Published in final edited form as:

Atherosclerosis. 2010 January ; 208(1): 155–160. doi:10.1016/j.atherosclerosis.2009.07.031.

The Effects of Sterol Structure upon Sterol Esterification

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Abstract

Cholesterol is esterified in mammals by two enzymes: LCAT (lecithin cholesterol acyltransferase) in plasma and ACAT₁ and ACAT₂ (acyl-CoA cholesterol acyltransferases) in the tissues. We hypothesized that the sterol structure may have significant effects on the outcome of esterification by these enzymes. To test this hypothesis, we analyzed sterol esters in plasma and tissues in patients having non-cholesterol sterols (sitosterolemia and Smith-Lemli-Opitz syndrome). The esterification of a given sterol was defined as the sterol ester percentage of total sterols. The esterification of cholesterol in plasma by LCAT was 67 percent and in tissues by ACAT was 64 percent. Esterification of nine sterols, (cholesterol, cholestanol, campesterol, stigmasterol, sitosterol, campestanol, sitostanol, 7-dehydrocholesterol and 8-dehydrocholesterol) was examined. (The relative esterification (cholesterol being 1.0) of these sterols by the plasma LCAT was 1.00, 0.95, 0.89, 0.40, 0.85, 0.82 and 0.80, 0.69 and 0.82 respectively. The esterification by the tissue ACAT was 1.00, 1.29, 0.75, 0.49, 0.45, 1.21 and 0.74 respectively. The predominant fatty acid of the sterol esters was linoleic acid for LCAT and oleic acid for ACAT. We compared the esterification of two sterols differing by only one functional group (a chemical group attached to sterol nucleus) and were able to quantify the effects of individual functional groups on sterol esterification. The saturation of the A ring of cholesterol increased ester formation by ACAT by 29 percent and decreased the esterification by LCAT by 5.9 percent. Esterification by ACAT and LCAT was reduced respectively by 25 percent and 11 percent by the presence of an additional methyl group on the side chain of cholesterol at the C-24 position. This data supports our hypothesis that the structure of the sterol substrate has a significant effect on its esterification by ACAT or LCAT.

Keywords

LCAT (lecithin cholesterol acyltransferase); ACAT (acyl-CoA cholesterol acyltransferase); free sterols; esterified sterols; plant and SLOS sterols

Introduction

Cholesterol is esterified in mammals by two major mechanisms in plasma and tissues (1, 2). In tissues, fatty acids are activated to form acyl-CoA, which reacts with cholesterol through the action of acyl-CoA cholesterol acyltransferase (ACAT). There are two ACAT enzymes: ACAT₁ is active in macrophages and many tissues, whereas ACAT₂ is found only in the liver and intestine (3, 4). In the second sterol ester pathway occurring in the plasma, fatty acids in position 2 of lecithin are transferred directly to cholesterol and esterified by lecithin

cholesterol acyltransferase (LCAT(5)). Recently, it was found in mice that ACAT₂ provides the cholesterol ester of VLDL in the liver, whereas LCAT adds cholesterol ester to LDL in the plasma (5).

The specificity of LCAT and ACAT toward the acyl acceptor (sterol substrate) has been reported only in vitro studies (6–8). In view of these in vitro data, we hypothesized that this substrate specificity may hold true in vivo as well. To test our hypothesis, it is essential to compare esterification of sterols with different structures. However, the practical difficulty to undertake this experiment is that cholesterol is the only sterol found in appreciable quantities in the blood or tissues in healthy humans. Fortunately, patients with the genetic diseases sitosterolemia and Smith-Lemli-Opitz syndrome (SLOS) have many other sterols in their blood and tissues and thus provide an opportunity for an in vivo experiment of nature.

Since sitosterolemia and SLOS are rare genetic diseases, summaries are presented as follows. In 1974 sitosterolemia with xanthomatosis was first described by Bhattacharyya and Connor (9). Since then additional patients with this rare inherited sterol storage disease have been diagnosed in the U.S. and around the world (10). The major clinical manifestations include tendon and tuberous xanthomas that involve Achilles and patellar tendons, extensor tendons of the hand and the skin of the elbows and knees. Other symptoms include recurrent arthritis and arthralgias of the knees and ankle joints, anemia and premature atherosclerosis. Chemically, increased amounts of plant sterols, such as sitosterol campesterol and stigmasterol are found in the plasma, erythrocytes and xanthomas (9). The genetic defect is mutations of the sterol transporters, ABCA5 and ABCA8 (10).

SLOS is an autosomal recessive disorder of impaired cholesterol synthesis (11–13). The defect in the cholesterol biosynthetic pathway occurs at the sterol Δ -7 reductase step, which converts 7-dehydrocholesterol (7-DHC) to cholesterol. This results in increased levels of 7-DHC and 8-DHC and low levels of cholesterol in the plasma and tissues. Clinical manifestations of this inherited syndrome include severe growth deficiency, malformations, neurological dysfunction and usually mental retardation. Varied multi-faceted and seemingly unrelated clinical manifestations can be accounted for by reduction in cholesterol concentrations and by the accumulation of potentially toxic cholesterol precursors (7 and 8-DHC) in the plasma and tissues.

In the present study, we analyzed the free and ester sterol content of the plasma of twelve sitosterolemic patients, xanthoma samples from six such patients and plasma samples of five SLOS patients. A total of nine different sterols were detected. From these data, we attempted to answer the following questions:

1. Do the LCAT and ACAT enzymes have different substrate specificity in vivo toward sterols with different chemical structures?
2. Is this substrate specificity different in vivo from the published in vitro data?
3. What is the effect of different functional groups on sterol esterification by the two enzymes
4. Which fatty acids form the sterol esters in plasma and tissues? Are there differences in the fatty acid moieties of sterol esters between plasma and tissues?

Methods

The plasma and xanthoma samples were obtained from patients evaluated by us. The sitosterolemic patients had typical elevations of plant sterols as recorded in previous publications (9). The ages of the sitosterolemia patients were from adolescents to the 40

years. Both sexes were included. Typical plasma values were sitosterol 17.7 mg/dl, campesterol 8.2 mg/dl and stigmasterol 0.5 mg/dl (9). The sitosterolemic patients were not treated with ezetimibe. The patients diagnosed as SLOS usually had low plasma cholesterol levels and elevated levels of 7 and 8 dehydrocholesterol. Typical values were: cholesterol 55 mg/dl, 7DHC 9.3 and 8 DHC 7.9. The ages of the SLOS patients were from stillborn to 14-years. Both sexes were included. The SLOS patients all had the characteristic clinical features of this disorder (12). Plant sterols in normal adults are less than 0.5 mg/dl or undetectable, and 7 dehydrocholesterol is undetectable by GLC in normal individuals.

Plasma and xanthoma sterols were analyzed by the methods described previously ((14)). Xanthoma samples were washed with saline to remove blood and blotted dry and freeze dried. Dried tissues were ground to powder and extracted with chloroform-methanol (15).

A tracer dose of [4- C^{14}] cholesterol and cholesteryl [C^{14}] stearate was added to the lipid extracts of plasma and xanthomas to monitor the recovery. The lipid extracts were chromatographed on silica gel G thin-layer plates. The developing solvent was hexane-chloroform-ether-acetic acid 80:10:10:1. The free sterols and sterol ester bands of the TLC plates were removed and extracted with ethyl ether. Ester sterols were saponified with alcoholic KOH, and the sterols were extracted with hexane. The sterol ester fatty acids were recovered by acidifying the aqueous phase and re-extracting with hexane. Their analyses will be described subsequently. The radioactivity of the sterol aliquots from both sterol and sterol ester bands was measured in a Packard tri-carb liquid scintillation counter equipped with an absolute activity analyzer (Packard instrument Downers Grove, Ill.). For samples from sitosterolemic patients, the sterol content in both free sterol and ester hands was determined by gas-liquid chromatography (Hewlett Packard 7610A, Avondale, PA) on a 3.8 percent SE-30 glass column. Cholestane was used as an internal standard according to the method of Miettinen, Ahrens and Grundy (16) Plasma sterols of SLOS patients were analyzed by the method described previously (17). The TMS-derivatives of the samples were analyzed by a GLC that was equipped with a hydrogen flame ionization detector (Perkin-Elmer Autosystem XL gas chromatography) and contained 25 m CP-Wax-57 capillary column (ChromPack, Vrian, Walnut Creek, CA) with 0.32 mm i.d. and 0.25 μ m film thicknesses. The column temperature was 205° C. Helium was used as carrier gas and cholestane was the internal standard. An aliquot of the sample was taken for radioactivity analysis.

The fatty acid composition was analyzed by the method reported previously (14). The fatty acids from sterol ester bands recovered after saponification were methylated with boron trifluoride ((18)). Their concentrations were determined by gas-liquid chromatography (Hewlett Packard 5830A, Avondale, PA) with a coiled-glass column packed with Supelco SP2330. The column temperatures were programmed from 180–200 C° at a rate of 2°c/min. Fatty acid standards were run daily.

Statistics

p-values were calculated by the student t test. Calculations were made with SPSS for Window Version 8.0 (SPSS).

The protocols for these patient studies had been approved by the Institutional Review Board at Oregon Health & Science University.

Results

In order to compare the esterification of various sterols, we first measured the free and esterified sterol content of plasma and xanthoma and then calculated the esterification of the different sterols.

Plasma sterol esterification

The plasma free and esterified sterol composition of twelve sitosterolemic and five SLOS patients is presented in Table 1. Besides cholesterol, a total of six different sterols in sitosterolemic patients were detected: cholestanol, campesterol, campestanol, stigmasterol, sitosterol and sitostanol. The free cholesterol was 71.1 ± 28.7 mg/dl. The esterified cholesterol was 147.5 ± 47.4 mg/dl. Free cholestanol was 1.7 ± 0.8 ; esterified cholestanol 2.7 ± 0.8 mg/dl. Free and esterified campesterol were 3.6 ± 1.5 and 5.5 ± 2.4 mg/dl respectively. Free campestanol was 0.6 ± 0.2 mg/dl and esterified campestanol was 0.8 ± 0.5 mg/dl. Stigmasterol was 1.2 ± 0.6 mg/dl in free form and 0.4 ± 0.2 mg/dl in ester form. Free sitosterol was 8 ± 3.5 mg/dl and esterified sitosterol was 10.8 ± 5.1 mg/dl. Sitostanol was 2.6 ± 1.1 mg/dl in free form and 3.0 ± 1.5 mg/dl in ester form.

In SLOS patients, three plasma sterols were identified: cholesterol, 7-dehydrocholesterol and 8-dehydrocholesterol. The free and esterified cholesterol in four SLOS children was 10.5 ± 5.2 mg/dl and 5.2 ± 14.7 mg/dl respectively. The free and esterified 7-dehydrocholesterol was 4.8 ± 2.1 mg/dl and 7.6 ± 2.3 mg/dl respectively. The free 8-dehydrocholesterol was 2.8 ± 1.9 mg/dl. The esterified 8-dehydrocholesterol was 6.4 ± 2.0 mg/dl. For one infant, free cholesterol was 1.7 mg/dl and esterified cholesterol was 3.9 mg/dl respectively. The free and esterified 7-dehydrocholesterol was 4.9 and 3.5 mg/dl respectively. The free 8-dehydrocholesterol was 3.0 mg/dl and esterified 8-dehydrocholesterol 5.1 mg/dl.

LCAT is mainly responsible for the formation of the ester sterols in plasma, though ACAT2 also makes rather a small contribution to plasma sterol esters. Hence, the ester content of a particular sterol represents sterol esterification by LCAT. In Fig. 1A, we have expressed the degree of esterification of each sterol as percent esterified sterol to total sterol. The esterification of cholesterol was 67.6 ± 6.1 percent, and of cholestanol was 63.9 ± 7.7 percent. Plant sterols were less significantly esterified than cholesterol ($p < 0.005$ and $p < 0.001$): the esterification of campesterol was 59.8 ± 7.0 percent; campestanol 55.0 ± 13.2 percent; sitosterol 57.2 ± 6.7 percent and sitostanol 53.9 ± 5.9 percent. Stigmasterol, with an additional double bond in the side chain, was the least esterified, only 26.8 ± 8.4 percent. The esterification for cholesterol, 7-dehydrocholesterol and 8-dehydrocholesterol was 83.2, 57.8 and 67.9 percent respectively (Fig. 1B). The esterification of 7-dehydrocholesterol was significantly lower than for cholesterol ($p < 0.004$).

Tissue free and esterified sterol content

Free and esterified sterol compositions of xanthomas from patients with sitosterolemia are given in Table 2. The concentration of free cholesterol was 23.3 ± 9.0 mg/g and esterified cholesterol was 49.9 ± 37.7 mg/g. The concentration of cholestanol was 0.6 ± 0.3 mg/g in the free form and 2.5 ± 0.4 mg/g in the ester form. The concentration of free and esterified campesterol was 2.2 ± 1.1 and 2.2 ± 1.5 mg/g. Campestanol was 0.3 ± 0.3 mg/g in the free form and 0.8 ± 0.6 mg/g in the ester form. Free stigmasterol was 1.1 ± 0.8 mg/g and esterified stigmasterol was 0.2 ± 0.2 mg/g. Sitosterol was 9.5 ± 5.9 mg/g in the free form and 3.1 ± 1.5 mg/g as esters. Free sitostanol was 1.5 ± 1.2 mg/g and esterified sitostanol was 1.4 ± 1.1 mg/g.

Since esterified sterols in tissues result from ACAT activity, we expressed the degree of esterification of each sterol as percent of esterified sterol to total sterol in xanthomas (Fig. 2). Cholesterol was 63.7 ± 18.8 percent esterified. Interestingly, cholestanol had higher esterification than cholesterol (82.4 ± 5.7 , $p < 0.025$). With the exception of campestanol, plant sterols, in general, were less esterified than cholesterol: campesterol 48.0 ± 20.1 percent; stigmasterol 31.0 ± 10.1 percent; sitosterol 28.6 ± 15.8 percent; sitostanol 46.9 ± 10.4 percent; campestanol 76.8 ± 11.3 percent. Saturated sterols (cholestanol and campestanol) were more esterified than unsaturated sterols.

Quantifying the effects of individual functional groups on esterification

Since these sterols have same basic sterol structure, by comparing the esterification of two sterols differing by only one functional group, we were able to delineate the effects of individual functional groups on sterol esterification (Table 3). We defined the functional groups as chemical groups attached to sterol moieties, in this case, cholesterol molecule. For LCAT activity, saturation of the C-5 double bond decreased esterification 5.9 percent. Addition of a cis-methyl group at C-24 reduced esterification 11 percent. There was a 15 percent decrease in esterification with addition of a cis-ethyl group at C-24 position. Adding methylene group at C-24 only decreased esterification by 4.5 percent. Adding a double bond at C-7 decreased esterification by 30 percent. Interestingly, the trans configuration had the most effect on esterification. For example, when adding double bond at C-22 and a trans ethyl group at C-24, the esterification was decreased 60 percent (cholesterol v.s. stigmasterol). When adding double bond at C-22 and modifying the ethyl group to a trans ethyl group at C-24, there was 52.9 percent decrease in esterification (sitosterol v.s. stigmasterol).

For ACAT activity, in contrast to LCAT, saturation of the double bond increased esterification 29 percent. Addition of cis-methyl and cis-ethyl groups at C-24 decreased esterification 25 and 55 percent respectively. Addition of methylene group at C-24 decreased esterification by 40 percent. Unlike LCAT, the trans-configuration did not have a special effect upon esterification. For example, the addition of a trans-ethyl group at C-24 decreased esterification by 51 percent which is similar to adding a cis-ethyl group at C-24. This decreased esterification by 55 percent. These data not only quantified the effects of individual functional group on sterol esterification but also demonstrated clearly the significant effects of sterol structure on esterification by both LCAT and ACAT.

Fatty acid composition of sterol esters

We compared the fatty acid composition of the sterol esters of the plasma and xanthoma of the sitosterolemia patients with the comparable values of hypercholesterolemia patients previously reported (Table 4) as seen in our previous study (14)). The major fatty acid of the plasma sterol esters was linoleic acid (C18:2 n-6) (Table 4), 56.7 ± 5.5 percent of total fatty acids. Oleic acid was 17.7 ± 2.5 percent. In xanthomas the reverse was noted. The major fatty acid was oleic acid, 45.6 ± 4.2 percent; linoleic acid was only 15.5 ± 1.6 percent of total fatty acids. These results thus clearly indicate that the major portion of the sterol esters in the tissues originate in tissues and not from plasma. They also clearly indicate that sterol esters in plasma occur from the activity of LCAT and in tissues from the activity of ACAT.

Discussion

In humans, two key enzymes control the synthesis of cholesterol esters. Acyl-CoA cholesterol acyltransferase (ACAT₁ and ACAT₂) is operative in the tissues, and lecithin-cholesterol acyltransferase (LCAT) is the plasma esterifying enzyme. The physiological and pathological importance of ACAT and LCAT has been reviewed previously (19–21), in

addition Asztalos et al demonstrated the role of LCAT in HDL remodeling (22). In our present studies, we had an opportunity to analyze the free and esterified sterol composition of the plasma and xanthoma from sitosterolemic patients and of plasma from SLOS patients, who have plasmas and tissues which uniquely contain sterols other than cholesterol. Our data demonstrated that the sterol (acyl acceptor) structure had a significant effect upon esterification by both ACAT and LCAT. Interestingly, our data is almost identical to the in vitro data by Piran and Nishida about LCAT esterification (6) and similar to in vitro data by Maculley and Tavani about ACAT esterification (7,8). Other groups (methyl, ethyl) on the side chain of sterol may make the structure more bulky and rigid and thus may reduce the mobility of sterols in the vesicles. The most notable effect was trans-ethyl group of stigmasterol on esterification. This special sterol-orientation exerted the most hindrance on esterification by LCAT. It is known that the shape of the molecule has a significant influence on its interactions with other molecules. Different structures may act differently as shown by our data.

We also found that sterol structure effects on esterification were different between LCAT and ACAT. The addition of methyl and ethyl groups on the side chain had reduced esterification more for ACAT than for LCAT. For ACAT, the cis and trans ethyl groups had similar effects. In contrast, the trans ethyl group had a greater effect than the cis ethyl for LCAT. The saturation of double bond at C-5 increased esterification for ACAT while this decreased esterification for LCAT. These differences in response to the structure of sterol (acyl acceptor) may be due to the differences of acyl donor (acyl-CoA for ACAT and lecithin for LCAT) and/or the nature of the enzymes itself. While mechanistic details remain to be investigated, these results will improve our understanding of the function of these esterifying enzymes.

The effects of sterol structure on esterification may have implications upon sterol absorption and atherogenesis. Esterification of sterols may be a necessary step for sterol absorption. This is based on the fact that the esterification of sterols in the mucosa is one of the several necessary processes leading to the formation of chylomicrons in which sterols are mostly in the ester form (23). The study by Compassi et al (24) indicated that discrimination between cholesterol and plant sterols occurred during intracellular processing involving the esterification and incorporation into lipoprotein particles. Temel et al showed that ACAT₂ displays the greatest capacity to differentiate cholesterol from sitosterol (25). These authors suggested that this sterol selectivity by ACAT₂ may reflect a role in the sorting of dietary sterols during their absorption by the intestine in vivo. In the present study, the esterification of sitosterol by ACAT was low (45 percent of cholesterol). The low esterification of sitosterol by ACAT may account for, in part, the much lower intestinal absorption of sitosterol and stigmasterol relative to cholesterol as has been shown by us and others in earlier studies (26). Incubating intestinal microsomal membranes with radioactive sitosterol, Field and Mather found that the esterification of sitosterol by ACAT was very low compared to cholesterol (27). They suggested that inadequate esterification of this plant sterol may play a role in the poor absorption of sitosterol by the gut. Interestingly, our data also showed that campestanol had a higher esterification than cholesterol. Hyperabsorption of both campestanol and sitostanol in sitosterolemic homozygotes has been reported (24, 28, and 29). This is important because these stanols may be used to reduce plasma cholesterol levels by decreasing cholesterol absorption but clearly should not be used in sitosterolemic patients (28, 29).

In the process of atherosclerosis, a large amount of the plasma LDL cholesterol is deposited into the artery wall (30, 31). The deposition of cholesterol from the plasma LDL attests to the important role of ACAT in cholesterol esterification which serves as a mechanism to divert the insoluble and potentially toxic crystalline free cholesterol into a less toxic ester

form when the free cholesterol exceeds the maximum saturation in the cellular membranes. As indicated from our data, sitosterol, which is less well esterified by ACAT in the tissues and less soluble than cholesterol, is likely to be more atherogenic than cholesterol in sitosterolemic patients. This deduction seems to fit the fact that sitosterolemic patients have normal ranges of plasma cholesterol, yet suffer premature atherosclerosis (32–34). However, a recent study showed that there was no association between plasma plant sterol levels and atherosclerosis in genetically modified mice and normal men (35). These mice did have very elevated plant sterol levels but did not have more aortic atherosclerosis than did control mice, both groups being fed an atherogenic diet. These data establish the point in mice but do not refute the well known predilection of sitosterolemic humans to have advanced atherosclerosis. The quoted human study did not involve sitosterolemic patients but a normal population with differing but normal plasma plant sterol levels (35). The variations of plant sterol levels did not correlate with a family history of coronary disease or with coronary calcification. It may be that plasma plant sterols have to be much higher as in sitosterolemia to affect atherosclerosis and xanthoma. There is also the lifetime exposure to these high sterol levels.

The role of LCAT in reverse cholesterol transport is well established (36). In that scheme, sterols must be esterified to be transported. In our study, sitosterol was much less esterified by LCAT than cholesterol. Therefore, sitosterol would be cleared more slowly from plasma than cholesterol in sitosterolemic patients. Incidentally, impaired clearance of sitosterol from the blood in humans has been documented (32, 37).

It is true that tendon xanthomas represent an unusual tissue, and the fact that esters from that tissue may not be representative of other tissues with a high ester content in which esters are synthesized by the action of ACAT. However, other tissues, such as liver, adrenal, etc., are not available for analysis. There are no sterol esters in erythrocytes. Rich in cellular lipids, xanthoma might also be compared to the atheroma of arteries whose sterol esters have already been discussed. Nonetheless, our in vivo analyses of xanthoma sterol esters are exactly what would be expected on the basis of the in vitro analyses (7, 8). Ginsberg and colleagues have shown that xanthomas have a good perfusion resulting in rapid uptake of labeled LDL after intravenous injection (38). Clinically, xanthomas grow but can regress over time with the treatment of sitosterolemic patients.

Despite the fact that patients with SLOS apparently had normal cholesterol esterification mechanisms in the plasma from LCAT, cultures of fibroblasts from SLOS patients did demonstrate abnormalities. Fibroblasts had increased free cholesterol as demonstrated by enhanced filipin staining in a previous study (39). When LDL was incubated with the SLOS fibroblasts, it was degraded poorly. These studies indicated that ACAT, the tissue esterifying enzyme, was less operative in fibroblasts from SLOS patients. The reason for this discrepancy is not clear and needs further investigation.

In summary, sterol esterification occurs both in plasma by LCAT and in tissues by ACAT₁ and ACAT₂. Sterol esters serve as a storage form of sterols, as in the adrenal cortex, and in xanthoma and atheroma. The amount of sterol esterified is affected by the structure of the sterol as shown in the two diseases, sitosterolemia and the Smith-Lemli-Opitz syndrome. Esterification ranged from 26.8 to 59.8 percent in the plant sterols of sitosterolemia and 57.8 to 67.9 percent in the cholesterol precursors, 7 and 8-dehydrocholesterol, found in the SLOS. Fatty acid preference is also clear with oleic acid the preferred fatty acid by the tissue ACAT enzyme and linoleic acid preferred by the plasma LCAT.

Acknowledgments

Supported by NIH grants DK29930, HL64618, HL073981 and RR00334 from The General Clinical Research Centers Program, Oregon Health & Science University Foundation

Acknowledgements to Jennifer Penfield, the staff of the General Clinical Research Center and referring physicians who sent us patients or plasma samples for analysis. Supported by the National Center for Research Resources(PHS 5 MO1 RR000334).

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Figure 1A **Figure 1B** **Fig. 1.**

Fig. 1-a The esterification of plasma sterols from patients with sitosterolemia by LCAT (expressed as the percent of sterol esters to total sterols)

Fig. 1-b The esterification of plasma sterols from patients with SLOS by LCAT (expressed as the percent of sterol esters to total sterols)

Fig. 2.

The esterification of xanthoma sterols from patients with sitosterolemia by ACAT (expressed as the percent of sterol esters to total sterols)

Table 1

The Free and Esterified sterols in the plasma of sitosterolemic and SLOS patients (mean $\pm$ SD).

(mg/dL)				
Patients (n)*	sterols	Free	Ester	Total
Sitosterolemia (12)	Cholesterol	71.1 $\pm$ 28.7	147.5 $\pm$ 47.4	218.6 $\pm$ 71.2
	Cholestanol	1.7 $\pm$ 0.8	2.7 $\pm$ 0.8	4.5 $\pm$ 1.5
	Campesterol	3.6 $\pm$ 1.5	5.5 $\pm$ 2.4	9.1 $\pm$ 3.7
	Campestanol	0.6 $\pm$ 0.2	0.8 $\pm$ 0.5	1.4 $\pm$ 0.6
	Stigmasterol	1.2 $\pm$ 0.6	0.4 $\pm$ 0.2	1.6 $\pm$ 0.8
	Sitosterol	8.0 $\pm$ 3.5	10.8 $\pm$ 5.1	18.0 $\pm$ 8.1
	Sitostanol	2.6 $\pm$ 1.1	3.0 $\pm$ 1.5	5.6 $\pm$ 2.6
SLOS children (4)	Cholesterol	10.5 $\pm$ 5.2	65.2 $\pm$ 14.7	75.8 $\pm$ 18.7
	7-Dehydrocholesterol	4.8 $\pm$ 2.1	7.6 $\pm$ 2.3	12.4 $\pm$ 2.2
	8-Dehydrocholesterol	2.8 $\pm$ 1.9	6.4 $\pm$ 2.0	9.2 $\pm$ 1.6
SLOS stillborn (1)	Cholesterol	1.7	3.9	5.6
	7-Dehydrocholesterol	4.9	3.5	8.4
	8-Dehydrocholesterol	3.0	5.1	8.1

Table 2

The Free and Esterified sterols of xanthomas from sitosterolemic patients (mean $\pm$ SD).

(mg/g dried wt)				
Sterols	No. of subjects	Free	Esters	Total
Cholesterol	6	23.3 $\pm$ 9.0	49.9 $\pm$ 37.7	73.2 $\pm$ 42.9
Cholestanol	3	0.6 $\pm$ 0.3	2.5 $\pm$ 0.4	3.1 $\pm$ 0.7
Campesterol	6	2.2 $\pm$ 1.1	2.2 $\pm$ 1.5	4.4 $\pm$ 1.5
Campestanol	3	0.3 $\pm$ 0.3	0.8 $\pm$ 0.6	1.1 $\pm$ 0.9
Stigmasterol	5	1.1 $\pm$ 0.8	0.2 $\pm$ 0.2	1.3 $\pm$ 0.8
Sitosterol	6	9.5 $\pm$ 5.9	3.1 $\pm$ 1.5	12.5 $\pm$ 5.4
Sitostanol	3	1.5 $\pm$ 1.2	1.4 $\pm$ 1.1	2.8 $\pm$ 2.2

Table 3

The effects of sterol structure on its esterification by LCAT and ACAT.

Sterol compared	Structure difference	LCAT % change in esterification (n)	ACAT % change in esterification
Sterol/stanol*	Saturation at C-5	↓ 5.9 [†] (12)	↑ 29.0 [‡] (3)
Cholesterol/campesterol	Addition of cis CH ₃ at C-24	↓ 11.0 [†] (12)	↓ 25.0 [‡] (6)
Cholesterol/sitosterol	Addition of cis CH ₂ CH ₃ at C-24	↓ 15.0 [†] (12)	↓ 55.0 [‡] (6)
Campesterol/sitosterol	Addition of -CH ₂ at C-24	↓ 4.5 [†] (12)	↓ 40.0 [‡] (6)
Sitosterol/stigmasterol	Double bond at C-22 change cis to trans -CH ₂ -CH ₃ at C- 24	↓ 52.9 [†] (12)	↑ 8.9 (3)
Cholesterol/stigmasterol	Addition of double bond at C-22 and trans CH ₂ -CH ₃ at C-24	↓ 60.0 [†] (12)	↓ 51.0 [‡] (3)
Cholesterol/7dehydrocholesterol	Addition of double bond at C-7	↓ 30.5 [†] (5)	

* sitosterol vs sitostanol (LCAT) and cholesterol vs cholestanol (ACAT)

[†] P<0.004 (paired t test)[‡] P<0.04 (paired t- test)

Table 4

Fatty acid composition of cholesterol esters of plasma and xanthomata of sitosterolemic and hypercholesterolemic patients (mean $\pm$ SD).

Fatty Acids	Sitosterolemia		Hypercholesterolemia [*]	
	Plasma (n=6)	Xanthomata(n=6)	Plasma (n=14)	Xanthomata(n=14)
16:0	11.2 $\pm$ 0.6	9.6 $\pm$ 1.5	12.0 $\pm$ 1.1	7.6 $\pm$ 2.0
18:0	1.1 $\pm$ 0.3	2.4 $\pm$ 1.0	1.4 $\pm$ 0.6	1.4 $\pm$ 1.0
16:1n-7	3.3 $\pm$ 1.1	6.4 $\pm$ 0.6	3.8 $\pm$ 1.1	7.6 $\pm$ 1.9
18:1n-9	17.7 $\pm$ 2.5	45.6 $\pm$ 4.2 ^{**}	20.2 $\pm$ 2.5	47.8 $\pm$ 4.4
18:2n-6	56.7 $\pm$ 5.5	15.5 $\pm$ 1.6 ^{**}	50.0 $\pm$ 5.2	17.4 $\pm$ 3.5
20:3n-6	0.5 $\pm$ 0.4	4.0 $\pm$ 1.4	0.4 $\pm$ 0.5	3.5 $\pm$ 1.7
20:4n-6	5.1 $\pm$ 3.1	4.1 $\pm$ 1.3	8.1 $\pm$ 2.5	4.3 $\pm$ 1.4

* From reference #14

** P<0.001 (paired t test)