester %	70 ± 0	70 ± 0	69 ± 1	69 ± 1
Squalene	T	686 ± 55	690 ± 117	692 ± 84	677 ± 85
	S	41 ± 3	41 ± 4	44 ± 3	38 ± 3

Mean ± SE. *Triads according to tissue total cholesterol as described in Techniques section; †*p* < 0.05 or less between triads by analysis of variance. Tissue cholesterol, mg/100 g of tissue, tissue squalene, $\mu\text{g}/100$ g of tissue; serum cholesterol, mg/dl, serum squalene, $\mu\text{g}/\text{dl}$. Lipids measured by gas-liquid chromatography; divide by 387 for conversion of mg or μg to respective mmol or μmol .

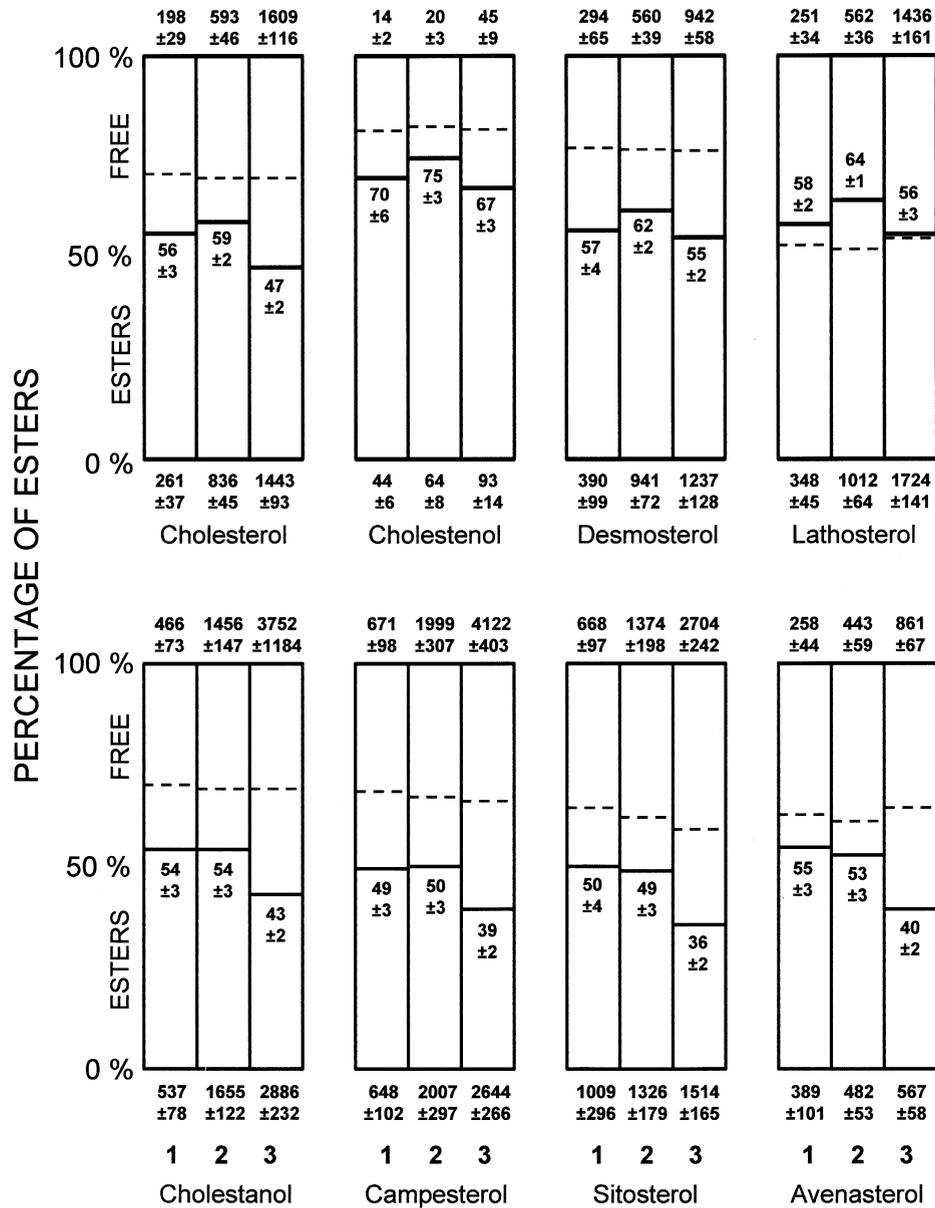


Figure 1. Cholesterol and non-cholesterol sterol concentrations and esterification in carotid artery wall in triads by tissue cholesterol concentration. Absolute values (mg/100 g of tissue for cholesterol and $\mu\text{g}/100$ g of tissue for non-cholesterol sterols) of free sterols are given on top, and those of esterified ones at bottom of each column. Esterification percentages of tissue sterols, mean \pm SE, are given by continuous horizontal lines, and those of respective serum values by dashed lines without given data.

respective triads (ranked by means of total cholesterol in tissue, Table 2) are given in Table 3. The ratios ($n = 68$) of total cholestanol, campesterol, and sitosterol were similar in the two sources, whereas those of desmosterol and avenasterol were higher, and free and ester lathosterol were lower and higher, respectively, in the tissue than serum samples.

In serum, the ratios of non-cholesterol sterols (Table 3) were similar in different triads. In tissues, with exception of cholestanol, all ratios of free, ester, and total non-cholesterol sterols decreased gradually with the increasing triads ($p < 0.05$ for all). The decrements with increasing triad resulted in that the ratios of the third triad for cholestanol were lower than those of serum, those for avenasterol were

similar, whereas those of sitosterol of the first triad were markedly higher in the tissue than serum.

Effects of statins. The subjects treated with statins had no difference in serum lipid (Table 1) or tissue cholesterol concentrations as compared with non-treated ones (Table 4). The ratios of the synthesis markers and cholestanol to cholesterol were also similar in the two groups. However, the ratios of plant sterols were higher in patients on than off statins, significantly so for tissue campesterol, sitosterol and avenasterol, and serum campesterol.

Serum versus plaque sterol correlation. The ratios of absorption sterols to cholesterol in serum were positively related to those in tissues as shown in Figure 2 for

Table 3. Ratios to Cholesterol ($10^2 \times \mu\text{g}/\text{mg}$ of Cholesterol) of Non-Cholesterol Sterols in Arterial Tissue (T) and Serum (S)*

Sterols	Source	Total	Triad 1	Triad 2	Triad 3
		(n = 66 for T, n = 25 for S)	(n = 22 for T, n = 14 for S)	(n = 22 for T, n = 13 for S)	(n = 22 for T, n = 12 for S)
Cholesterol	T free	5 ± 1	9 ± 2	4 ± 1	3 ± 0
	T ester	11 ± 2	21 ± 5	8 ± 1	6 ± 1
	T total	8 ± 1	15 ± 3	6 ± 1	4 ± 0†
	S free	6 ± 1	7 ± 2	5 ± 1	7 ± 2
	S ester	10 ± 1	11 ± 2	10 ± 1	12 ± 2
	S total	9 ± 1	10 ± 2	8 ± 1	11 ± 2
Desmosterol	T free	113 ± 13	170 ± 34	106 ± 1	63 ± 5
	T ester	118 ± 10	156 ± 28	112 ± 6	87 ± 7
	T total	111 ± 9	151 ± 24	107 ± 6	74 ± 6†
	S free	39 ± 2	40 ± 3	40 ± 3	40 ± 3
	S ester	55 ± 3	56 ± 4	58 ± 4	58 ± 4
	S total	50 ± 2	51 ± 4	52 ± 3	52 ± 3
Lathosterol	T free	114 ± 7	149 ± 18	104 ± 9	88 ± 6
	T ester	140 ± 12	176 ± 33	123 ± 7	122 ± 8
	T total	119 ± 4	141 ± 9	112 ± 6	104 ± 6†
	S free	200 ± 17	208 ± 30	199 ± 18	214 ± 31
	S ester	98 ± 8	92 ± 8	91 ± 5	112 ± 14
	S total	129 ± 8	127 ± 14	125 ± 8	143 ± 14
Cholestanol	T free	236 ± 5	231 ± 10	239 ± 10	236 ± 9
	T ester	206 ± 7	221 ± 16	197 ± 8	200 ± 10
	T total	218 ± 5	221 ± 11	215 ± 8	219 ± 8
	S free	158 ± 5	162 ± 6	158 ± 6	158 ± 8
	S ester	156 ± 5	162 ± 7	155 ± 7	155 ± 8
	S total	157 ± 5	162 ± 7	156 ± 7	156 ± 8
Campesterol	T free	313 ± 17	346 ± 21	333 ± 38	259 ± 23
	T ester	223 ± 11	257 ± 16	228 ± 25	185 ± 15
	T total	263 ± 13	296 ± 17	270 ± 30	222 ± 18†
	S free	309 ± 28	310 ± 41	308 ± 41	309 ± 47
	S ester	270 ± 22	284 ± 33	274 ± 35	255 ± 30
	S total	282 ± 23	292 ± 35	284 ± 36	272 ± 34
Sitosterol	T free	258 ± 19	375 ± 40	229 ± 22	171 ± 13
	T ester	219 ± 37	400 ± 101	151 ± 15	104 ± 8
	T total	233 ± 27	376 ± 69	184 ± 17	139 ± 10†
	S free	223 ± 35	191 ± 40	188 ± 26	274 ± 70
	S ester	141 ± 13	138 ± 19	132 ± 14	149 ± 24
	S total	166 ± 19	154 ± 24	149 ± 16	188 ± 38
Avenasterol	T free	90 ± 7	138 ± 15	75 ± 7	56 ± 4
	T ester	94 ± 16	183 ± 43	58 ± 6	40 ± 5
	T total	86 ± 10	145 ± 23	65 ± 6	48 ± 4†
	S free	52 ± 5	52 ± 6	52 ± 7	50 ± 6
	S ester	39 ± 4	36 ± 4	34 ± 3	42 ± 7
	S total	43 ± 3	41 ± 4	39 ± 4	44 ± 6

Cholesterol and noncholesterol sterols were measured by gas-liquid chromatography as presented in Techniques section. Ratios ($\mu\text{g}/\text{mg}$) were calculated dividing concentrations of noncholesterol sterols by respective cholesterol concentrations. The change of $\mu\text{g}/\text{mg}$ to $\mu\text{mol}/\text{mol}$ does not change the values. *Triads according to tissue total cholesterol as described in Techniques section; † $p < 0.05$ for decreasing triad values for free and ester sterols by analysis of variance.

campesterol with $r = 0.683$ and $p < 0.001$. The correlation was highest in the second ($r = 0.801$, $p < 0.001$) and lowest in the first ($r = 0.555$, $p < 0.01$) triad, and it was detectable both with and without statins (data not shown). On the other hand, the ratios of synthesis markers were not related between serum and tissues. However, the ratios of synthesis markers in serum were negatively correlated with those of absorption markers in tissue (for instance $r = -0.503$, $p < 0.001$ for lathosterol vs. campesterol) (Fig. 2).

DISCUSSION

The major new observation of the present study was that the higher the ratio of the dietary absorption sterol (cholestanol,

campesterol, sitosterol, and avenasterol) to serum cholesterol, the higher the ratio was also in the carotid artery wall. In addition, despite undetectable changes in serum and tissue cholesterol concentrations with statins, the ratios of serum synthesis markers tended to decrease, and those of absorption markers to increase in agreement with earlier observations (4). An additional important novel finding was that the statin treatment increased the ratios of absorption sterols also in arterial atheromata.

Cholesterol contents of arterial atheromatous tissues are believed to rise in proportion to serum cholesterol concentration. The transfer of LDL particles, possibly partly in oxidized form, through the arterial endothelium is believed

Table 4. Cholesterol (Tissue mg/100 g, Serum mg/dl) and Ratios to Cholesterol ($10^2 \times \mu\text{g}/\text{mg}$ of Cholesterol) of Squalene and Noncholesterol Sterols in Arterial Tissue (T) and Serum (S) Off and On Statin Treatment

Variable	Source	Statin – (n = 36 for T, n = 13 for S)	Statin + (n = 32 for T, n = 12 for S)
Cholesterol	T	1,745 ± 223	1,542 ± 189
	S	135 ± 8	125 ± 10
Squalene	T	109 ± 30	92 ± 19
	S	35 ± 2	29 ± 7
Cholestenol	T	9 ± 2	8 ± 1
	S	9 ± 1	9 ± 2
Desmosterol	T	97 ± 9	118 ± 16
	S	54 ± 3*	46 ± 3*
Lathosterol	T	125 ± 8	118 ± 7
	S	142 ± 12	115 ± 10
Cholestanol	T	204 ± 7	221 ± 7
	S	156 ± 9*	157 ± 4*
Campesterol	T	233 ± 17	280 ± 15†
	S	239 ± 30	329 ± 33†
Sitosterol	T	169 ± 16	290 ± 51†
	S	139 ± 16	196 ± 36
Avenasterol	T	63 ± 8	107 ± 17†
	S	39 ± 5	47 ± 5

Mean ± SE. *p < 0.05 vs. respective tissue value; †p < 0.05 vs. statin –. Divide mg by 387 to get mmol; $\mu\text{g}/\text{mg}$ when expressed as $\mu\text{mol}/\text{mol}$ does not change the values.

to increase the extracellular matrix of subendothelial space, probably by binding to glucosaminoglycans in subendothelial tissues, causing finally the development of atheromatous plaques with markedly increased cholesterol contents ending ultimately in crystallization of cholesterol in the most tight atheromata (1,11). The crystallization can explain the marked variation in cholesterol concentration in various parts of removed carotid arterial pieces in Table 2 and Figure 1. It has been shown that cholesterol is frequently esterified with locally synthesized fatty acids, but, in crystalline areas of atheromata, cholesterol can be mainly in free form. This may be the reason that the esterification percentage of cholesterol was lowest in the third triad with the highest cholesterol concentration including probably most of the crystalline cholesterol. Ester percentage of serum cholesterol was similar in each triad and clearly higher than that of tissue cholesterol in all triads.

It is clear that plant sterols in atheromatous tissues are totally of dietary origin transported mainly with LDL from circulation. Plant sterols have been found in human tissues, including normal aortic wall and atheromatous tissues, in several earlier studies (6,12–14). The sitosterol concentration was similar to that of campesterol in postmortem normal aorta, as in triad 1 of Figure 1 of the present study, but twice as high in the one available calcified atheroma (6). In triad 2, and especially triad 3 of Figure 1 of the present study, both the free and esterified campesterol concentrations exceeded those of sitosterol. The markers of cholesterol synthesis may also be transported by circulating lipoproteins to arterial walls, even though macrophages, smooth muscle cells, and other cells in arterial walls are

apparently able to synthesize cholesterol and could, accordingly, contribute locally to these precursor sterols. Non-cholesterol sterol and cholesterol concentrations in atheromatous tissues, in contrast to serum, had a highly significant relation to each other. Thus, the standardization by the cholesterol concentrations showed that the ratios of free, ester, and total non-cholesterol sterols were decreased in proportion to increased respective cholesterol concentration in tissues, but not in serum. In addition, as cholesterol, also non-cholesterol sterols were less esterified in most atheromatous tissue samples, suggesting that some of them were also in a crystalline non-esterified state. However, despite relatively low ratios of non-cholesterol sterols in the highest tissue cholesterol triads, the ratios of the absorption sterols in serum were positively related to those in atheromatous tissues. Thus, the higher the absorption efficiency of intestinal sterols, especially of plant sterols, the higher their serum level will be, suggesting that the plant sterol content in the atheromata will also be higher. Provided that cholesterol were a factor responsible for formation of atheromata, accumulation of plant sterols first in serum followed by an increase in arterial walls may have contributed to the development of atheromata. No gray-scale analysis of

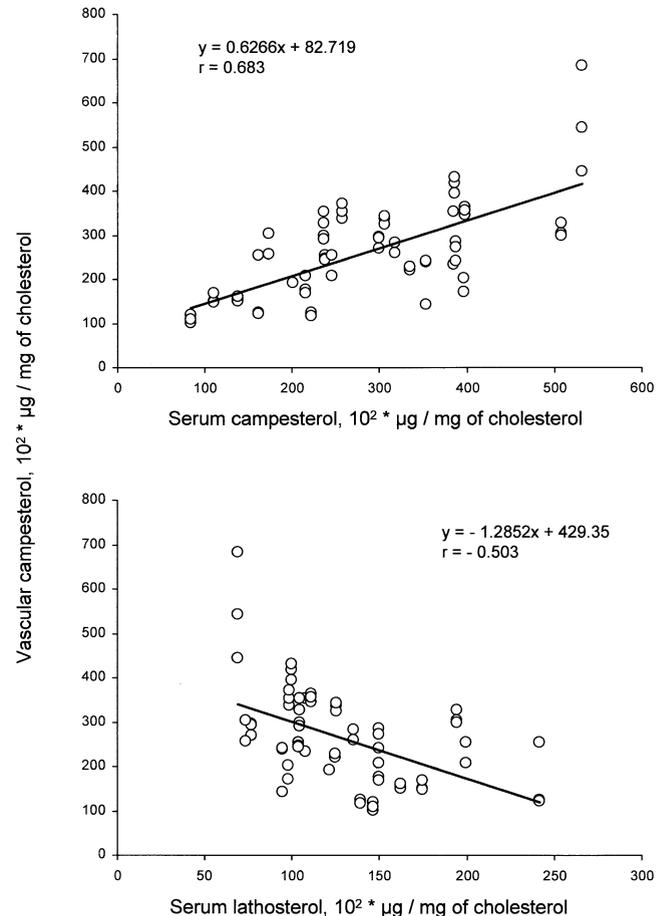


Figure 2. Correlation of serum ratios of campesterol (upper panel) and lathosterol (lower panel) to cholesterol with those of tissue campesterol (p < 0.001 for both).

plaque echolucency was made preoperatively, but, in the plaques studied, non-cholesterol sterol concentrations were highest in the third triad.

The question now arises whether there is any evidence that dietary plant sterols could contribute to the development of atherosclerotic plaques in human subjects. The question may sound naive, because vegetarianism is considered to be healthy as far as arteriosclerotic arterial disease is considered, mainly because body weight of the subjects is low, hypertension is rarely observed, occurrence of type 2 diabetes is rare, and hyperlipidemia, especially hypercholesterolemia, is infrequent. In addition, serum plant sterol concentrations, despite relatively high plant sterol intake, are not excessively high (15). The nature may have considered that high plant sterol concentrations in animal cell membranes offer a possible danger for well being of the cell owner, limiting intestinal absorption of plant sterols from diet to roughly one-fifth to one-tenth of that of cholesterol. However, mutation of ABCG5/8 genes in intestinal enterocytes and liver results in a marked increase of serum plant sterol concentrations, which, in patients with sitosterolemia (16), is associated with severe coronary artery atheromatosis at a young age almost like in homozygous familial hypercholesterolemia. Glueck *et al.* (17) showed a relationship between elevated serum plant sterol levels and incidence of CHD in a large study population. Higher serum plant sterol levels have also been found in patients with rather than without CHD (18), and coronary angiographies in patients with CHD revealed that the higher the plasma sitosterol concentration or ratio to cholesterol, the more the extent and severity of CHD, especially in women (19). In a group of postmenopausal coronary women and their random controls, the concentrations and ratios to cholesterol of plasma cholestanol and plant sterols independently predicted CHD, whereas those of squalene and cholesterol precursor sterols exhibited a negative correlation (18). Increased absorption sterols in the previously mentioned studies even after adjustment for cholesterol suggest that effective cholesterol absorption in the subjects with high plant sterol ratios was probably not the sole factor for CHD. A ten-year follow-up study of a random population showed an increased incidence of CHD in subjects with the highest serum sitosterol concentration as compared with the three other quartiles (20). The incidence was in fact even five-fold in the highest global risk group (20). Patients with a positive family history of CHD had significantly higher plasma levels of campesterol, sitosterol, and their ratios to cholesterol (21). In the Scandinavian Simvastatin Survival Study (4S) placebo group, the baseline non-cholesterol sterol concentrations or ratios to cholesterol were not related to recurrences of CHD events (4,22). Thus, the significance of serum plant sterols in the development of coronary atheromatosis needs further studies, even though a recent investigation in middle-aged men and women did not support an association between elevated plasma levels of serum plant sterols and atherosclerosis (23).

Statin treatment decreases serum ratios to cholesterol of cholesterol precursor sterols and increases those of absorption marker sterols, indicating that the drug decreases cholesterol synthesis and increases absorption of plant sterols (4,7–9). The decreased serum ratios of the synthesis sterols with statins tended to be detectable also in the present study, but the resulting reduced cholesterol synthesis was not associated with any change in the respective tissue sterol values. The tissue or serum cholesterol concentrations were not consistently different in subjects with or without the statin treatment, but the increased serum and tissue plant sterol ratios to cholesterol indicate that enhanced intestinal sterol absorption contributed to arterial wall sterol composition. The finding raises a question, whether increased plant sterol content in serum and arterial wall with statins should be considered harmful for prevention of development of CHD in some patients. In long-term studies with statin treatment, prevention of CHD events is usually around 30%, while 70% of the recurrent events should need more effective prevention (2,3). In 4S, no reduction was observed in recurrence of CHD with simvastatin in patients with high baseline plant sterol contents and with marked increase of serum plant sterols during the five-year treatment period (22). Additional treatment with inhibition of sterol absorption (e.g., with plant stanol esters) was suggested for this particular group of patients (4,22).

Reprint requests and correspondence: Dr. Tatu A. Miettinen, Biomedicum Helsinki C 4 22, University of Helsinki, P.O. Box 700, FIN-00029 HUS, Finland. E-mail: tatu.a.miettinen@helsinki.fi.

REFERENCES

1. Camejo G, Hurt-Camejo E, Olsson U, Wiklund O, Bondjers G. The role of the extracellular matrix on atherosclerosis. In: Stemme S, Olsson AG, editors. *Atherosclerosis XII*. Amsterdam: Excerpta Medica, International Congress Series 1215. 2000:41–52.
2. Heart Protection Study Collaborative Group. MRC/BHF Heart Protection study of cholesterol lowering with simvastatin in 20,536 high-risk individuals: a randomised placebo-controlled trial. *Lancet* 2002;360:7–22.
3. Doggrel SA. Atorvastatin versus pravastatin: intensive versus moderate lipid lowering. *Expert Opin Pharmacother* 2004;5:2025–8.
4. Miettinen TA, Strandberg TE, Gylling H, for the Finnish Investigators of the Scandinavian Simvastatin Survival Study Group. Noncholesterol sterols and cholesterol lowering by long-term simvastatin treatment in coronary patients: relation to basal serum cholestanol. *Arterioscler Thromb Vasc Biol* 2000;20:1340–6.
5. Kesäniemi YA, Miettinen TA. Cholesterol absorption efficiency regulates plasma cholesterol level in the Finnish population. *Eur J Clin Invest* 1987;17:391–5.
6. Mellies MJ, Ishikawa TT, Glueck CJ, Bove K, Morrison J. Phytosterols in aortic tissue in adults and infants. *J Lab Clin Med* 1976;88:914–21.
7. Uusitupa MI, Miettinen TA, Happonen P, *et al.* Lathosterol and other noncholesterol sterols during treatment of hypercholesterolemia with lovastatin alone and with cholestyramine or guar gum. *Arterioscler Thromb* 1992;12:807–13.
8. Miettinen TA, Gylling H, Lindbohm N, Miettinen TE, Rajaratnam RA, Relas H, Finnish Treat-to-Target Study Investigators. Serum

- noncholesterol sterols during inhibition of cholesterol synthesis by statins. *J Lab Clin Med* 2003;141:131–7.
9. Miettinen TA, Gylling H. Synthesis and absorption markers of cholesterol in serum and lipoproteins during a large dose of statin treatment. *Eur J Clin Invest* 2003;33:976–82.
 10. Miettinen TA. Cholesterol metabolism during ketoconazole treatment in man. *J Lipid Res* 1988;29:43–51.
 11. Hansson GK. Inflammation, injury and atherosclerosis—the Russels? Ross Memorial Lecture. In: Stemme S, Olsson AG, editors. *Atherosclerosis XII*. Amsterdam: Excerpta Medica, International Congress Series 1215. 2000:53–66.
 12. Bhattacharyya AK, Connor WE. Beta-sitosterolemia and xanthomatosis. A newly described lipid storage disease in two sisters. *J Clin Invest* 1974;53:1033–43.
 13. Gould RG. Absorbability of beta-sitosterol. *Trans N Y Acad Sci* 1955;18:129–34.
 14. Gould RG, Jones RJ, LeRoy GV, Wissler RW, Taylor CB. Absorbability of beta-sitosterol in humans. *Metabolism* 1969;18:652–62.
 15. Vuoristo M, Miettinen TA. Absorption, metabolism, and serum concentrations of cholesterol in vegetarians: effects of cholesterol feeding. *Am J Clin Nutr* 1994;59:1325–31.
 16. Berge KE. Sitosterolemia: a gateway to new knowledge about cholesterol metabolism. *Ann Med* 2003;35:502–11.
 17. Glueck CJ, Speirs J, Tracy T, Streicher P, Illig E, Vandegrift J. Relationships of serum plant sterols (phytosterols) and cholesterol in 595 hypercholesterolemic subjects, and familial aggregation of phytosterols, cholesterol, and premature coronary heart disease in hyperphytosterolemic probands and their first-degree relatives. *Metabolism* 1991;40:842–8.
 18. Rajaratnam RA, Gylling H, Miettinen TA. Independent association of serum squalene and noncholesterol sterols with coronary artery disease in postmenopausal women. *J Am Coll Cardiol* 2000;35:1185–91.
 19. Sutherland WHF, Williams MJA, Hye ER, Restieaux NJ, de Jong SA, Walker HL. Association of plasma noncholesterol sterol levels with severity of coronary artery disease. *Nutr Metab Cardiovasc Dis* 1998;8:386–91.
 20. Assmann G, Cullen P, Erbey J, Ramey DR, Kannenberg F, Schulte H. Elevation in plasma sitosterol concentration is associated with an increased risk for coronary events in the PROCAM study. *Circulation* 2003;108 Suppl:3300.
 21. Sudhop T, Gottwald BM, von Bergman K. Serum plant sterols as a potential risk factor for coronary heart disease. *Metabolism* 2002;51:1519–21.
 22. Miettinen TA, Gylling H, Strandberg T, Sarna S, for the Finnish 4S Investigators. Baseline serum cholestanol as predictor of recurrent coronary events in subgroup of Scandinavian Simvastatin Survival Study (4S). *Br Med J* 1998;316:1127–30.
 23. Wilund K, Yu L, Xu F, et al. No association between plasma levels of plant sterols and atherosclerosis in mice and men. *Arterioscler Thromb Vasc Biol* 2004;24:2326–32.