HMG-CoA reductase is not the site of the primary defect in phytosterolemia

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Abstract **Phytosterolemia is an autosomal recessive disorder characterized by the excessive absorption, reduced excretion, and consequent high tissue and plasma levels of plant sterols, by the presence of tendon xanthomas, and by premature atherosclerosis. Low HMG-CoA reductase (HRase) activity and mass have been reported in liver and mononuclear leucocytes and low mRNA levels in liver from phytosterolemic subjects. These results led to the proposal that the primary defect in this condition involves the HRase gene locus. We examined this hypothesis in phytosterolemic subjects and heterozygous parents from four unrelated families. A variable number tandem repeat (VNTR) polymorphism of the HRase gene in the three informative families and a ScrFI restriction fragment length polymorphism (RFLP) within intron 2 of the gene in one of these families, segregated independently of the disease phenotype. Biological parentage was confirmed in the family in whom both polymorphisms failed to segregate with the disorder. These results conclusively exclude the HRase gene locus as the site of the primary defect in phytosterolemia. The study was extended by examining plasma levels of mevalonic acid and lathosterol, both markers of cholesterol biosynthesis, in response to cholestyramine, a bile acid sequestrant that is known to up-regulate HRase. Oral administration of cholestyramine resulted in a substantial (7.7-fold) increase in mevalonic acid levels in two phytosterolemic subjects, compared with a 2.2-fold rise in their obligate heterozygote parents and a 2.3-fold increase in three healthy control subjects. The lathosterol/cholesterol (L/C) ratio showed a quantitatively similar response. Baseline levels of mevalonate and the L/C ratio were low in the phytosterolemic patients in conformity with reports of reduced cholesterol biosynthesis and HRase activity in this disorder. These functional data provide support for the concept that the primary defect in phytosterolemia does not affect a *trans* gene locus responsible for the constitutive expression or regulation of HMG-CoA reductase.—**Berger, G. M. B., R. J. Pegoraro, S. B. Patel, P. Naidu, L. Rom, H. Hidaka, A. D. Marais, A. Jadhav, R. P. Naoumova, and G. R. Thompson. **HMG-CoA reductase is not the site of the primary defect in phytosterolemia. J. Lipid Res. 1998.** 39: **1046–1054.**

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Phytosterolemia (sitosterolemia) is an autosomal recessive disorder characterized by excessive absorption of plant sterols (PS), early xanthomatosis, and premature coronary artery disease (1–3). To date at least 34 cases have been reported in the literature (3–5). Studies have demonstrated that absorption of sitosterol in phytosterolemic patients is 20–30% in contrast to approximately 5% in normal subjects (3, 6, 7). Shellfish sterols are also absorbed in excess (8). The concentration of PS in plasma from phytosterolemic patients varies between 15 and 27% of total sterol content (3) and increased concentrations are also found in cell membranes, xanthomas, and atherosclerotic plaques, although cholesterol remains the predominant lipid (3, 8–10). Isotopic studies indicate a marked (up to 80-fold) expansion of the plant sterol body pool in phytosterolemic subjects but very little difference between obligate heterozygotes and normals (6, 11, 12).

The pathophysiological basis for these findings involves abnormalities of both the absorption and excretion of PS. In addition to the markedly enhanced absorption of PS in phytosterolemic patients and to a lesser extent in heterozygotes, several studies have shown a significantly reduced rate of sitosterol excretion in homozygotes (6, 11, 12). The ability to concentrate sitosterol in bile is reduced (8, 11) and sitosterol is unable to undergo 7α-hydroxylation, the first step in bile acid synthesis (13–15). In heterozy-

Abbreviations: HMG-CoA, 3-hydroxy-3-methylglutaryl coenzyme A; HRase, HMG-CoA reductase; PS, plant sterols; MVA, mevalonic acid; RFLP, restriction fragment length polymorphism; VNTR, variable number tandem repeats; PCR, polymerase chain reaction; LDL, low density lipoprotein; L/C, lathosterol/cholesterol.

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gotes, however, excretion is almost normal, thus accounting for the absence of PS accumulation and clinical manifestations (16).

In vivo and in vitro studies have uniformly demonstrated diminished rates of cholesterol synthesis in this disorder (6, 11, 12). Nguyen and co-workers (17, 18) have reported low levels of HMG-CoA reductase (HRase) mass and activity in mononuclear leucocytes, and in liver biopsy and postmortem material. Levels of HRase mRNA were also reduced in liver (18). Catalytic efficiency was normal or increased, with a greater proportion of the total HRase activity in the active form in phytosterolemic microsomes, suggesting reduced enzyme synthesis rather than an abnormal enzyme protein. HRase in mononuclear leucocytes anomalously decreased in response to cholesterol lowering by cholestyramine in phytosterolemic patients (17). These results suggested the possibility that HRase regulation may be the site of the primary defect in phytosterolemic patients but to date no mutational basis for the disease has been reported. A number of alternative explanations have been proposed (3) but conclusive evidence is lacking.

We examined the basis for the reduced HRase activity using two different approaches. In one we determined the segregation of the disease phenotype with DNA polymorphisms within the HRase gene in linkage studies performed on three informative phytosterolemic families. The second approach involved administration of cholestyramine, a bile acid sequestrant, to two phytosterolemic sisters, to four obligate heterozygotes, and to three healthy, unrelated control subjects. Fasting plasma mevalonic acid (MVA) and lathosterol were measured prior to and during the administration of bile acid sequestrant to assess indirectly changes in the rate of cholesterol biosynthesis and hepatic HRase activity. Additionally, the diurnal rhythm and response to an HRase inhibitor, simvastatin, were monitored by determining plasma MVA levels in a phytosterolemic patient and her mother.

METHODS

Subjects

Of the five phytosterolemic families investigated (3 from South Africa, 1 Japanese, and 1 American) in this study, four (families C, K, N and R) have been reported previously (5, 19). The phytosterolemic subjects had sitosterol values ranging between 0.35 and 1.21 mmol/l whereas levels in their obligate heterozygote parents were undetectable or less than 0.05 mmol/l. Xanthomatosis was prominent in most of the homozygous phytosterolemic subjects. Some of the patients were being treated with cholestyramine in addition to a low phytosterol diet with a substantial fall in plasma sitosterol levels and regression of xanthomatosis. **Table 1** summarizes the biochemical and clinical data on the three families and controls involved in the physiologic studies.

Family linkage study

Segregation of the HRase loci was studied using previously described polymorphisms (20, 21): a variable number tandem repeat (VNTR) Alu sequence-related polymorphism situated 10 kb 3′ of exon 2, and a ScrFI restriction fragment length polymorphism (RFLP) in intron 2 of the gene. Genomic DNA was salt-extracted from the white cell fraction of EDTA–blood collected from members of four families, three of which were informative. The R kindred was not studied as it comprised only mother and daughter. The VNTR region was PCR amplified as described by Zuliani and Hobbs (20); one of the primers (GZ-1) was ^{32}P-endlabeled by polynucleotide kinase. The products were denatured with formamide, separated by denaturing 8% acrylamide/urea gels, and subjected to autoradiography. Allele sizes were assigned relative to each run and all three families (14 individuals) were analyzed on the same gel. To determine the RFLP alleles in family K, DNA was amplified using previously reported PCR conditions and primers (21). The product was restricted with the enzyme ScrFI, and the multiple fragments ranging from 430 to 42 base pairs were separated on a 12% polyacrylamide gel. The two polymorphic alleles, **a** and **b**, were identified by bands of 165 (absence of the cutting site, **a**) or 120 + 45 (presence of the cutting site, **b**) base pairs. Parentage in this family was confirmed using a set of five polymorphic markers.

Cholestyramine provocation study

At least 2 weeks prior to the commencement of this study bile acid sequestrant therapy was stopped in the homozygote subjects, HK and ZK, and in the heterozygote, AK. Baseline blood samples were taken from the homozygotes and a second set of baseline blood samples 2 weeks later. The four obligate heterozygote parents (families K and C) were sampled on a single occasion only in the baseline period, whereas the three controls were sampled twice at an interval of 1 week. Immediately after the baseline collections, cholestyramine (Questran®, Mead Johnson) was given twice daily (8 g/day) to all subjects. After 3 weeks blood samples were collected and the intake of bile acid sequestrant was reduced to 6 g/day before taking a further blood sample 2 weeks later from all subjects except BeC. These studies were not carried out under metabolic ward conditions but both homozygotes remained on their low plant sterol diets throughout.

HMG-CoA reductase inhibition study

Acute mevalonic acid suppression test with simvastatin (Zocor, MSD). Patient JR and her heterozygote mother (PR) had plasma MVA levels measured at 09:00, 10:00, 12:00, 14:00, and 16:00 h to analyze diurnal rhythm (day 1). The next day (day 2) the same protocol was followed except that 40 mg simvastatin was administered immediately after the 09:00 h sample. Both subjects were

TABLE 1. Personal and clinical data on experimental subjects

	Age	Sex	CHOL	TRIG	SITO	Xanthomas
	yr		*mmol/l*	*mmol/l*	*mmol/l*	
Phytosterolemics						
HK	12	F	8.30	0.88	1.15	yes
ZK	8	F	6.14	0.83	0.63	no
JR	19	F	6.60	0.90	1.21	yes
Heterozygotes						
AK	45	M	8.63	1.66	0.05	no
MK	35	F	5.95	0.98	ND	no
BaC	40	M	5.61	1.64	0.03	no
BeC	37	F	4.88	1.25	ND	no
PR	53	F	7.90	1.90	0.01	no
Controls						
DP	28	M	4.04	0.43	—	no
BC	21	M	3.61	0.27	—	no
ED	23	M	4.37	0.71	—	no

CHOL, cholesterol; TRIG, triglyceride; SITO, sitosterol.

kept on a cholesterol-free diet containing less than 5% fat on each of these days.

Short-term administration of simvastatin in obligate heterozygotes and normolipidemic subjects. After baseline blood samples were collected, simvastatin (20 mg/daily) was administered for 8 days to the four obligate heterozygote parents in the K and C families and to four normolipidaemic subjects. At the same time plant sterol intake was increased to roughly 400 mg daily by including a variety of foodstuffs rich in plant sterols in the diet of the participants. Blood samples were collected after 4 and 8 days for analysis.

Blood was sampled between 8:00 and 9:00 h after an overnight fast in all the experimental subjects. Samples for cholesterol and PS assay were collected into tubes without anticoagulant and allowed to clot for 1 h before centrifugation at 3000 rpm for 15 min. The serum was stored at −70°C prior to analysis. Blood for MVA and lathosterol assay was collected into EDTA-containing tubes on ice, spun in a refrigerated centrifuge, and the plasma was stored at −70°C or on dry ice prior to analysis.

The methods used for cholesterol and plant sterol assay were essentially as described previously (5), except that the sensitivity for the detection of plant sterols was increased 6-fold by altering the attenuation on the gas chromatograph and by increasing the injection volume of the methylene chloride extract 3-fold. Mevalonic acid was determined by gas chromatography electron capture mass spectrometry. The method is fully detailed elsewhere (22, 23) and is summarized briefly below. After addition of [$^2H_3$]MVA to each sample as internal standard, MVA was converted into the lactone form using Dowex 50 (H^+) and then extracted into dichloromethane-propan-2-ol. After purification and conversion back to the acid form, MVA was esterified to the 3,5 *bis* (trifluor-methyl)benzylester and the trimethylsilyl derivative was prepared using *bis* (trimethylsilyl)-trifluoroacetamide. Derivatized samples were analyzed on a Finnigan 4500 quadropole mass spectrometer. This instrument uses electron capture and selected ion monitoring of ions at m/z ratios of 291 and 294 for detection of the derivatized MVA and [$^2H_3$]MVA, respectively; the intra-assay and the interassay coefficients of variation were 3.5% and 6.0%, respectively.

Serum concentrations of 7-lathosterol were determined by the method described by Wolthers et al. (24), using a PYE 4500 analytical Gas Chromatograph (Pye Unicam, Cambridge, UK) equipped with a fused capillary column (SAC-5, Sigma-Aldrich, Poole, Dorset). Helium was used as carrier gas. Lathosterol is reported in μmol/l and is also expressed as the L/C ratio so as to correct for changes in serum levels attributable to decreases in low density lipoprotein during treatment with cholesterol (25).

Statistical analysis

Analysis of variance was used to compare results obtained in the phytosterolemic patients, heterozygotes and control subjects. Results within each group were analyzed using the paired *t*-test.

Ethical approval

Approval for this study was obtained from the Ethics Committee of the Faculty of Medicine, University of Natal. The subjects participated on a voluntary basis and were free to withdraw at any time.

RESULTS

Family linkage study

When the VNTR bands were ranked in terms of mobility, five distinct size alleles were detected in the three informative families (**Fig. 1**). In all three families the VNTR alleles segregated independently of the disease phenotype. In family K this was confirmed by an informative ScrFI RFLP which also segregated independently of the disease phenotype (**Fig. 2**). The one phytosterolemic sister inherited the **a** allele from her father and the **b** allele from her mother whereas the second affected sib, HK, was homozygous (**b/b**) which implies that she must have inherited the **b** allele from her father. Biological parentage in this kindred was confirmed by tracing the segregation of five polymorphic loci: the apolipoprotein E locus on chromosome 19 (26), the myotonic dystrophy locus (a trinucleotide repeat) on chromosome 19 (27), the platelet glycoprotein IIIa polymorphism on chromosome 17 (28), and the p53 Hae III and p53 MspI RFLPs on chromosome 17 (29, 30). In four of the five loci, at least one of the parents was heterozygous. The distribution of the polymorphic alleles was compatible with biological parentage in each case. The fourth family was uninformative for both polymorphisms.

Cholestyramine study

The baseline MVA levels and changes in response to cholestyramine are shown in **Fig. 3**. In the two phytosterolemic patients, the mean baseline level was 2.4 μg/l, in the

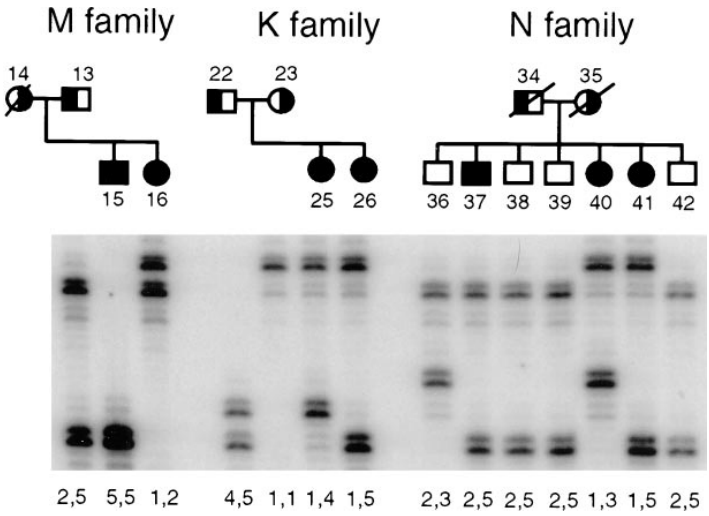

Fig. 1. Autoradiograph showing segregation of a VNTR in intron 2 of the HRase gene in three families. A total of five alleles differing in size were detected; in each family the affected children, M15 and M16, K25 and K26, and N37, N40 and N41, inherited different alleles. Although the parents were not available for genotyping in families M and N, the presence of at least three differing alleles within each family allows for informative segregation and exclusion.

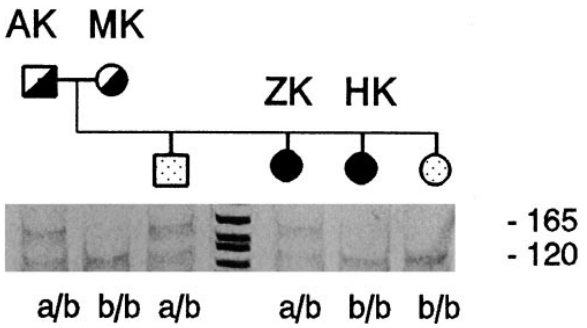

Fig. 2. Polyacrylamide gel (12%) electrophoresis showing segregation of ScrFI digested products of an intron 2 segment of the HRase gene in the K family. Heterozygosity for the restriction site within the 165 base pair fragments is indicated by **a/b** while **b/b** indicates homozygosity for the presence of this cutting site. The two affected siblings are ZK and HK.

four obligate heterozygotes the mean was 5.4 μg/l (range 2.5–8.3 μg/l), and in the three control subjects the MVA level was 4.5 μg/l (range 4.1–4.9 μg/l). The mean MVA concentration in 21 healthy adults was 6.1 μg/l (SD, 2.6 μg/l), as reported previously (31). Administration of cholestyramine resulted in an increase in MVA in all groups: 7.7-fold in the homozygote patients (range 7.2–8.2), 2.2-fold (range 0–3.0) in the heterozygotes, and 2.3-fold (range 1.6–2.7) in the control subjects. The relative changes in MVA were more pronounced ($P = 0.0009$) in the two phytosterolemics, who had lower baseline concentrations. The changes in the other two groups when pooled were less marked, but statistically significant ($P = 0.019$). Treatment with cholestyramine (Fig. 3) also induced increases in the L/C ratio in the two sitosterolemic patients (3.8- and 7.2-fold), in the three controls (2.1- to 2.8-fold), and in the four heterozygotes (0- to 1.5-fold) which were comparable to the changes observed in plasma MVA. As with the latter, the changes in the L/C ratio were more marked in the patients than in the other subjects ($P = 0.017$). The treatment was continued for a further 2 weeks using a lower concentration of cholestyramine (see Methods) resulting in a fall in MVA levels, which, however, remained above baseline in the two homozygotes and heterozygotes.

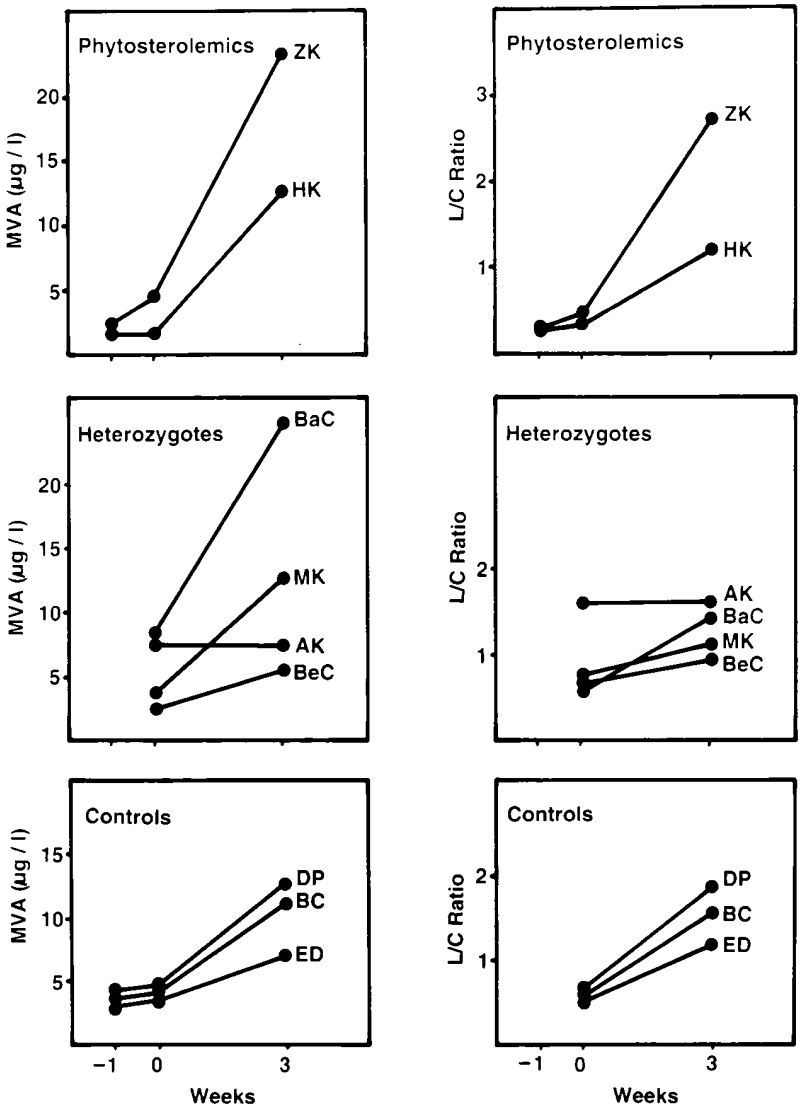

Fig. 3. Changes in baseline MVA levels (μg/l) and L/C ratios in response to treatment with cholestyramine in patients with phytosterolemia, heterozygotes and controls.

TABLE 2. Results of cholestyramine provocation study

	Lathosterol			Cholesterol			Sitosterol			
	Basal		Week 3	Basal		Week 3	Week 5	Basal		Week 3	Week 5
	μmol/l			*mmol/l*				*mmol/l*			
Phytosterolemics											
HK	0.4	0.8	3.8	5.05	6.20	3.20	3.10	0.91	1.01	0.69	0.62
ZK	1.6	2.4	9.6	4.95	5.56	3.52	3.78	0.57	0.60	0.50	0.50
Heterozygotes											
AK	—	12.4	11.3	—	7.60	7.00	6.40				
MK	—	4.2	5.7	—	5.42	5.00	5.24				
BaC	—	4.3	8.4	—	7.47	5.80	6.30				
BeC	—	3.4	4.1	—	5.10	4.45	—				
Controls											
DP	—	2.7	6.5	—	4.17	3.47	3.96				
BC	—	2.5	5.7	—	4.23	3.60	3.76				
ED	—	2.6	4.6	—	4.50	3.87	4.19				

Cholesterol levels fell in all subjects after 3 weeks of cholestyramine administration (**Table 2**), indicating reasonable compliance with the protocol. The relative fall in cholesterol was greater in the phytosterolemic patients than in the other subjects (as previously reported). Sitosterol concentrations diminished markedly in HK but only slightly in ZK. In the heterozygote and control subjects, baseline sitosterol levels were extremely low and the changes after cholestyramine intake were inconsistent.

HMG CoA reductase inhibition study

Results of the acute MVA suppression test with simvastatin in patient JR and her heterozygote mother are presented in **Fig. 4**. The mother showed normal fasting plasma MVA levels and a normal diurnal rhythm (day 1) whereas patient JR had low baseline levels. The single 40-mg dose of simvastatin reduced plasma MVA levels by 70% in the mother but had no effect in JR. Eight days administration of simvastatin to heterozygotes and controls caused a slight decrease in cholesterol levels ($P = 0.04$) but no change in either sitosterol or campesterol (**Table 3**).

DISCUSSION

The polymorphism linkage data conclusively establish that in the three informative families studied phytosterolemia was not inherited through a genetic defect at the HRase gene locus. The VNTR data were further strengthened in family K using a second polymorphism (ScrFI RFLP), together with biological proof of parentage. It is reasonable to assume that the consistency of the data across all three families renders this interpretation applicable to the majority of phytosterolemic kindreds in South Africa, Japan, and the United States.

The implications of the genetic data were further studied and extended by measuring changes in MVA and lathosterol concentrations in response to cholestyramine administration. Measurement of plasma levels and urinary excretion of MVA have been shown to be good indices of the in vivo rate of cholesterol synthesis (32, 33). A diurnal rhythm of plasma MVA has been described in humans (34) that correlated closely with the incorporation of deuterium into plasma free cholesterol (35). Whole body cholesterol synthesis as measured by sterol balance has been shown to correlate closely with fasting plasma MVA (32) under metabolic ward conditions and with urinary MVA

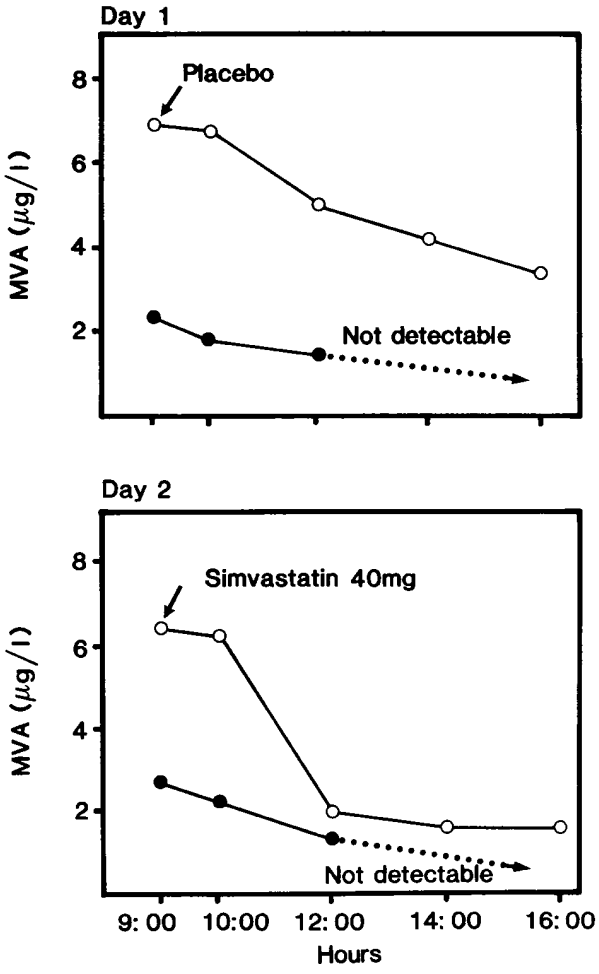

Fig. 4. MVA levels (μg/l) after suppression with simvastatin. Phytosterolemic patient (●) and her obligate heterozygote mother (○). Day 1, placebo; day 2, single dose of simvastatin 40 mg administered at 09:00 h.

TABLE 3. Effect of simvastatin on plasma sterol levels

	Baseline			Day 4			Day 8		
	Chol	Sito	Camp	Chol	Sito	Camp	Chol	Sito	Camp
	mmol/l	*μmol/l*	*mmol/l*	*mmol/l*	*μmol/l*	*mmol/l*	*mmol/l*	*μmol/l*	*mmol/l*
Heterozygotes									
AK	7.52	40.7	34.4	7.82	45.5	39.5	6.39	41.2	39.5
MK	6.24	25.4	22.1	5.63	22.3	19.3	4.67	25.3	22.8
BaC	5.32	12.0	5.5	5.03	13.2	0.0	4.15	15.7	8.5
BeC	5.21	4.6	3.9	4.91	9.6	7.7	4.39	11.5	7.5
Mean	6.07	20.7	16.5	5.85	22.6	16.6	4.90	23.4	19.6
Controls									
SM	5.55	13.3	23.0	5.05	12.0	20.1	4.74	8.5	20.9
PN	6.3	9.7	9.3	5.19	15.1	6.8	4.36	15.3	15.9
RR	4.28	12.4	11.4	3.63	6.2	7.7	3.77	9.6	10.7
GMB	5.39	11.3	10.1	4.67	11.0	10.3	4.29	11.1	7.5
Mean	5.38	11.7	13.4	4.63	11.1	11.2	4.29	11.1	13.7

excretion (36). In addition, plasma MVA levels correlate well with HRase activity in human liver (37). Furthermore, plasma levels and urinary excretion of MVA have been shown to decrease after treatment with HMG-CoA reductase inhibitors and to increase after bile acid sequestrants (33, 38). Thus there is good evidence that fasting plasma MVA levels provide a valid albeit semi-quantitative index of cholesterol synthesis. The concentration of 7-lathosterol in plasma is another well-established index of whole body cholesterol synthesis in humans (25, 39, 40) that has been shown to correlate closely with hepatic HRase activity (41). Serum lathosterol concentration and the L/C ratio both increase during treatment with cholestyramine or colestipol (42, 43), which stimulate cholesterol biosynthesis in the liver, and decrease during treatment with an HMG-CoA reductase inhibitor (25, 42, 44).

In the present study intra-individual variation in plasma MVA levels was minimized by sampling at the same time in the morning to avoid the effects of the diurnal rhythm previously reported (32, 34), and by maintaining the same diet over the period of investigation. The increase in MVA was considerably greater in the phytosterolemic patients (Fig. 3) than in either the obligate heterozygote or control group. The within-subject variability of plasma MVA estimated from data obtained in 20 hypercholesterolemic patients, from whom fasting blood samples were obtained at 9 am on three separate occasions in 1 week (45), was 12.4% (range 5.7–18.5%). By comparison, plasma MVA increased during treatment with cholestryramine by 7.2- to 8.2-fold in the phytosterolemic patients and up to 3-fold in heterozygous relatives and normolipidemic controls. Lathosterol levels (Table 2) and especially the L/C ratio (Fig. 3) increased pari passu with the plasma MVA changes. The changes in the MVA levels and the L/C ratio in both phytosterolemic subjects were considerably greater than could be accounted for by intra-individual variability.

Plasma MVA and lathosterol, measured in our study as markers of early and late steps of the cholesterol biosynthesis pathway, respectively, were both comparably increased. This excludes the unlikely event that MVA levels increased in response to inhibition of one or more steps in the conversion of mevalonate to cholesterol. In particular, both indices reflect predominantly hepatic HRase activity (37, 41). These results are compatible with the genetic data that conclusively demonstrate that the primary defect in sitosterolemia does not involve the HRase locus. They also support, but do not unambiguously prove, the stronger hypothesis that the primary defect does not down-regulate HRase synthesis at a genetic site remote from (*trans* to) the gene locus. The regulation of HRase activity is complex and is exerted at transcriptional and various post-transcriptional levels (46). Whatever the mechanism underlying the observed reduction in HRase mRNA and enzyme mass levels in sitosterolemia, it did not prevent the apparent increase in HRase activity elicited by cholestyramine in this study. As cholestyramine normally exerts its well-documented stimulation of HRase synthesis by reducing the intrahepatic level of the free sterol pool or pools responsible for the negative feedback control of HRase gene expression, it is reasonable to conclude that the same mechanism is operative in the two sitosterolemic homozygotes. Cobb et al. (47) reported a paradoxical decrease in the 24-h urinary excretion of MVA in a 10-year-old sitosterolemic girl given cholestyramine. As the subject also suffered from heterozygous familial hypercholesterolemia, the functional significance of this observation is difficult to determine. Considering the most plausible interpretaion of our data, two further questions arise. First, what further evidence exists in favor of suppression of basal HRase activity and cholesterol biosynthesis in phytosterolemia, secondary to the biochemical consequences of the primary defect? Second, what role, if any, does the diminished HRase activity play in accounting for the accumulation of plant sterols characteristic of this disorder?

It is not clear whether increased quantities of sitosterol alone can account for the suppression of HRase activity. In rat ileum and rat liver, increased sitosterol concentrations induced by feeding and infusion experiments, respectively, failed to inhibit HRase activity or reduce mRNA levels at concentrations similar to those found in phytosterolemic homozygotes (48–50). At these concentrations 7α-hydrox-

ylase activity was inhibited by 30% in the liver of the infused rats, a reduction similar to that observed in phytosterolemic patients. In a separate study, LDL-receptor expression was normal in phytosterolemic homozygotes (18). These data were taken to exclude secondary inhibition of HRase synthesis in this disorder, thus strengthening the hypothesis that the reduction in HRase activity is primary in nature. Contrary to these results, the inclusion of micellar sitosterol has been reported to inhibit cholesterol synthesis in cultured cells (51).

It is also far from clear that artificial elevation of sitosterol alone mimics the biochemical status of phytosterolemic patients. In this disorder other plant sterols as well as 5α-sterol alcohols are also present in excess. Perhaps more importantly, little is known of the levels of the isoprene pathway intermediates or of other sterol-derived molecules. In addition to 25-hydroxycholesterol, HRase activity is affected by a number of other compounds including mevalonate (52, 53), other phosphorylated isoprenoid substances (54), sterols, such as 24(S), 25-epoxycholesterol (55), endotoxins and cytokines (56). In addition, although genes coding for HRase, 7α-hydroxylase, farnesyl pyrophosphate synthase, and the LDL-receptor are often coordinately regulated, this is not universal. Experiments on T-lymphocytes (57) and leukemic cells (58) have demonstrated independent regulation of HRase and LDL-receptor activity. Although a number of genes involved in controlling intracellular cholesterol levels are regulated by a common set of steroid responsive elements (59), HRase activity is also regulated by a variety of post-transcriptional and post-translational mechanisms (52, 54, 55, 60, 61). There is thus ample scope for secondary inhibition of HRase expression in sitosterolemia that is wholly or partially alleviated by cholestyramine administration.

The failure of the phytosterolemic subject, JR, in contrast to her heterozygous mother, to respond to an HRase inhibitor with a fall in MVA levels (Fig. 4) is compatible with the proposal that basal suppression of HRase activity is close to maximum in this disorder. This is in accord with the markedly reduced expression of HRase mRNA and the reduced mass and activity previously reported as well as with the well-known poor response of patients with phytosterolemia to treatment with HRase inhibitors (3). The greater response to cholestyramine in the two homozygote sitosterolemic patients relative to the obligate heterozygote parents and the control subjects is also compatible with the above interpretation. Alternative explanations for this observation do exist. The sitosterolemic patients were younger (hence smaller) than the controls, which was unavoidable for ethical reasons. A reduced post-mevalonate flux along the cholesterogenic pathway has also been reported (18) and could account for the marked increase in MVA seen in the sitosterolemic homozygotes given cholestyramine, though the concurrent substantial increase in the L/C ratio militates against this explanation.

It is also unlikely, though not impossible, that the reduced HRase activity and diminished rate of cholesterol biosynthesis in phytosterolemic patients can account for the increased absorption of plant sterols and their accumulation in plasma and tissues observed in this disorder. The administration of an HRase inhibitor did not result in increased levels of plant sterols despite a significant fall in plasma cholesterol (Table 3). This has been previously reported (3) and has been further confirmed by us in a trial of atorvastatin in hypercholesterolemic subjects (unpublished data). The diminished rate of cholesterol production may, however, account for the tissue levels of this sterol being normal despite high absorptive rates and reduced rates of clearance.

In conclusion, the genetic results preclude a primary defect in phytosterolemia at the HRase gene locus. The functional data support this conclusion and suggest that the reduced enzyme activity and mass previously observed in this disorder are secondary events resulting in relatively normal cholesterol levels in the face of elevated cholesterol absorption and reduced excretion rates. Any inherited defect must account, directly or indirectly, for the observed hyperabsorption of plant sterols and down-regulation of cholesterol synthesis. Further study of the molecular and mechanistic basis of this disorder should provide further insight into these unresolved issues.

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