High levels of plant sterols and cholesterol precursors in cerebrotendinous xanthomatosis

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Abstract We measured the cholestanol, cholesterol precursor (lathosterol), and plant sterol (campesterol and sitosterol) concentrations of serum and bile in 11 patients with cerebrotendinous xanthomatosis. The mean values of serum cholestanol, lathosterol, campesterol, and sitosterol were, respectively, 8.4-, 2.5-, 2.7-, and 1.4-times higher in the patients than in normal control subjects (n = 26). Cholestanol (6.7-fold) and campesterol (3.7-fold) levels in bile (n = 4) were also elevated in the patients. There was no significant difference of serum sterol levels between patients with coronary artery disease and those without it. Chenodeoxycholic acid treatment for periods ranging from 6 months to 3 years and 4 months lowered serum lathosterol (57.7% reduction) and campesterol (57.8%) levels in parallel with cholestanol (70.8%) level, but the sitosterol level (19.7%) decreased less. Thus, increased levels of cholesterol precursor (lathosterol), plant sterols (campesterol and sitosterol), and cholestanol were found in the serum and bile in cerebrotendinous xanthomatosis. Chenodeoxycholic acid treatment effectively reduced the levels of these sterols, except for sitosterol. —**Kuriyama, M., J. Fujiyama, T. Kasama, and M. Osame.** High levels of plant sterols and cholesterol precursors in cerebrotendinous xanthomatosis. *J. Lipid Res.* 1991. **32:** 223–229.

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The combination of tendon xanthomas and atherosclerosis is common in a few types of familial hyperlipoproteinemia, and especially in type IIa hyperlipoproteinemia or familial hypercholesterolemia (FH), which involves a defect of the receptor-mediated endocytosis of low density lipoprotein (LDL). In addition, this combination frequently occurs in two rare inherited sterol storage diseases, cerebrotendinous xanthomatosis (CTX) and sitosterolemia with xanthomatosis (phytosterolemia) (1). CTX is characterized by the accumulation of cholesterol and cholestanol in various tissues, particularly xanthomas and nervous tissue (1). The underlying biochemical defect is a deficiency of hepatic mitochondrial hydroxylation, which is involved in the biosynthesis of bile acids (2). This disturbance results in reduced production of bile acids, especially chenodeoxycholic acid (CDCA), and in increased formation of intermediates of bile acid biosynthesis, such as cholestanol and several kinds of bile alcohols. Cholesterol biosynthesis is enhanced through the activation of 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase as a consequence of decreased feedback regulation by the lowered bile acid pool (3, 4). The clinical manifestations include premature cataracts and various neurological symptoms, as well as tuberous and tendon xanthomas and premature atherosclerosis (1). Phytosterolemia is another sterol storage disease in which plant sterols such as campesterol and sitosterol accumulate in the plasma, erythrocyte membranes, and xanthomas. Plasma cholesterol levels are normal or slightly elevated. Intestinal absorption of plant sterols in these patients is 5- to 10-times greater than in controls and is associated with reduced clearance of sitosterol and cholesterol from the plasma. These two factors apparently cause the elevation of serum plant sterol levels, but not all the biochemical defects are known and the mechanism underlying the tissue deposition of plant sterols and cholesterol remains to be defined. The major clinical manifestations include tuberous and tendon xanthomas, recurrent arthritis and arthralgia, and marked premature atherosclerosis, but there are no neurological manifestations in this condition (1, 5)

Abbreviations: CTX, cerebrotendinous xanthomatosis; FH, familial hypercholesterolemia; LDL, low density lipoprotein; CDCA, chenodeoxycholic acid; CAD, coronary artery disease; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; HPLC, high performance liquid chromatography; GLC-MS, gas-liquid chromatography-mass spectrometry. The systemic names of sterols referred to by their trivial names are: cholesterol, cholest-5-en-3β-ol; sitosterol, 24-ethylcholest-5-en-3β-ol; campesterol, 24-methylcholest-7-en-3β-ol; cholestanol, 5α-cholestan-3β-ol; lathosterol, 5α-cholest-7-en-3β-ol.

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Thus, a combination of tendon xanthomas and premature atherosclerosis commonly occurs in CTX, phytosterolemia, and FH. In another study we found that coronary atherosclerosis was more frequent in CTX patients than has been reported previously (6). An increased level of serum cholestanol is essential for the diagnosis of CTX, but it is not specific to CTX. Increased cholestanol levels have also been reported in several other diseases, including phytosterolemia, FH, primary biliary cirrhosis and hypothyroidism (7-9). In addition, Wang et al. (10) have reported the unique case of a patient with both CTX and sitosterolemia, who showed defective CDCA synthesis and a marked increase of serum plant sterols such as sitosterol and campesterol. Furthermore, some patients with FH and clinical features mimicking those of CTX have been reported (11, 12). These findings suggest that a similar or common pathological mechanism may exist in these three diseases—CTX, phytosterolemia, and FH. In this study, we investigated the serum levels of plant sterols and cholesterol precursor in patients with CTX in an attempt to shed more light on the pathogenesis of this disease.

Recently, Berginer, Salen, and Shefer (13) reported that long-term treatment with CDCA inhibited abnormal bile acid synthesis, reduced plasma cholestanol concentrations, and appeared to prevent disease progression in patients with CTX. Accordingly, we also investigated the effects of CDCA on serum cholestanol, cholesterol precursor, and plant sterol levels in this study.

MATERIALS AND METHODS

Patients

Eleven patients with CTX (8 males and 3 females from seven families) were studied (**Table 1**). Their average age was 38.5 ± 5.7 years, with a range of 31 to 50 years. The diagnosis of CTX was established by the presence of characteristic clinical features together with high serum cholestanol levels. The clinical features and laboratory findings of 8 patients will be described in detail in separate reports (6). Four patients had ischemic heart disease (Cases 1, 5, 6, 7) and showed significant coronary arterial stenosis and/or occlusion on coronary angiography. One exceptional patient (Case 1) had no involvement of the Achilles tendon nor any xanthomatous lesions on the joints or skin, despite having ischemic heart disease and other clinical manifestations and high serum cholestanol and bile alcohol levels that were compatible with CTX. The control group was composed of 27 subjects (15 males and 12 females) who had no hypercholesterolemia and no evidence of atherosclerotic disease. Their average age was 39.3 ± 17.9 years, with a range from 10 to 74 years.

Methods

Venous blood was collected from the 11 CTX patients and 27 normal controls in the fasting state. All subjects continued their usual diets. In CTX patients (Cases 1, 3,

TABLE 1. Comparison of serum sterol concentrations in patients with cerebrotendinous xanthomatosis and control subjects

Patients	Age	Sex	ChE	ChA	Latho	Campe	β-Sito	% of Cholesterol			
								ChA	Latho	Campe	β-Sito
			mg/ml	*μg/ml*	*μg/ml*	*μg/ml*	*μg/ml*				
Case 1 Y. K.	39	M	1.39	29.36	16.85	11.42	9.60	1.52	0.87	0.59	0.50
Case 2 I. S.	37	F	1.59	21.02	9.51	8.14	8.11	1.32	0.60	0.51	0.51
Case 3 H. M.	36	M	1.49	28.14	17.34	12.17	9.15	1.89	1.16	0.82	0.61
Case 4 N. Y.	31	F	1.40	27.44	10.64	10.20	9.75	1.96	0.76	0.73	0.70
Case 5 S. S.	48	M	1.66	17.04	9.91	5.75	6.45	1.03	0.60	0.35	0.39
Case 6 K. S.	50	M	1.41	45.06	14.03	10.81	10.49	3.20	1.00	0.77	0.74
Case 7 Y. S.	37	M	1.98	19.95	7.21	7.29	8.66	1.01	0.36	0.37	0.44
Case 8 K. O.	35	M	1.29	42.10	12.69	26.14	8.15	3.26	0.98	2.02	0.63
Case 9 M. N.	37	M	1.34	24.25	5.33	7.51	7.06	1.81	0.40	0.56	0.53
Case 10 A. T.	40	M	1.47	26.55	12.21	7.26	6.39	1.81	0.83	0.49	0.43
Case 11 H. T.	34	F	1.77	31.75	14.71	12.76	14.63	1.80	0.83	0.72	0.83
Mean ± SD	38.5 ± 5.7		1.58 ± 0.32	28.42 ± 8.68	11.86 ± 3.79	10.86 ± 5.57	8.95 ± 2.31	1.87 ± 0.75	0.76 ± 0.25	0.72 ± 0.46	0.57 ± 0.14
Controls (n = 27)	39.33 ± 17.9		1.66 ± 0.32	3.37 ± 1.55	4.81 ± 1.83	3.99 ± 2.18	6.29 ± 1.71	0.20 ± 0.07	0.30 ± 0.11	0.24 ± 0.12	0.41 ± 0.09
P	NS		<0.001	<0.001	<0.005	<0.001	0.001	<0.001	<0.01	<0.001	

No difference at all sterol levels was noted between males (n = 15) and females (n = 12) in control subjects. The results were analyzed statistically by means of Student's *t*-test or Wilcoxon's test; NS, not significant. Abbreviations are the same as those in Fig. 1.

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5, 6, 7, 8), blood collection was also performed after CDCA treatment (daily administration of 300 mg of CDCA) for periods ranging from 6 months to 40 months. Samples of bile were collected from 4 CTX patients before treatment (Cases 1, 3, 6, 7), and from 6 normal controls. Tendon xanthomas were biopsied in one patient with CTX (Case 6) and one patient with FH.

Cholesterol, cholestanol, lathosterol, campesterol, sitosterol, and 5β-cholestan-3α-ol, which were used as standards, were obtained from Sigma Chemical Co., St. Louis. All other chemicals were from Nacarai-Tesque, Inc., Japan. All organic solvents used in the experiments were redistilled.

The total lipid content was extracted from the xanthoma biopsies with 20 volumes of chloroform–methanol 2:1 (by volume) (14). Sterol concentrations were measured by high-performance liquid chromatography (HPLC) according to the method of Kasama, Byun, and Seyama (15). After the addition of 5β-cholestan-3α-ol as the internal standard, serum (100 μl), bile (100 μl), or total lipid solution (100–200 μl) were saponified in 1 N ethanolic potassium hydroxide for 1 h at 80°C. The unsaponified material was extracted with hexane. The extracted sterols were then converted into their benzoyl derivatives by addition of 0.3 ml benzoyl chloride reagent (benzoyl chloride–pyridine–1,2-dichloroethane 0.3:1.0:10 (by volume)). After the addition of 2 ml of 1,2-dichloroethane, the reaction mixture was washed once with 2 ml of 0.1 M hydrochloric acid and twice with 2 ml of water. The organic layer was evaporated under nitrogen, and then the residue was dissolved in 0.5 ml of acetronitrile–1,2-dichloroethane 2:1 (by volume). The liquid chromatography apparatus used for sterol assays was an LKB 2125 system (LKB- Produkter AB, Sweden) equipped with a column oven (LKB 2155) and a chromatogram data processor (SIC Chromatocorder 12, System Instrument Co., LTD, Japan). The column used was an ODS-H (15 cm × 4.6 mm, Shimadzu, Japan) and the column-oven temperature was maintained at 50°C. The solvent was acetonitrile–water–acetic acid 100:3:0.2 (by volume), and the flow-rate was 1.0 ml/min. Steryl benzoates were detected by their absorbance at 228 nm, and were automatically identified and quantitated by the Chromatocorder. A typical chromatogram that illustrates the separation of the sterols achieved under the above conditions is shown in **Fig. 1**. Cholesterol, cholestanol, lathosterol, campesterol, and sitosterol, as well as the 5β-cholestan-3α-ol added as an internal standard, were all clearly separated (Fig. 1). Plasma sterols after trimethylsilyl derivatization were also analyzed qualitatively by GLC–MS (TSQ-70, Finmigan MAT) using a capillary column DB-5 (30 m × 0.25 mm, J & W, Folson) (16).

RESULTS

Sterol levels in serum, bile, and xanthoma biopsies

The serum cholestanol level was markedly elevated in all CTX patients. The mean value was 8.4 times higher than that in normal control subjects. Among the remaining serum sterols, the levels of lathosterol (2.5-fold), campesterol (2.7-fold), and sitosterol (1.4-fold) were also found to be increased in the CTX patients in comparison with the control group (Table 1). Similar changes of sterol concentrations were also found in the bile samples from CTX patients (**Table 2**).

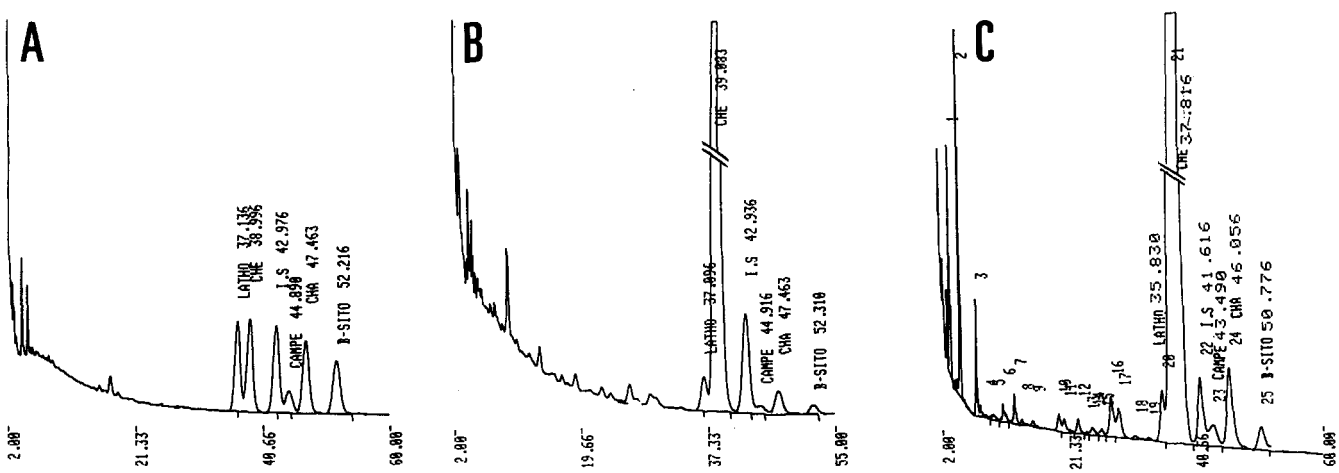

Fig. 1. High performance liquid chromatography of standard sterols (A) and serum from a control subject (B) and from a patient with cerebrotendinous xanthomatosis (C). (ChE) cholesterol, (ChA) cholestanol, (Latho) lathosterol, (Campe) campesterol, and (Sito) sitosterol were clearly separated.

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TABLE 2. Comparison of bile sterol concentrations in patients with cerebrotendinous xanthomatosis and control subjects

	% of Cholesterol			
	ChA	Latho	Campe	β-Sito
CTX (n = 4)	7.94 ± 3.89	4.78 ± 2.49	4.11 ± 2.03	1.45 ± 0.63
Controls (n = 6)	1.19 ± 0.54	2.13 ± 1.91	1.11 ± 0.25	1.13 ± 0.26
P	<0.01	NS	<0.05	NS

The results were analyzed by means of Wilcoxon's test; NS, not significant. Abbreviations are the same as those in Fig. 1.

The relative sterol concentrations were determined in CTX and FH xanthomas. Cholestanol accumulation was 8.8 times greater and campesterol levels were also slightly increased in the CTX xanthoma, while the lathosterol level was 6.5 times higher in the FH xanthoma (**Table 3**).

Serum sterol levels in the CTX patients with coronary artery disease (CAD)

Serum sterol levels were compared among three groups of CTX patients. CTX I patients had xanthomas and CAD (n = 3, Cases 5, 6, and 7), while CTX II patients had xanthomas but no CAD (n = 7, Cases 2, 3, 4, 8, 9, 10, and 11). Group CTX III was just one patient with CAD but no xanthoma (Case 1). There were no significant differences for any of the sterol levels among these three groups (**Table 4**).

Effect of CDCA treatment

The effect of CDCA was evaluated on serum sterol concentrations in 6 CTX patients (**Table 5**). The cholestanol level was markedly reduced by CDCA treatment, as previously reported (13). Lathosterol, campesterol, and sitosterol levels also improved. Interestingly, the levels of lathosterol and campesterol were reduced in parallel with the cholestanol level, while the change in the sitosterol level was only slight.

DISCUSSION

Plant sterols such as campesterol and sitosterol are a group of noncholesterol sterols that can be detected in the blood and tissues of humans. These sterols are not synthesized in human tissues, but are derived exclusively from dietary sources (17). Normally they are poorly absorbed from the intestine (e.g., sitosterol, 5% or less), and thus only low levels are detected in serum (17, 18). Serum plant sterol concentrations have been shown to be positively related to cholesterol absorption in healthy subjects (19) or in patients with hyperlipidemia (20, 21). On the other hand, lathosterol is a cholesterol precursor in the cholesterol biosynthesis pathway of humans. The serum concentrations of cholesterol precursor sterols show a correlation with overall cholesterol synthesis under both experimental and clinical conditions (22, 23). In particular, the serum lathosterol concentration and the lathosterol/cholesterol ratio are good indicators of whole-body cholesterol synthesis in humans (24). Cholesterol absorption and synthesis are inversely interrelated, i.e., the serum plant sterol and cholesterol precursor levels show a significant inverse correlation with each other (19, 21, 25).

The serum cholestanol concentration was found to be markedly increased in all our CTX patients. In addition, the serum levels of lathosterol, campesterol, and sitosterol were also high in all the CTX patients. We found that plant sterols were also increased in the bile of CTX patients. Phytosterolemia is characterized by the marked accumulation of plant sterols in serum (% of total sterols or cholesterol; 15.5–37.7%) (5, 8, 26, 27), RBC membranes (12.1–13.6%) (5), bile (9.6–30.7%) (26, 27), and xanthomas (12.3–17.5%) (5). The serum cholestanol level (% of total sterols; 1.3%) is also increased about 10-fold in phytosterolemia patients, in comparison with normal control subjects (7). However, although our CTX patients showed increased plant sterol levels, the accumulation was not so marked in the serum (the ratio of the total amount of campesterol and sitosterol/cholesterol was 1.3%) and bile (5.6%) or xanthomas (0.9%).

TABLE 3. Compositions of sterols in biopsied xanthomas from a patient with familial hypercholesterolemia (FH) and a patient with cerebrotendinous xanthomatosis (CTX)

Patient	ChE	ChA	Latho	Campe	β-Sito
		μg/mg of wet weight			
FH xanthoma	12.23	0.065	0.071	0.025	0.065
CTX xanthoma	9.01	0.419	0.011	0.034	0.046
		% of Cholesterol			
FH xanthoma		0.529	0.580	0.204	0.535
CTX xanthoma		4.650	0.121	0.377	0.513

Abbreviations are the same as those in Fig. 1.

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TABLE 4. Serum sterol concentrations in patients with cerebrotendinous xanthomatosis associated with cardiovascular disease

Patients	n	Age	ChE	ChA	Latho	Campe	β-Sito
		yr	*mg/dl*	*μg/ml*	*μg/ml*	*μg/ml*	*μg/ml*
CTX I	3	27.4 ± 15.4	168 ± 29	27.35 ± 15.40	10.38 ± 3.43	7.95 ± 2.59	8.53 ± 2.02
CTX II	7	35.7 ± 2.8	147 ± 16	28.75 ± 6.76	11.78 ± 3.84	12.03 ± 6.60	9.03 ± 2.72
CTX III	1	39	193	29.36	16.85	11.42	9.60
				% of Cholesterol			
CTX I	3			1.74 ± 1.26	0.65 ± 0.32	0.50 ± 0.24	0.52 ± 0.19
CTX II	7			1.98 ± 0.60	0.79 ± 0.25	0.84 ± 0.54	0.61 ± 0.13
CTX III	1			1.52	0.87	0.59	0.50

CTX I: patients with xanthomas and coronary artery disease (CAD): CTX II: patients with xanthomas, but without CAD; CTX III: patients with CAD, but without xanthomas. Abbreviations are the same as those in Fig. 1.

CDCA treatment lowered serum levels of lathosterol (57.7% reduction) and campesterol (57.8%) in parallel with the level of cholestanol (70.8%), but the level of sitosterol (19.7%) was not reduced as much. The principal route of excretion of absorbed plant sterols in humans is via the bile. Salen, Ahrens, and Grundy (17) found that only about 20% of absorbed dietary sitosterol was converted to CDCA and cholic acid before excretion, and the remainder was excreted as unconjugated sterol in the bile. The detailed metabolic pathways of other plant sterols, including campesterol, remain unclear at present. In CTX, the synthesis of bile acids is impaired (1), and this may enhance the accumulation of plant sterols. However, the possibility that the elevation of serum plant sterol levels is secondary to increased absorption cannot be excluded. The discrepancy noted between the reduction in campesterol and sitosterol levels by CDCA treatment suggests differences in the metabolism of these plant sterols, such as a different rate of conversion to CDCA and cholic acid or even a different metabolic pathway.

In CTX, cholesterol biosynthesis is enhanced mainly through the activation of HMG-CoA reductase as a consequence of decreased feedback regulation by the lowered bile acid pool (3). Nicolau et al. (4) have reported that the

TABLE 5. Effects of chenodeoxycholic acid (CDCA) treatment on serum sterol concentrations in patients with cerebrotendinous xanthomatosis

Patients	Treatment (Duration in Months)	ChA	Latho	Campe	β-Sito
			μg/ml		
Case 1	None	29.36	16.85	11.42	9.60
	CDCA (11)	11.16	9.58	2.67	4.70
% Reduction		62.0	43.2	76.6	51.0
Case 3	None	28.14	17.34	12.17	9.15
	CDCA (13)	9.88	7.80	6.83	8.73
% Reduction		64.9	55.0	43.9	4.6
Case 5	None	17.04	9.91	5.75	6.45
	CDCA (40)	3.00	3.10	2.12	5.07
% Reduction		82.3	68.7	63.1	21.4
Case 6	None	45.06	14.03	10.81	10.49
	CDCA (14)	11.99	6.53	6.45	9.36
% Reduction		73.4	53.5	40.3	10.8
Case 7	None	19.95	7.21	7.29	8.66
	CDCA (10)	7.69	3.25	3.57	6.04
% Reduction		61.5	54.9	51.0	30.3
Case 8	None	42.10	12.69	26.14	8.15
	CDCA (6)	8.18	3.65	7.28	8.19
% Reduction		80.6	71.2	72.1	+ 0.5
% Reduction (Mean ± SD)		70.8 ± 9.3	57.7 ± 10.5	57.8 ± 15.0	19.7 ± 18.9

Abbreviations are the same as those in Fig. 1.

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HMG-CoA reductase activity determined in liver biopsy specimen from CTX patients was 4-fold higher than that in normal control subjects. We found that the serum lathosterol level was 2.7-times greater in CTX patients than in controls, indicating that overall cholesterol biosynthesis was increased in CTX, as would be expected. In general, serum levels of plant sterols and cholesterol precursors are inversely related to each other (17, 19, 23). The elevation of both serum plant sterol and cholesterol precursor levels is unique to CTX.

The mechanism of atherosclerosis in CTX has not yet been clarified. In this study, we compared serum sterol levels between the CTX patients with and without CAD, but no significant difference of any of the serum sterols was found. In a separate paper, we have reported that no "clustering" of known atherogenic risk factors occurred in CTX patients, and that the serum of CTX patients was actually "anti-atherogenic." These findings were based on the analysis of serum lipids, lipoproteins, apolipoproteins, and indices of atherosclerosis (J. Fujiyama, M. Kuriyama, S. Takenaga, et al., unpublished data). Almost all patients with CTX have been found to have normal or relatively low serum cholesterol levels (1), despite the increase in cholesterol biosynthesis. Recently, Ballantyne et al. (28) and Tint et al. (29) reported that both the rate of production and the fractional catabolic rate of LDL and VLDL-apolipoprotein B were markedly increased in patients with CTX. The mechanism of the accumulation of cholesterol and cholestanol in the arterial wall and nervous tissue in CTX remains unclear, but the disturbance of VLDL/LDL lipoprotein metabolism may explain at least part of the atherogenesis occurring in CTX.

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