Lethal atherosclerosis associated with abnormal plasma and tissue sterol composition in sitosterolemia with xanthomatosis


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Abstract Tissue sterol composition was determined in an 18-year-old male with sitosterolemia with xanthomatosis who died suddenly and whose coronary and aortic vessels showed extensive atherosclerosis and, for comparison, in an 18-year-old male with minimal atherosclerosis who died accidently. Sterols in the control tissues (plasma, erythrocytes, cardiac muscle, lung, liver, aorta, and brain) contained cholesterol with only trace amounts of cholestanol. In contrast, sterols in corresponding tissues of the sitosterolemic subject (except brain) were composed of cholesterol, increased amounts of plant sterols, campesterol and sitosterol, and 5α-saturated stanols, cholestanol, 5α-campestanol, and 5α-sitostanol, that were deposited in approximately the same ratio as present in plasma. However, sitosterolemic brain sterol composition resembled that of the control brain with cholesterol and only trace amounts (<1%) of cholestanol and phytosterols. The sitosterolemic aorta was extensively atherosclerotic and contained more than twice the quantity of sterols as the control aorta (5.6 mg/g versus 2.6 mg/g) with increased amounts of cholesterol, plant sterols, and 5α-saturated stanols. These results indicate that cholesterol, plant sterols, and 5α-stanols are deposited prematurely and are associated with accelerated atherosclerosis in subjects with sitosterolemia with xanthomatosis. — Salen, G., I. Horak, M. Rothkopf, J. L. Cohen, J. Speck, G. S. Tint, V. Shore, B. Dayal, T. Chen, and S. Shefer. Lethal atherosclerosis associated with abnormal plasma and tissue sterol composition in sitosterolemia with xanthomatosis. J. Lipid Res. 1985. 26: 1126-1133.

Supplementary key words cholesterol • 5α-sitosterol • sitosterolemia • atherosclerosis

The association of high plasma cholesterol concentrations and atherosclerosis is best documented in the inherited lipid disorder familial hypercholesterolemia, where absent (homozygous) or reduced (heterozygous) functional tissue low density lipoprotein receptors result in extraordinarily high plasma low density lipoprotein cholesterol levels (1). As a consequence, these individuals show extensive tendon and tuberous xanthomatosis and develop atherosclerosis rapidly and often die at young ages because of myocardial infarctions.

Recently, premature atherosclerosis and extensive xanthomatosis have been described in two rare lipid storage diseases, cerebrotendinous xanthomatosis (CTX) and sitosterolemia with xanthomatosis (2). In cerebrotendinous xanthomatosis, severe neurologic dysfunction, cataracts, pulmonary abnormalities, and endocrine hypofunction are found, while in sitosterolemia with xanthomatosis, arthritis and episodic erythrocyte hemolysis are noted occasionally. However, both diseases are characterized chemically by increased plasma and tissue concentrations of sterols other than cholesterol. In cerebrotendinous xanthomatosis, cholestanol, the 5α-dihydro derivative of cholesterol, is present in all tissues with particularly high amounts in brain, nerve, xanthomas, and bile. In distinction, large quantities of phytosterols (campesterol and sitosterol) along with 5α-saturated stanols, cholestanol, 5α-campestanol, and 5α-sitostanol accumulate in plasma of subjects with sitosterolemia with xanthomatosis. Although the mechanism for the enhanced sterol accumulation is not known, abnormal lipoprotein sterol transport has been suggested to play a role (3, 4).

In this report tissue sterol measurements were correlated with the degree of atherosclerosis in an 18-year-old male with sitosterolemia with xanthomatosis who died suddenly of an acute myocardial infarction. For comparison, sterol composition was determined in similar tissues of an 18-year-old male who died accidently. The findings indicate that atherosclerosis occurs prematurely in sitosterolemia with xanthomatosis and probably results from

Abbreviations: CTX, cerebrotendinous xanthomatosis; GLC, gas-liquid chromatography.

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accelerated plasma sterol deposition. The presence of substantial amounts of abnormal sterols in plasma may promote their uptake by vascular endothelial cells.

METHODS

Clinical

The subject was an 18-year-old white male who died suddenly of an acute myocardial infarction. For the past 8 years, he had noted enlarging tuberous and tendon xanthomas that began on the left elbow and back and then subsequently in the Achilles tendons, knees, and wrists. Other than recurrent arthralgias of the ankles and knees and tenderness at the xanthoma sites, the subject was asymptomatic. He denied chest pain on exertion and participated in school athletics without difficulty.

Measurements of plasma sterols by gas-liquid chromatography 4 years prior to death revealed high concentrations of plant sterols with mild hypercholesterolemia, and a diagnosis of sitosterolemia with xanthomatosis was established. An exercise electrocardiogram and cardiac scintiscan at that time showed no ischemic changes. Three female siblings, ages 24, 22, and 19 years, were also found to have elevated plasma phytosterol levels and one also developed tendon and tuberous xanthomas, establishing the diagnosis of sitosterolemia with xanthomatosis. The mother and father were not related and did not exhibit xanthomas or elevated plasma phytosterol concentrations. However, heart disease was prevalent in maternal relatives, and all males in the family had suffered from symptomatic coronary artery disease; one uncle died at age 44 of a myocardial infarction.

A physical examination performed 2 months before death revealed resting systolic hypertension (180/80), a grade II/VI early systolic ejection murmur heard at the pulmonic area, and tendon and tuberous xanthomas in the wrists, knees, ankles, and skin of elbows.

Because of the concern for possible subclinical cardiac disease, the patient underwent further cardiac evaluation including echocardiography, systolic time interval, and exercise electrocardiography. The exercise testing was augmented with thallium 201 scintigraphy. The cardiac scintiscans were obtained at maximal stress and following a 3-hr recovery period. The exercise electrocardiogram was abnormal and disclosed 2 mm horizontal ST segment depression 14 min into the test at a heart rate of 199 beats/min. This finding persisted after 3 min of rest but returned to normal after 10 min. The cardiac scintiscan taken during the period of maximal ST depression revealed markedly diminished perfusion to the apico-anterior and inferior left ventricular wall segments. This defect reversed to normal after redistribution.

Since the exercise electrocardiogram and cardiac scinti-
scan were abnormal, plans were undertaken to admit the subject for coronary angiography. However, before these studies could be performed, the subject collapsed while participating in a basketball game. Despite resuscitation efforts, the subject died.

A postmortem examination performed 20 hr after death revealed extensive coronary artery atherosclerosis with about 60% occlusion of the proximal left main and proximal right coronary arteries (Fig. 1). Diffuse atherosclerotic lesions were present also in the right and left anterior descending and circumflex arteries as well as the thoracic and abdominal aortas (Fig. 2 and Fig. 3) and iliac vessels. Many areas of myocardial fibrosis suggestive of previous infarction (Fig. 4) were noted. Death was attributed to cardiac arrhythmia and myocardial infarction secondary to severe atherosclerotic occlusive disease of the coronary arteries, although histologic evidence of acute infarction was absent. Brain, cardiac muscle, liver, lung, xanthomas, and atherosclerotic aorta were removed for chemical examination.

Fig. 1. Photomicrograph of anterior descending branch of left coronary artery showing almost 60% occlusion of the lumen by sub-intimal fibrosis. (H & E, × 40)
flash heater 250°C, N₂ gas flow 30 cc/min. The retention times (RRT) relative to 5α-cholestanol for 10 consecutive determinations were: cholestanol 6.3 ± 0.1, cholesterol 7.1 ± 0.1, 5α-campestanol 8.3 ± 0.1, campesterol 9.2 ± 0.1, 5α-sitostanol 10.2 ± 0.1, and sitosterol 11.3 ± 0.2. The identity of the sterols was checked by co-chromatography with authentic reference sterols that were prepared according to Dayal et al. (6). The values were expressed as mg sterol per dl of plasma or gram of tissue.

RESULTS

In Table 1 are listed plasma and tissue sterol compositions from the sitosteroicemic and control subjects. The tissues include plasma, erythrocytes, liver, cardiac muscle, lung, aorta, and xanthoma. Only cholesterol (99.8%) and trace amounts of cholestanol (0.2%) were present in the control plasma and tissues; neither plant sterols nor

For comparison, specimens of brain, cardiac muscle, liver, lung, thoracic aortic, and plasma were removed at postmortem from an 18-year-old male who died accidentally. Microscopically, the tissues were judged to be normal and only minimal atherosclerosis was seen in the coronary vessels and aorta.

Chemicals

Plasma and tissue sterol concentrations were measured by gas—liquid chromatography (GLC) according to the method of Ishikawa et al. (5). Briefly, plasma (1.0 ml) or tissue (1.0 g) was saponified in 1 N NaOH for 1 hr. After extraction of neutral sterols with hexane, aliquots that contained 100 μg of sterols were dissolved in 200 μl of hexane that contained 140 μg of 5α-cholestanol as an internal standard; 3 μl was analyzed by GLC. The underivatized free sterols were separated on 180 cm × 4 mm glass column packed with 1% SP-1000 on 80/100 mesh Gas Chrom Q (Supelco Inc., Bellefonte, PA). Column conditions: temperature 230°C, flame detector 260°C,
their 5α-saturated derivatives were detected. In contrast, large amounts of phytosterols, campesterol (6%) and sitosterol (11%), and 5α-saturated stanols, cholestanol (1.4%), 5α-campestanol (0.6%), and 5α-sitostanol (1.1%), were deposited along with cholesterol (80%) in the sitosterolemic plasma and tissues. The abnormal sterols were present in approximately the same proportion as found in plasma. Although the total sterol concentration was higher in sitosterolemic plasma, the liver, lung, and heart muscle from both subjects contained about the same amount of sterols. However, aorta from the sitosterolemic subject, which was extensively atherosclerotic (Figs. 2 and 3), had more than twice the total sterol concentration as control with increased amounts of cholesterol, phytosterols, and 5α-stanols. The ratio of unsaturated to saturated sterols was more than 9 times greater in the control tissues than in sitosterolemic tissues, which reflected the net increase of 5α-stanols in sitosterolemic tissues.

In Table 2 the brain sterol composition for the sitosterolemic individual is compared with the brain sterol composition from a subject with cerebrotendinous xanthomatosis (CTX). Data for CTX brain is included for comparison since it has been established that cholestanol preferentially accumulates in brain tissue in this disease (2). As expected, cholestanol accounted for 18.2% of the total sterols in the CTX brain, with the remainder cholesterol. In distinction, the control brain contained only cholesterol with trace amounts of cholestanol (0.3%). However, despite the high amounts of phytosterols and 5α-saturated stanols in plasma and other tissues, brain sterols from the sitosterolemic subject were composed almost exclusively of cholesterol with only trace amounts

![Fig. 4. Photomicrograph of trabecular muscle of the left myocardium, showing foci of replacement fibrosis. (H & E, × 40)](image)

<table>
<thead>
<tr>
<th>Table 1. Plasma and tissue sterol composition</th>
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<tbody>
<tr>
<td>Tissue (mg/dl)</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>Plasma (mg/dl)</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>RBC (mg/dl)</td>
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<tr>
<td>Control</td>
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<tr>
<td>Liver (mg/g)</td>
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<tr>
<td>Control</td>
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<tr>
<td>Lung (mg/g)</td>
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<tr>
<td>Control</td>
</tr>
<tr>
<td>Heart muscle (mg/g)</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>Thoracic aorta (mg/g)</td>
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<tr>
<td>Control</td>
</tr>
<tr>
<td>Xanthoma (mg/g)</td>
</tr>
<tr>
<td>Mean ± SD</td>
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<tr>
<td>Control (n = 6)</td>
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</table>

*Plasma and erythrocytes were obtained from a healthy 18-year-old male volunteer.

*Not detected.

*Sterol composition was determined in a section of the atherosclerotic coronary artery from the sitosterolemic subject and the percentage composition of sterols and 5α-stanols was almost identical to that found in atherosclerotic aorta.

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of cholestanol and unsaturated plant sterols. No 5α-saturated plant sterols were detected. Thus the sterol composition of the sitosterolemic brain differed significantly from that of plasma and other tissues.

Table 3 lists the proportion of free and esterified sterols in the sitosterolemic and control tissues. Aorta and xanthoma sterols from the sitosterolemic subject contained a greater proportion of esterified cholesterol and cholestanol than liver, lung, or cardiac muscle. Plant sterols and 5α-saturated plant stanol derivatives were less esterified than cholesterol in these tissues. Of interest, sterols from the aorta of the control subject were also esterified to a greater extent than in other tissues.

**DISCUSSION**

The results of this investigation, in which the diagnosis of sitosterolemia with xanthomatosis was established ante-mortem and in which detailed tissue biochemical and pathologic examinations were made at autopsy, emphasize the lethal nature and risk of cardiovascular complications in this disease. Four years earlier the stress electrocardiogram and cardiac scintiscan were normal; 2 weeks before death, however, both tests had become abnormal and showed myocardial ischemia. Moreover, examination of the coronary and aortic vessels and myocardium at postmortem revealed extensive arteriosclerosis and microscopic infarctions. Thus, degenerative lesions developed progressively and prematurely in the coronary and aortic vessels (Figs. 1–3) resulting in coronary artery obstruction, myocardial infarction (Fig. 4), and death. Of the twenty known affected subjects (five males and fifteen females), three males (ages 10, 42, and 18 years) and one female (age 39 years) have died as a consequence of coronary atherosclerosis (2–4, 7) and one female and one male who are alive exhibit symptomatic vascular disease (8, 9) (Table 4). Therefore, males seem predisposed to develop significant vascular complications at an early age and are at great risk. Skin and tendons also accumulate sterols with the development of tendon and tuberous xanthomas and all symptomatic subjects (Table 4) have manifested xanthomas. The mechanism of sterol deposition is unclear but apparently involves the uptake of circulating sterols from low density lipoproteins since tissue sterols resemble plasma (Table 1) and low density lipoprotein sterol composition (10, 11). Thus, besides cholesterol, large amounts of phytosterols (campesterol and sitosterol) and 5α-stanols (cholestanol, 5α-campestanol, 5α-sitostanol) are deposited in all tissues except brain in approximately the same proportion as found in plasma. However, it was noteworthy that, despite the greater number of sterols in liver, lung, and cardiac muscle from the sitosterolemic patient, the total quantity of sterols was not different from that found in these control tissues (Table 1). This finding may indicate a limit for sterol accumulation in these tissues, whereas sitosterolemic aorta, tendons, and skin contained greatly increased amounts of sterols.
### Table 4. Sitosterolemic subjects with symptomatic atherosclerosis

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Condition</th>
<th>Clinical Findings</th>
<th>Biochemical</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. R.C. (present case)</td>
<td>18 M</td>
<td>Deceased</td>
<td>Coronary arteries and aorta contained extensive atherosclerosis at postmortem. Tendon and tuberous xanthomas were present.</td>
<td>Increased plasma phytosterols and 5α-saturated stanols noted before death</td>
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<tr>
<td>2. C.L. (7)</td>
<td>42 M</td>
<td>Deceased</td>
<td>Extensive coronary atherosclerosis noted by coronary angiography. Tendon and tuberous xanthomas were present.</td>
<td>Increased plasma phytosterols and 5α-stanols noted before death.</td>
<td></td>
</tr>
<tr>
<td>3. Z. (4)</td>
<td>13 M</td>
<td>Deceased</td>
<td>Died suddenly. Neither the cause of death nor vascular condition established. Tendon and tuberous xanthomas were present.</td>
<td>No biochemical data but four siblings with similar clinical findings have elevated plasma phytosterol and 5α-stanol levels.</td>
<td></td>
</tr>
<tr>
<td>4. L.B. (4)</td>
<td>39 F</td>
<td>Deceased</td>
<td>Sister of Case 3, died of myocardial infarction. Tendon and tuberous xanthomas were present.</td>
<td>Increased plasma phytosterols and 5α-stanols were measured before death.</td>
<td></td>
</tr>
<tr>
<td>5. H.R. (9)</td>
<td>29 M</td>
<td>Alive</td>
<td>Extensive coronary atherosclerosis determined by coronary angiography associated with angina pectoris. Triple coronary artery bypass performed. Tendon and tuberous xanthomas were present.</td>
<td>Increased plasma phytosterol levels.</td>
<td></td>
</tr>
<tr>
<td>6. P.M. (8)</td>
<td>45 F</td>
<td>Alive</td>
<td>Vascular bruit over abdominal aorta; intermittent claudication. Tuberous and tendon xanthomas were present.</td>
<td>Increased plasma phytosterol and 5α-stanol levels.</td>
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</tr>
</tbody>
</table>

Thus certain tissues (aorta, skin, tendon) have a predilection for enhanced uptake and accumulation of plasma sterols, resulting in atherosclerosis and xanthoma formation.

Major biochemical abnormalities in this disease are the increased plasma and tissue levels of phytosterols and 5α-stanols. In their original report, Bhattacharyya and Connor (10) found increased amounts of unsaturated plant sterols (campesterol, stigmasterol, and sitosterol) in the plasma, erythrocytes, lipoproteins, skin, and adipose tissue (10). Similar cholesterol and phytosterol composition was found in plasma and lipoproteins by Shulman et al. (8) and Kwiterovich et al. (4), while Miettinen (9) and Whittington et al. (12) found an additional plant sterol, avenosterol (24-ethylidene cholesterol), present in plasma and lipoproteins of their patients. The origin of the tissue and plasma plant sterols was in the diet since there is convincing evidence that plant sterols are not produced by mammalian tissues (13). Further, Bhattacharyya and Connor (10) and Miettinen (9) showed enhanced intestinal absorption of sitosterol in their patients. In other studies, Lin and colleagues (14) measured sitosterol turnover by the isotope kinetic method after an intravenous injection of a tracer dose of [3H]sitosterol and found not only greater sitosterol production rates (absorption) but decreased turnover as compared to data from a control study. Thus two mechanisms play a role in the pathogenesis of this disease: increased intestinal absorption of plant sterols, and probably cholesterol, combined with decreased removal. In other words, the intestinal mucosa and liver of these subjects presumably have lost the capacity to discriminate between cholesterol and plant sterols with the effect that increased amounts of sterols (cholesterol and phytosterols) are absorbed and transported in the plasma and deposited in all tissues except brain. The fact that plasma campesterol and sitosterol levels were similar to tissue levels of these sterols is further evidence that these sterols were deposited rather than synthesized locally. Also significant, however, is the failure of liver to preferentially excrete the plant sterols into the bile (13), which also contributes to their retention.

Of importance was the demonstration that increased amounts of 5α-saturated stanols, cholestanol, 5α-campes-tanol, and 5α-sitostanol were also present in plasma and were deposited in the same proportion in all tissues but brain. These 5α-stanols are produced endogenously from their respective Δ5-unsaturated precursors, cholesterol, campesterol, and sitosterol, because 5α-saturated stanols were not found in the diet (11). Further, by using improved gas–liquid chromatographic techniques, increased amounts of these 5α-stanols have been detected in the plasma and lipoproteins of seventeen other subjects with sitosterolemia with xanthomatosis (11). Thus, the increased formation of these 5α-stanols constitutes an additional significant biochemical abnormality in this disease.

The pathway for the conversion of cholesterol to cholesterol in animals and humans has been investigated and...
includes 4-cholesten-3-one as an intermediate. Further, the key rate-determining step in the cholestanol biosynthetic pathway is the transformation of cholesterol into 4-cholesten-3-one and has been demonstrated in hepatic microsomes from control and CTX subjects (15). Also, the specific in vivo 5α-reduction of 4-cholesten-3-one to 5α-cholestanol has been shown in these same subjects. Thus, it is likely that the production of 5α-campestanol and 5α-sitostanol from campesterol and sitosterol probably involves an analogous pathway with the formation of 3-keto precursors. However, it is still unclear why 5α-stanols are overproduced in sitosterolemia or why this biochemical pathway is activated.

It is noteworthy that despite increased amounts of plasma and tissue phytosterols and 5α-saturated stanols, abnormal sterols were discriminated by the blood–brain barrier and thus sterol composition in the sitosterolemic brain resembled that of the control. Further, in support of the chemical determinations, no sitosterolemic subject has exhibited neurologic dysfunction. Thus, in contrast to CTX where cholestanol preferentially accumulates in brain tissue with the development of neurologic abnormalities (2), sitosterolemic brain functions normally without abnormal sterol accumulation. Clearly, these two diseases with increased 5α-stanols are different. The blood–brain barrier remains intact in sitosterolemia but obviously is impaired in CTX. This difference has also been noted in the xanthoma sterol composition, which showed that sitosterolemic xanthoma contained cholestanol, 5α-saturated plant sterol derivatives, and unsaturated phytosterols in the same proportion as found in plasma.

With regard to the mechanism of cholestanol formation, we have previously shown that in cholestanol CTX arises as a by-product of abnormal bile acid synthesis (2). In other words, the block in bile acid synthesis that characterizes CTX results in the increased conversion of cholesterol into cholestanol. Feed-back inhibition of abnormal bile acid synthesis in CTX by the administration of chenodeoxycholic acid reduces plasma cholestanol levels (16). In sitosterolemia, we postulate that increased cholestanol and 5α-saturated plant sterols are produced from an acquired abnormality of bile acid synthesis that results from the abundance of plant sterols in the bile acid synthetic pathway. Although trace amounts of cholic acid and chenodeoxycholic acid may be formed from sitosterol (13), the large amounts of plant sterols found in the liver may compete with cholesterol for key enzyme sites and interfere with normal bile acid synthesis and thus divert cholesterol and phytosterols into their respective 5α-saturated derivatives. In favor of this hypothesis is the observation by Miettinen (9) that total bile acid synthesis was reduced in sitosterolemia. Also Dayal et al. (17) have shown that sitosterolemic subjects excrete in their feces bile acid precursors that have been identified as 5β-

cholestan-3α,7α,12α,25-tetrol and 27-nor-5β-cholestan-3α,7α,12α,24R,25-E-pentol. The increased excretion of these bile alcohols may reflect a partial block in bile acid synthesis.

Finally, cholestyramine, which enhances hepatic bile acid synthesis presumably by increasing the conversion of plasma sterols into bile acids, lowers plasma cholesterol, phytosterol, and 5α-saturated stanol levels in this disease (12). Activation of bile acid synthesis, which in turn lowers plasma sterol levels, is one factor that may be beneficial in slowing or reversing the atherosclerotic process in sitosterolemia.

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