Clinical and metabolic findings in a patient with phytosterolaemia

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Additional key phrases: coronary heart disease; aortic stenosis; LDL cholesterol; cholestyramine

A patient with premature coronary heart disease and aortic stenosis is described in whom the diagnosis of phytosterolaemia was made by gas chromatographic analysis of plant sterols in plasma and aortic tissue. The clinical presentation mimicked familial hypercholesterolaemia. The plasma concentration of low density lipoprotein (LDL) cholesterol was elevated due to both reduced catabolism and increased synthesis of LDL-apolipoprotein B. Phytosterolaemia was responsive to cholestyramine but not to a low plant sterol diet alone.

CLINICAL REPORT

A 33-year-old man presented with progressive dyspnoea on exertion and painful swelling of both heels; he did not admit to chest or calf pain, nor to haemoptysis. He was a non-smoker and had not previously suffered from rheumatic or scarlet fever. There was no history of systemic hypertension. He had large Achilles tendon xanthomata, small xanthomata of the extensor tendons of the hands, xanthelasma palpebrarum and an arcus corneae (Fig. 1). The body mass index was $27 \cdot 1 \, kg/m^2$, the systemic blood pressure 120/90 mmHg and there was a moderately loud ejection systolic murmur; he was not in cardiac failure and all peripheral pulses were present. Deep venous thrombosis, pulmonary embolism and pneumothorax were excluded clinically. He was not anaemic and there was no organomegaly. Neurological examination was normal. There was no proteinuria on dipstick urinalysis. The parents were consanguinous, the family history being negative for premature cardiovascular disease. An electrocardiogram showed sinus rhythm at a rate of 80/min, left

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ventricular hypertrophy and ST depression in the anterior chest leads. Pulmonary venous hypertension was noted on a chest X-ray. He was referred for cardiac angiography. This revealed tight supravalvular aortic stenosis and extensive atheromatous disease of the right and left coronary arteries.

Venous blood was collected in the fasting state (on at least three occasions) into tubes containing Na_2EDTA (final concentration 1 mg/mL) for lipid, lipoprotein, apolipoprotein and sterol analyses. Samples were also obtained to exclude secondary causes of hyperlipidaemia. First degree relatives (both parents, six siblings and four offspring) were initially screened for hyperlipidaemia alone.

Because of rapidly progressive symptoms the patient proceeded to aortic root replacement and coronary artery bypass surgery, from which he made an excellent recovery. A biopsy of aortic tissue was obtained at operation for sterol analysis. Three months post-operatively a radiokinetic study of the metabolism of LDL-apo B was carried out using the study protocol of Sigurdsson *et al*;[1] blood was also collected for the *in vitro* assessment of LDL receptor activity. The patient was on an *ad libitum* diet throughout the above investigations.

ANALYTICAL METHODS

The plasma concentration of cholesterol was measured by the CHOD-PAP enzymatic colorimetric method (Boehringer, Lewes, UK), with an interassay coefficient of variation (CV) of $< 3 \cdot 5\%$. Plasma triglyceride was also measured by an enzymatic method (Wako Chemicals, Neuss, Germany); interassay CV $< 3\%$. High density lipoprotein (HDL) cholesterol was assayed after the precipitation of apo B with $MnCl_2$ and heparin. LDL cholesterol was measured after isolation of LDL at plasma density $d = 1 \cdot 063 \, g/mL$ using preparative ultracentrifugation. Apo B was
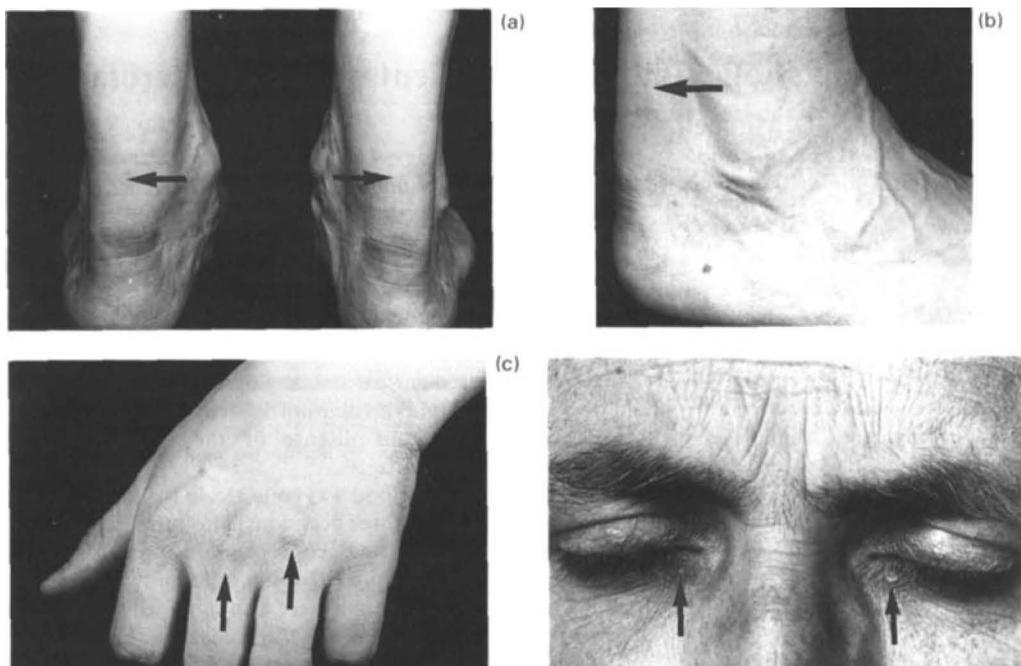
FIGURE 1. *Xanthomata of the Achilles tendons (a, b) and extensor tendons of hands (c), and xanthelasma palpebrarum (c) in a patient with phytosterolaemia. Lesions are indicated by arrows.*

assayed by direct immunoturbidimetry,[2] mean interassay CV < 3·5%. The fractional catabolic rate (FCR) of LDL was estimated from the half-life (two compartment model) of an intravenously injected bolus of autologous ^{125}I-labelled-apo B;[1] absolute synthetic (turnover) rate was calculated as the FCR × apo B pool size (mg/kg body weight/day). The degradation of ^{125}I-labelled human LDL by freshly isolated blood mono-nuclear cells was measured as described by Bilheimer *et al.*[3] Plant sterols in plasma were quantitated by gas chromatography (GC) after hydrolysis and extraction.[4] Coprostanol (Stera-loids Ltd, Croydon, UK) was used as the internal standard and taken through the complete procedure. The ratios of chromatographic peak heights of plant sterols to coprostanol in the test sample were compared with those from a set of sterol standards (Sigma Chemicals, Dorset, UK). Cholestanol was measured by GC as the ketone derivative. GC employed 3% OV-17 (glass column 4 mm i.d., 1·52 m), an oven temperature of 280°C, a detector temperature of 330°C and an argon gas flow rate of 40 mL/min. The CV of the patient's plasma level of plant sterols measured on four separate occasions whilst on no treatment was 6%, in excess of the imprecision of the routine GC methods (β-sitosterol:

CV = 4·2%; campesterol: CV = 4·4%). The tissue concentrations (μg/mg wet weight) of cholesterol, plant sterols and cholestanol were measured in an aortic biopsy using the same method as described for plasma.

RESULTS

The concentration of lipids, lipoproteins, apo B, plant sterols and cholestanol in plasma, and the kinetic parameters of LDL apo B and the LDL receptor activity are given in Table 1; reference values were obtained from the literature[2,5-8] and for conventional measurements should be distinguished from so-called 'desirable' values.[9,10] Cholesterol, LDL cholesterol and apo B concentrations were all elevated. This was consistent with the low FCR and high synthetic rate of LDL-apo B and low LDL receptor activity. Beta-sitosterol and campesterol were both markedly elevated in plasma; the concentration of cholestanol was also increased, but to a lesser extent than the plant sterols. Stigmasterol was not identified. The plant sterols in the patient's plasma are demonstrated in the GC tracings shown in Fig. 2a; Figs 2b and c are control and standards, respectively. Table 2 shows that the aortic content of cholesterol, plant

TABLE 1. *The concentration of lipids, lipoproteins, apolipoprotein B, plant sterols (β-sitosterol, campesterol) and cholestanol in plasma, and kinetic parameters of LDL apo B and LDL receptor activity in a patient with phytosterolaemia*

Metabolic variable	Patient	Reference range[2,5-8]
Cholesterol (mmol/L)	7·5	4·1–6·9*
Triglyceride (mmol/L)	1·4	0·3–2·7*
HDL cholesterol (mmol/L)	1·2	0·8–1·7*
LDL cholesterol (mmol/L)	6·1	2·2–4·7*
Apolipoprotein B (mg/dL)	157	50–118
FCR of LDL apo B (pools/day)	0·20	0·25–0·35
Synthetic rate of LDL apo B (mg/kg body weight/day)	13·2	5·0–10·9
LDL degraded by mononuclear cells (mg LDL/mg cell protein/8 h)	160	165–342
Beta-sitosterol (mg/dL)	30·9	0·3–1·7
Campesterol (mg/dL)	21·1	0·15–0·8
Cholestanol (mg/dL)	2·2	0·1–0·6

*For corresponding 'desirable values' see EAS and BHA policy statements.[9,10]
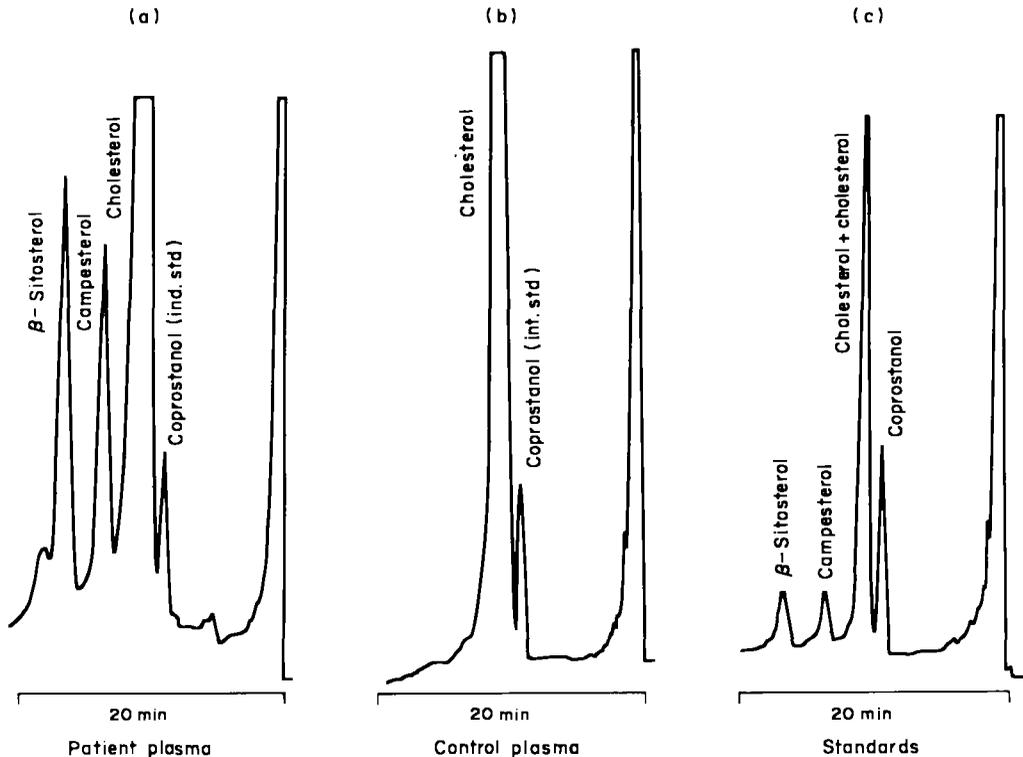

FIGURE 2. *Gas chromatographic tracings of the plasma sterols of a patient with phytosterolaemia (a) and a control subject (b); the standard tracings are also shown (c).*

sterols and cholestanol was markedly increased compared with control material.[11,12]

The above findings were diagnostic of phytosterolaemia associated with reduced catabolism and increased synthesis of LDL apo B. Heterozygous familial hypercholesterolaemia, the most likely diagnosis at initial presentation, was excluded since all relatives studied were normolipidaemic (plasma cholesterol <6·0, LDL cholesterol <4·5, triglyceride <2·5 mmol/L) and none had xanthomatosis. The patient did not show biochemical evidence of diabetes mellitus, hypothyroidism, renal insufficiency or abnormal liver function.

TABLE 2. *Aortic content of cholesterol, plant sterols and cholestanol (µg/mg of wet tissue) in a patient with phytosterolaemia*

Tissue sterol (µg/mg)	Patient	Reference material[11,12]
Cholesterol	9·50	2·5
Beta-sitosterol	0·46	Undetectable
Campesterol	0·29	Undetectable
Cholestanol	0·08	0·008

The plasma phytosterol and cholesterol levels did not alter significantly after a period of 3 months on isocaloric, low plant sterol diet (60 mg/day) with regular review by a dietitian. However, the addition of cholestyramine (16 g/day), a bile acid-binding resin, resulted in a significant fall in phytosterols (-23%), cholesterol (-36%) and LDL cholesterol (-43%).

DISCUSSION

Phytosterolaemia, originally termed 'sitosterolaemia and xanthomatosis',[13] is a rare autosomal recessive disorder in which plant sterols accumulate in the plasma and tissues.[8,13,14] Only twenty-three cases have hitherto been reported.[14,15] The external clinical signs in this patient are typical of the disorder, but may also be seen in familial hypercholesterolaemia and cerebotendinous xanthomatosis.[16] Premature coronary heart disease is a well-recognized complication, particularly in men.[14] A novel finding was the extensive supravalvular aortic stenosis due to accumulation of cholesterol and plant sterols in aortic tissue. Unlike cerebotendinous xanthomatosis neurological lesions are not seen.[16]

Elevated plasma and tissue levels of β-sitosterol and campesterol, the most common plant sterols, are diagnostic of phytosterolaemia.[13,14] Hypercholesterolaemia and hypercholestanolaemia are less consistent findings.[14,17] The plasma level of cholestanol may be similar to that seen in cerebotendinous xanthomatosis,[16] but the latter is distinguished by accumulation of cholestanol in the brain, neurological abnormalities and absence of phytosterolaemia. The bases for premature atherosclerosis in phytosterolaemia have not yet been established, but are likely to relate to the increased uptake of plant sterols by endothelial cells and macrophages.[12] In this patient the elevated plasma concentration of LDL apo B must have also contributed to the vascular injury.[18] We provide the first evidence that the elevation in plasma apo B previously described in phytosterolaemics[19] is due to a combination of increased synthesis and reduced clearance of LDL apo B. Both mechanisms are compatible with a reduction in LDL receptor activity,[6] although we cannot exclude that increased synthesis of LDL apo B was also due to increased hepatic secretion of VLDL apo B.[20] Since there was no evidence of hypercholesterolaemia or xanthomatosis in the patient's immediate relatives, it is more probable that reduction in LDL receptor activity was due to the metabolic defect in phytosterolaemia than to a primary hyperlipidaemia, such as polygenic or familial hypercholesterolaemia. Absence of familial combined hyperlipidaemia also militates against hepatic oversecretion of apo B.[6]

The metabolic bases for phytosterolaemia have not been fully elucidated. The available evidence suggests increased intestinal absorption coupled with reduced hepatic excretion of plant sterols.[14] Normally less than 5% of plant sterols in the diet are absorbed, but in phytosterolaemia this can increase to 30%.[13] Marked reduction in the biliary excretion of sterols has also been demonstrated,[21] consistent with a low turnover rate of intravenously injected [^{3}H]-sitosterol.[22] An additional defect might be an impaired formation of bile acids.[12] In humans 70% of phytosterols circulate in plasma in LDL particles. The reduced FCR of LDL apo B noted in our patient concurs with a defect in the hepatic handling and excretion of sterols.[12,20,22] LDL receptor activity is reciprocally related to the intrahepatic pool of cholesterol.[23] The poor response of phytosterolaemia to dietary restriction of plant sterols compared with the significant fall following addition of cholestyramine also suggests that in this patient reduced biliary excretion of sterols was probably a more important lesion than an increase in their absorption. Cholestyramine, a resin which binds bile acids in the gut, enhances the intrahepatic conversion of sterols to bile acids[24] and lowers cholesterol and phytosterols in plasma by increasing LDL receptor activity.[14,25] The mechanism for the hypercholestanolaemia remains unexplained, but might also involve the removal defect proposed above. This defect will need to be confirmed using sterol balance and radiokinetic studies.

Phytosterolaemia must be considered in all patients with xanthomatosis and hypercholesterolaemia, especially if there is a negative family history of premature cardiovascular

disease and relatives are normolipidaemic. Since its inheritance is autosomal recessive, the diagnosis should also be suspected with parental consanguinity.[13,14] The biochemical diagnosis is readily made by GC analysis of plant sterols in plasma, with definitive evidence derived from direct assay of affected tissue.

Establishing the diagnosis is important because the dietary treatment is radically different from that where an elevation in LDL cholesterol is the sole abnormality.[14] Bile acid-binding resins should be used when there is a poor response to a diet low in, or free from, plant sterols. Since lowering plasma cholesterol and phytosterols has been shown to result in regression of xanthomata,[15] phytosterolaemia must be considered a treatable cause of premature atherosclerosis.

Acknowledgements

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