Feedback inhibition of the cholesterol biosynthetic pathway in patients with Smith-Lemli-Opitz syndrome as demonstrated by urinary mevalonate excretion

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Abstract **Smith-Lemli-Opitz syndrome (SLOS) is a genetic disorder characterized by low plasma cholesterol and high 7-dehydrocholesterol (7-DHC). Synthesis of cholesterol and 7-DHC and its metabolites is regulated by HMG-CoA reductase, whose activity can be measured by 24-h excretion of its product mevalonate. We devised a simple, non-invasive method for collecting 24-h urine in our subjects. With a background of a very low cholesterol diet, mean mevalonate excretion did not differ between controls and SLOS children, indicating that SLOS subjects have normal HMG-CoA reductase activity. In a short term feeding study, the effects of a high cholesterol diet in SLOS subjects include a significant 55% increase in plasma cholesterol levels and 39% decrease in mevalonate excretion and no change in plasma 7-DHC levels. However, in four SLOS subjects, fed a high cholesterol diet for 2–3 years, plasma cholesterol levels continued to increase, urinary mevalonate excretion remained low and total 7-DHC decreased significantly, likely from decreased total sterol synthesis. Thus, in SLOS subjects, HMG-CoA reductase activity was normal and was subject to normal cholesterol induced feedback inhibition. However, total sterol synthesis in SLOS may still be decreased because of increased diversion of mevalonate into the shunt pathway away from sterol synthesis.—** Pappu, A. S., R. D. Steiner, S. L. Connor, D. P. Flavell, D. S. Lin, L. Hatcher, D. R. Illingworth, and W. E. Connor. **Feedback inhibition of the cholesterol biosynthetic pathway in patients with Smith-Lemli-Opitz syndrome as demonstrated by urinary mevalonate excretion. *J. Lipid Res.* 2002.** 43: **1661–1669.**

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The Smith-Lemli-Opitz Syndrome (SLOS) is an autosomal recessive disorder with an incidence of at least one in 20,000 and a carrier frequency of 1 in 30 (1–4). This metabolic disorder is characterized clinically by specific facial dysmorphism, severe growth and feeding abnormalities, multiple congenital malformations, and endocrine and neurological dysfunction, including often-severe mental retardation (2–4). Elevated levels of plasma 7-dehydrocholesterol (7-DHC) and low or low-normal plasma cholesterol levels (5, 6) confirm the diagnosis of SLOS. The biochemical defect in SLOS subjects is a deficiency of sterol-delta-7-reductase (E.C.1.3.1.21) in the cholesterol biosynthetic pathway, which converts 7-DHC to cholesterol (7). Mutations in the gene encoding sterol-delta-7-reductase on chromosome 11q12-q13 cause SLOS (8–10). Although the biochemical and genetic basis for SLOS has been elucidated, the underlying pathogenesis is not well understood.

The multifaceted nature of the syndrome may be the consequence of a deficiency in blood and tissues of cholesterol (11) or accumulation of 7-DHC and its metabolites (12). Deficiency of cholesterol may interfere with structural/functional integrity of cellular membranes (13) with synapse formation (14), with production of steroid hormones and bile acids (15), and with maturation of the hedgehog family of morphogens involved in developmental patterning (16). Deficiency of cholesterol interferes with the response of frontal cortex neurons to

Abbreviations: 7-DHC, 7-dehydrocholesterol; 8-DHC, 8-dehydrocholesterol; SLOS, Smith-Lemli-Opitz syndrome.

[1] Drs. Pappu and Steiner shared equally in the writing of the manuscript. Dr. Pappu supervised and carried out the key laboratory work. Dr. Steiner carried out the clinical investigation.

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glutamate in a genetic mouse model (17). Accumulation of 7-DHC, and its oxidized byproducts, has been shown to inhibit HMG-CoA reductase activity in tissue culture studies and in a second genetic mouse model (18, 19). To date, no studies of the regulation of HMG-CoA reductase have been evaluated in vivo in subjects with SLOS.

One approach to assessing abnormalities in sterol synthesis in vivo is by measurement of mevalonate, the direct product of HMG-CoA reductase, which is a rate-limiting enzyme in cholesterol synthesis. Two pathways of mevalonate metabolism have been demonstrated (20, 21). The major pathway, called the Kandutsch-Russell pathway (20) (**Fig. 1**), leads to cholesterol synthesis. A second, normally minor pathway proposed by Edmond and Popjak (21), shunts mevalonate away from sterol production and ultimately results in oxidation to CO_2. Studies in our laboratory and others have shown that changes in the concentrations of mevalonate in plasma and urine reflect parallel changes in the activity of hepatic HMG-CoA reductase and thus normally reflect changes in the rates of whole body cholesterol biosynthesis (22–25). While plasma concentrations of mevalonic acid exhibit diurnal variations with peak values occurring at night (25–27), the mean 24-h plasma concentration is an accurate indicator of the rates of hepatic (whole body) cholesterol synthesis under different metabolic conditions. The 24-h urinary excretion of mevalonate reflects the integrated plasma concentration and provides a more practical and non-invasive way of assessing whole body cholesterol synthesis. Urinary mevalonate is also a good surrogate for measurement of HMG-CoA reductase activity in vivo.

The objective of treatment of patients with SLOS is to raise plasma cholesterol levels and to reduce the production of 7-DHC. Treatment with simvastatin, an HMG-CoA reductase inhibitor, has been shown to decrease HMG-CoA reductase activity and lower the production of 7-DHC in SLOS (28). Supplementation with dietary cholesterol is another option for potentially treating SLOS (29–31). We hypothesized that measurement of urinary mevalonate excretion in subjects with SLOS can be used to monitor HMG-CoA reductase activity in vivo to determine whether feedback inhibition of the enzyme occurs in SLOS and whether the rationale for using dietary cholesterol to inhibit synthesis of 7-DHC and its metabolites is sound.

In the present study, we determined urinary mevalonate excretion and plasma sterol levels in children with SLOS as compared with matched controls. The effectiveness of a high cholesterol diet in lowering urinary mevalonate and influencing plasma sterol composition was determined in patients maintained on a high cholesterol diet for a period of 1 to 3 months. Finally the ability of a high cholesterol diet to sustain reduction in urinary mevalonate, increase plasma cholesterol levels, and reduce 7-DHC and its metabolites was monitored in four patients so treated for more than 2 years. The results from these experiments would answer two questions: Do SLOS patients have normal activity of the rate-limiting enzyme (HMG-CoA reductase) in cholesterol biosynthesis and is this enzyme regulated normally by feedback inhibition from dietary cholesterol?

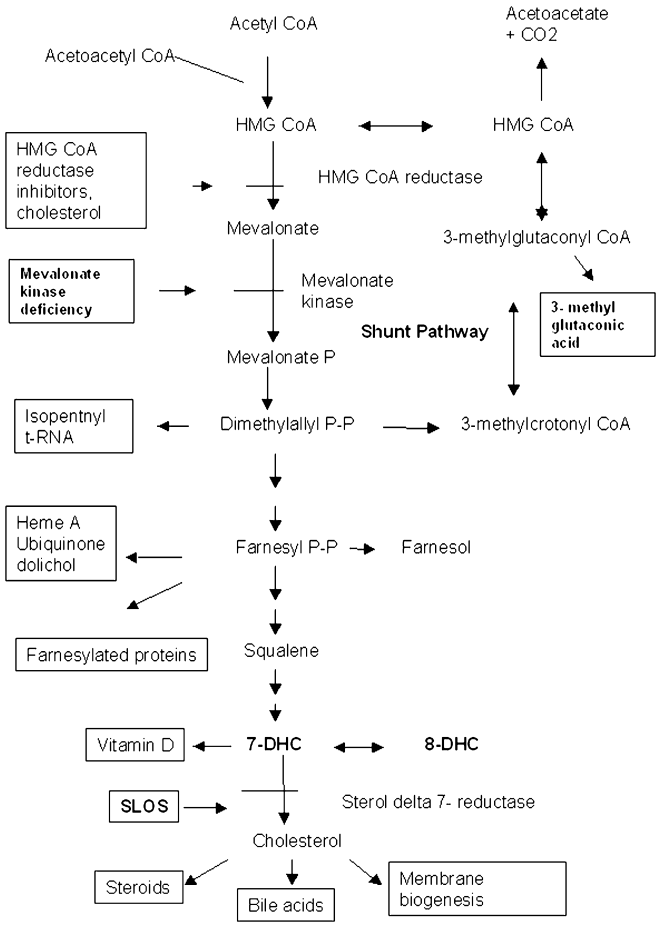

Fig. 1. Pathways of mevalonate metabolism for isoprenoid and sterol synthesis and the mevalonate shunt linking the isoprenoid pathway and mitochondrial acetyl-CoA pathway.

MATERIALS AND METHODS

Subjects

All studies were conducted in the General Clinical Research Center at Oregon Health & Science University. Informed consent was obtained from the parents of each patient and the OHSU Institutional Review Board approved the protocol. **Table 1** shows the sex, age, and weight of the 14 SLOS patients enrolled in the study, together with values for 11 healthy normolipidemic children. The SLOS patients ranged in age from 1 month to 15 years. Controls ranged from 2 months to 17 years of age. While the mean age of subjects with SLOS was slightly higher than the control group, the body weights were comparable.

Study design

Fourteen children with SLOS and 11 healthy normolipidemic children were maintained on very low cholesterol diets for a period of 3 to 4 weeks to approximate a uniform steady state of sterol homeostasis. Parents were given both oral and written instructions on institution of proper diet by registered dietitians and were asked to maintain intake records. All patients exhibited low to normal plasma cholesterol levels and a diagnostic accumulation of 7-DHC and its metabolites (8-DHC and cholestatriene-3β-ol) (**Table 2**). The plasma cholesterol levels varied in these patients between 40 and 109 mg/dl with an average of 70 ± 24.

TABLE 1. Description of experimental subjects

Control Subjects				SLOS Subjects				
No	Sex	Age	Body Wt	No	Sex	Feeding	Age	Body Wt
		years	*kg*				*years*	*kg*
1[a]	M	2.5	14.3	1[a]	F	G-tube	2.9	9.8
1[a]	M	0.3	7.7	2[b]	M	G-tube	0.6	5.4
3[b]	M	4.3	15.4	3	F	N	5.7	12.0
4[c]	F	5.0	26.7	4[c]	M	N	0.1	3.9
5	M	0.3	6.1	5	M	G-tube	14.8	29.2
6[d]	F	3.6	14.1	6	F	G-tube	9.4	25.4
7[d]	M	16.6	72.5	7	F	G-tube	3.3	10.4
8	F	0.2	5.6	8	F	N	13.4	61.8
9	F	1.8	14.5	9	F	N	13.0	36.9
10	F	1.5	11.0	10	F	G-tube	8.5	17.4
11	F	2.0	14.0	11	F	N	3.2	10.9
				12	F	N	3.8	11.3
				13	M	N	0.5	5.5
				14[d]	F	N	2.0	7.4

The controls consisted of normal recruited volunteers. Some children with SLOS were fed by gastrostamy (G-tube) while others took nourishment orally (N).

[a,b,c,d] Sibling groups.

The total 7-DHC and its metabolites constituted 5–40% of total plasma sterols in SLOS patients. Three SLOS patients had total plasma cholesterol levels above 100 mg/dl. The range of plasma cholesterol in control infants in this study was 140–189 (mean 166 ± 24 mg/dl), significantly higher than the mean total sterols (89 ± 22 mg/dl) in patients with SLOS. None of the controls had detectable 7-DHC or its metabolites.

Subjects were admitted to the metabolic ward of the General Clinical Research Center (GCRC) for 1-week periods for more precise dietary control and 24 h urine collection. Some control subjects were outpatients and some were siblings of SLOS subjects.

For studies comparing very low and high cholesterol diets, GCRC dietitians and cooks prepared specialized diets that met all nutritional requirements recommended by the National Research Council. Infants were fed breast milk and/or commercially available infant formulas and older infants were fed commercially available infant formula or breast milk plus pureed cereals, fruits and vegetables. Older children were fed mixed general foods. In all diets, protein contributed 15–20%, fat 20–30%, and carbohydrate 45–55% of total caloric intake. The high cholesterol diets consisted of the very low cholesterol diet supplemented with egg yolks. After hard-boiling the egg, the yolk was shelled out, mashed and then blended with formula, breast milk, or food according to the child's routine diet (31). Prepared in this way, the egg yolk readily passed through a nipple or a feeding tube. The potentially allergenic egg white was completely avoided. A portion of the prepared food was analyzed to determine the precise cholesterol intake. Cholesterol intake of the study patients ranged from 0–19 mg/day on the very low cholesterol diet and 190–270 mg/day on the high cholesterol diet.

To study the long-term effects of a high cholesterol diet, GCRC dieticians trained the parents of children with SLOS in the preparation of a hard-boiled egg yolk supplemented diet for use at home.

TABLE 2. The composition of the plasma sterols in patients with SLOS fed a very low cholesterol diet

	Plasma Sterols							
No	Chol	7-DHC	8-DHC	Cholestatriene-3β-ol	Total Sterols	7-DHC + its Metabolites Total	% Total Sterols	Ratio 7-DHC/Chol
	mg/dl							
1[a]	46.7	9.5	13.9	2.0	72.1	25.4	35.2	0.20
2[a]	88.3	13.2	12.9	2.1	116.3	28.2	24.2	0.15
3	57.5	3.7	4.9	0.5	66.6	9.1	13.7	0.06
4	75.5	9.4	4.5	1.2	90.6	15.1	16.7	0.12
5[a]	39.8	12.6	9.3	1.8	63.5	23.7	37.3	0.32
6[a]	49.5	11.9	8.8	1.6	71.8	22.3	31.1	0.24
7[a]	59.5	11.6	9.7	1.5	82.3	22.8	27.7	0.19
8	104.5	13.6	11.8	2.6	132.5	28.0	21.1	0.13
9	78.1	5.2	5.8	1.6	90.7	12.6	13.9	0.07
10[a]	39.8	14.0	12.1	1.5	67.4	27.6	40.9	0.35
11	101.6	2.3	2.5	0.3	106.7	5.1	4.8	0.02
12	109.4	8.0	2.5	0.6	120.5	11.1	9.2	0.07
13	52.8	11.0	7.9	1.9	73.6	20.8	28.3	0.21
14	80.3	3.0	4.7	0.0	88.0	7.7	8.8	0.04
Mean ± SD	70.2 ± 24.2	9.2 ± 4.1	8.0 ± 3.9	1.4 ± 0.8	88.8 ± 22.2	18.5 ± 8.2	22.3 ± 11.5	0.13 ± 0.17

Plasma sterol levels were determined by GLC as described in Materials and Methods. 7-DHC+ its metabolites include 7-DHC, its isomer 8-DHC, and its metabolite cholestatriene-3β-ol.

[a] SLOS subjects fed by G-tube.

Collection of samples and biochemical analysis

Collection of urine. We report a novel technique for 24-h urine collection in infants. Obtaining 24-h urine collections from some subjects was problematic, as some of the subjects were infants and many of the older subjects with SLOS had cognitive and physical limitations and could not assist in urine collection. Hence, we devised and standardized a simple non-invasive method to collect urine. Twenty four-hour urine collections from infants were obtained using pre-weighed cloth diapers. All diapers used in a period of 24-h for each individual child were collected in labeled plastic bags and stored at 4°C. When urine and stool were mixed on the diaper, the dried stool was scraped off the diapers. The weight difference between wet and dry diapers allowed for calculation of urine weight and volume over a 24-h period.

Urine-containing diapers were soaked overnight in a measured amount of distilled water (2–3 liters) in one-gallon paint cans (3 to 4 diapers/can). The cans were shaken for 5 min using a paint shaker. Aliquots of the diluted diaper extract were taken for creatinine and mevalonate estimations and stored at −20°C. Total extract volumes were calculated by adding the urine weight and the volume of distilled water added for extraction of urine. The urine extraction was found to be 98% complete using ^{3}H-labeled mevalonate as a tracer. Toilet trained subjects collected 24-h urine. After noting the volume, aliquots were frozen at −20°C.

Urinary mevalonate

Aliquots of urine from toilet trained and other subjects were thawed and centrifuged at 2,000 *g* for 30 min at 4°C to remove insoluble residues. Mevalonic acid in the supernatant was phosphorylated using ^{32}P-γ-ATP (NEN, MA) by purified pig liver mevalonate kinase. [^{14}C]mevalonate phosphate was extracted with ethanol and quantified using a modification of the isotope dilution chromatography method of Popjak et al. (32, 33). The coefficient of variation within experiment and between experiments was less than 5%. The presence of minute amounts of stool in the urine did not interfere with mevalonate estimations. The GCRC Core Laboratory determined creatinine concentrations in the urine samples. Urinary mevalonate excretion in a given individual may vary by 35% under stable metabolic conditions (24). Hence, we measured mevalonate in 24-h urine samples collected for 5 consecutive days of each dietary period. Each urinary meval-

onate value reported is the mean of mevalonate measurements on five independent determinations for each individual patient.

Plasma sterols

Venous blood samples were collected in EDTA-containing tubes from each individual when they were admitted to the GCRC. Plasma was separated by centrifugation of collected blood. Sterols were extracted from plasma saponified with alcoholic KOH and then converted to trimethylsilyl ether derivatives, which were quantitated using gas liquid chromatography (GC) as previously described (34, 35). Cholestatriene-3β-ol found in the blood of SLOS patients has been shown to be a GC artifact formed in fractions containing 8-DHC (36).

Statistical analysis

Statistical analysis was performed by two-tailed Student's *t*-tests, paired *t*-tests, or ANOVA using SPSS statistical software package 10.00 SPSS (Inc.) (37). Results were expressed as mean ± SD.

RESULTS

The 24-h urinary mevalonate excretions in children with SLOS and control children during very low cholesterol diet period were very similar (**Table 3**). In SLOS subjects urinary mevalonate excretion varied from 0.22 to 1.6 (mean 0.83 ± 0.47 μmol/day), while in the control subjects urinary mevalonate excretion varied from 0.2 to 2.3 (mean 0.76 ± 0.66 μmol/day). The wide variation in the observed urinary mevalonate excretion may be due to differences in age, sex, and body weights. Urinary mevalonate excretion expressed as nmol/day/kg body weight varied in SLOS subjects from 26.3 to 144.8 and in control children from 18 to 101.6 (mean 57.8 ± 31 vs. 48.8 ± 24). Urinary mevalonate excretion was not statistically different between control and SLOS subjects ingesting a very low cholesterol diet. These data indicate normal activity of HMG-CoA reductase.

TABLE 3. Urinary mevalonate excretion in SLOS subjects and controls fed a very low cholesterol diet

	Urinary Mevalonate				Urinary Mevalonate		
	Control		SLOS		Control		SLOS
	μmol/day				*nmol/day/kg body wt*		
1	0.85	1	0.48	1	59.1	1	49.1
2	0.78	2	1.32	2	101.6	2	144.8
3	0.66	3	0.61	3	42.5	3	51.2
4	0.80	4	0.38	4	30.0	4	97.4
5	0.20	5	1.47	5	33.1	5	50.3
6	0.43	6	1.08	6	30.6	6	42.4
7	2.34	7	0.58	7	32.3	7	55.6
8	0.24	8	1.63	8	43.2	8	26.3
9	0.26	9	1.09	9	18.0	9	29.5
10	0.59	10	1.22	10	53.5	10	69.9
11	1.26	11	0.32	11	90.2	11	29.6
		12	0.80			12	70.6
		13	0.22			13	39.6
		14	0.39			14	53.2
Mean ± SD	0.76 ± 0.61		0.83 ± 0.47	Mean ± SD	48.5 ± 26.2		57.8 ± 31.4

Each value is a mean of five independent determinations. Urinary mevalonate was determined by a radioenzymatic method as described in Materials and Methods.

Variations in the methods of urine collection may result in incomplete urine collections and wide variation in 24-h urinary mevalonate values. The completeness of 24-h urine collection from each patient was determined by measuring urinary creatinine concentration (38). In SLOS subjects, urinary creatinine varied from 8.5 to 15.2 (mean 11.6 ± 2.2 mg/day/kg body weight). In controls, urinary creatinine varied from 7.3 to 23 mg/day/kg body weight (mean 12.4 ± 5.9). These results are in agreement with creatinine values reported by Foman (39). There were no significant differences in creatinine excretion between SLOS subjects and controls. There were no differences in creatinine values whether urine was collected from toilet-trained subjects or extracted from diapers. The completeness of urine collection was further confirmed by the significant correlation ($r = 0.77$, $P = 0.0003$) observed between urinary creatinine excretion and age (months) of a given individual. In these children, creatinine excretion increased with muscle mass that increased with age, similar to the pattern observed in young adults. (38)

We found no correlation between urinary mevalonate excretion and plasma cholesterol levels or total sterol levels. We found a significant correlation ($r = 0.59$, $P < 0.03$) between μmoles of urinary mevalonate excreted per day and total plasma 7-DHC and its metabolites (**Fig. 2**). When urinary mevalonate was expressed as nmol/day/kg body wt, however, there was no significant correlation between urinary mevalonate excretion and 7-DHC and its metabolites. Body weight is a confounder affecting both 7-DHC and its metabolites and urinary mevalonate. With the inclusion of body weight in multivariate linear regression analysis of 7-DHC and its metabolites, urinary mevalonate in the patients with SLOS is an independent predictor of total 7-DHC and its metabolites ($P < 0.03$). The inclusion of age and sex as independent variables did not affect the analysis.

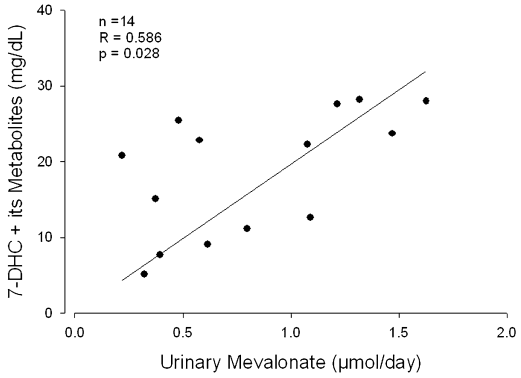

Fig. 2. Correlation between urinary mevalonate excretion and plasma 7-DHC and its metabolites (8-DHC and cholestatriene-3β-ol) in SLOS patients. Total plasma 7-DHC and its metabolites are expressed as mg/dl and is a single determination. Urinary mevalonate expressed as μmol/day is an average of five independent determinations.

TABLE 4. Effects of dietary cholesterol on plasma sterols and mevalonate excretion

Diet	Body Wt	Urinary Mevalonate	Plasma Sterols						Ratio
			Chol	7-DHC	8-DHC	Cholestatriene-3β-ol	Total Sterols	7-DHC + its Metabolites	7-DHC/Chol
	kg	*nmol/day/kg body wt*	*mg/dl*						
Very low cholesterol	8.3 ± 3.2	69.3 ± 38	68.8 ± 20.2	8.7 ± 4.1	8.0 ± 4.4	1.4 ± 0.7	86.8 ± 18.8	18.1 ± 8.6	0.14 ± 0.1
High cholesterol	8.6 ± 1.8	42[a] ± 15	106.6[a] ± 49.4	10.3 ± 4.6	7.9 ± 3.7	2.1 ± 1.2	126.9[a] ± 46.8	20.2 ± 8.8	0.13 ± 0.1

Cholesterol content of low chol diet was 0–19 mg/day. Cholesterol content of high chol diet was 190–270 mg/day. High chol diet was given for a period of 1 to 3 months. 7-DHC and its metabolites include 7-DHC, 8-DHC, and cholestatriene-3β-ol. Each value is expressed as a mean ± SD of seven individual values. Plasma sterols were single determinations for each individual. Urinary mevalonate were average of five determinations for each individual.

[a] Significantly different from corresponding value on very low cholesterol diet. $P < 0.05$.

A subset of subjects with SLOS (n = 7) were fed a high cholesterol diet for 1 to 3 months. The plasma sterol levels and urinary mevalonate excretion in this group of subjects fed a very low and high cholesterol diets are given in **Table 4**. In these subjects, the administration of dietary cholesterol in the form of half to two egg yolks per day depending on body weight increased plasma cholesterol levels by 55%, from a baseline mean of 68.8 ± 20.2 to 106.6 ± 49 mg/dl on the high cholesterol diet ($P < 0.05$). The amount of total 7-DHC and its metabolites remained unchanged over this short time period even when the cholesterol content in the diet was increased. Individual 7-DHC and its metabolites levels (8-DHC or Cholestatriene-3β-ol) were also unchanged. However, cholesterol supplementation decreased urinary mevalonate excretion in these patients by 39% ($P < 0.05$) from a baseline mean of 69.3 ± 38 to 42 ± 15 (nmol/day/kg body wt) on a high cholesterol diet. Thus, dietary cholesterol administered for a short duration to subjects with SLOS reduced biosynthesis of the cholesterol precursor mevalonate, demonstrating feedback inhibition of HMG-CoA reductase.

Studies from our laboratory have shown that long-term supplementation of cholesterol with egg yolk increased plasma cholesterol and decreased 7-DHC in subjects with SLOS (31). We measured plasma sterols and urinary mevalonate excretion in four patients at baseline and at the end of 2 months and 2 years of high cholesterol feeding. (**Table 5**). As previously reported, with a high cholesterol diet, the plasma cholesterol levels in these patients increased significantly at the end of 2 months from the baseline mean of 76.5 ± 23 mg/dl to 120.6 ± 55 and increased further to171.5 ± 73.2 at the end of 2 years. Total 7-DHC and its metabolites and individual 7-DHC and its metabolites did not decrease at the end of 2 months but did decrease significantly at the end of 2 years from 17.6 ± 9.5 mg/dl to 8.3 ± 7.8 ($P < 0.05$). Urinary mevalonate excretion in these four patients decreased significantly at the end of 2 months from the baseline value of 81.8 ± 49. nmol/day/kg body weight to 49.2 ± 23.9 and remained low (32.6± 15.5 nmol/day/kg body weight) during high cholesterol feeding.

Early diagnosis of SLOS in one male child led to institution of a high cholesterol diet at 1.5 months. Plasma sterol and urinary mevalonate measurements were carried out over a 42-month period in this growing child with SLOS maintained continuously on a high cholesterol diet. (**Fig. 3**). The increase in body weight in kilogram is also depicted. The plasma cholesterol concentrations increased by 3-fold, from 75 to 218 mg/dl at the end of 3 years. The total plasma 7-DHC and its metabolites did not decrease at the end of 2 months. However, with continued high cholesterol diet, total 7-DHC and its metabolites decreased from 15.1 mg/dl to 4.1 mg/dl. The urinary mevalonate excretion in this child decreased at the end of 2 months of diet intervention and remained low.

DISCUSSION

In subjects with SLOS, cholesterol synthesis is impaired because of a defect in the enzyme sterol-delta-7-reductase

TABLE 5. Long-term effects of dietary cholesterol on plasma sterols and mevalonate excretion in patients with SLOS (n = 4)

Duration of Treatment	Body Wt	Urinary Mevalonate	Plasma Sterols						Ratio
			Chol	7-DHC	8-DHC	Cholestatriene-3β-ol	Total Sterols	7-DHC + its Metabolites	7-DHC/Chol
	kg	*nmol/day/kg body wt*	*mg/dl*						
Baseline	8.4 ± 3.1	81.8 ± 49.2	76.5 ± 23.0	8.5 ± 4.4	7.8 ± 5.2	1.4 ± 0.8	94.2 ± 16.6	17.6 ± 9.6	0.13 ± 0.08
Two months	6.8 ± 2.7	49.2[a] ± 23.9	120.6 ± 55.0	10.5 ± 6.5	8.5 ± 5.0	2.2 ± 1.5	141.8 ± 47.1	21.2 ± 12.1	0.11 ± 0.07
Two years	13.0[a] ± 1.5	32.6[a] ± 15.5	171.5[a] ± 73.2	4[a] ± 3.1	3.9[a] ± 3.7	0.3[a] ± 0.3	179.8 ± 42.2	8.3[b] ± 7.8	0.03[a] ± 0.02

Patients were treated with high chol diet for a period of 2 years. Cholesterol content of the diet was 190–270 mg/day. 7-DHC and its metabolites include 7-DHC, 8-DHC, and cholestatriene-3β-ol. Each value is expressed as a mean ± SD of four individual values. Plasma sterols were single determinations for each individual. Urinary mevalonate were average of five determinations for each individual.

[a] Significantly different from corresponding value at baseline on a very low cholesterol diet. $P < 0.05$.

[b] Significantly different from corresponding value at 2 months on high cholesterol diet. $P < 0.05$.

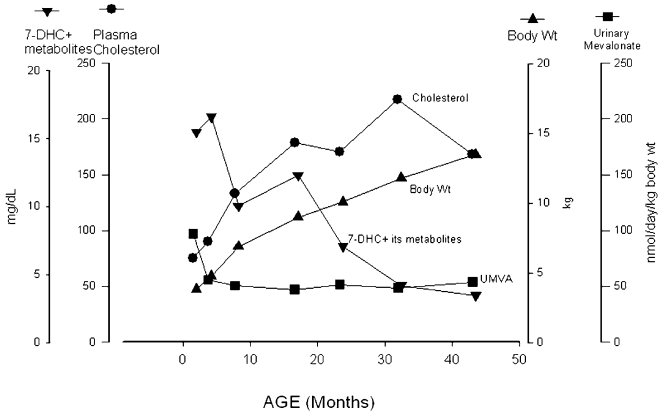

Fig. 3. Plasma sterols and urinary mevalonate excretion in a growing child with SLOS (Patient # 4) maintained on a high cholesterol diet. Body weight (upright triangle) is expressed as kg. Plasma cholesterol (circle) and total 7-DHC and its metabolites (7-DHC, 8-DHC and cholestatriene-3β-ol) (inverted triangle) are expressed as mg/dl. Urinary mevalonate excretion (UMVA) (square) expressed as nmol/day/kg body wt is an average of five independent determinations.

which catalyzes a distal step in cholesterol biosynthesis. This blockage of cholesterol synthesis results in low plasma cholesterol levels and high levels of 7-DHC. This impairment in cholesterol biosynthesis can usually be measured by determining urinary mevalonate which reflects the rate of HMG-CoA reductase activity and hence the rate of whole body cholesterol synthesis (22–26, 33, 40). Twenty-four hour urinary mevalonate excretion was the same in SLOS patients as in normal controls ingesting a very low cholesterol diet, indicating normal HMG-CoA reductase activity despite low plasma cholesterol levels. A second significant finding in this study was that in SLOS subjects, as in normal subjects, dietary cholesterol down regulated the activity of HMG-CoA reductase, supporting the therapeutic rationale for dietary cholesterol in the SLOS. This reduction in mevalonate continued for as long as 2 years when measured periodically in SLOS patients maintained on a high cholesterol diet.

Twenty-four hour urinary mevalonate excretion is a measure of HMG-CoA reductase activity and is specifically useful to study feedback regulation of the enzyme in vivo by cholesterol and other inhibitors. Under normal metabolic conditions, urinary mevalonate excretion is a measure of total sterol and cholesterol synthesis, though at first glance it is difficult to understand why HMG-CoA reductase activity is the same in SLOS subjects with very low plasma cholesterol levels as in controls. Patients with inherited defects in the synthesis of apo-B containing lipoproteins (abetalipoproteinemia) that result in very low plasma cholesterol concentrations, have been shown to have higher rates of cholesterol synthesis by sterol balance studies and high urinary mevalonate excretions (33). Hence, one could logically expect that low plasma cholesterol levels in SLOS might stimulate HMG-CoA reductase activity, increasing the production of 7-DHC and its metabolites. The finding of normal urinary mevalonate excretion in SLOS patients fed the very low cholesterol diet indicates normal hepatic HMG-CoA reductase activity in SLOS, despite low plasma and tissue cholesterol levels. One cannot rule out the possibility that HMG-CoA reductase activity may be up regulated in both controls and SLOS subjects, probably more in SLOS than control, due to the presence of very low levels of cholesterol in their tissues, (lack of feedback inhibition).

Our data are in are in agreement with those reported by Nissinen et al. (41). These authors have shown that in seven patients with SLOS, levels of plasma lathosterol, another marker of cholesterol biosynthesis and of flux of precursors through cholesterol pathway proximal to the blockage, were comparable with those observed in a control group. Lathosterol levels did not decrease below controls, indicating that the inhibition of lathosterol-C5-desaturase by the product 7-DHC may be minimal. Our results, however, are in contrast to those reported by Honda et al. (42), who have shown that plasma mevalonate levels in patients with SLOS were significantly lower than in age matched controls. However, plasma mevalonate was determined in single blood samples in these patients who were not studied in a steady state or under metabolic ward conditions. The measurement of a single plasma mevalonate concentration may not be a true reflection of HMG-CoA reductase activity, as plasma mevalonate concentrations exhibits diurnal periodicity (25–27). The observed discrepancy in these results may be in part due to differences in the sources and measurement of mevalonate, severity of SLOS, and cholesterol content of the diet. Fitzky et al. (19) have also shown that 7-DHC causes enhanced proteolysis of HMG-CoA reductase in a dose dependent manner in tissue culture cells lacking DHCR7. 7-DHC and its derivatives may not be as effective in vivo as in vitro in inhibiting HMG-CoA reductase or degrading the enzyme. 7-DHC and its oxidized products were very toxic when added in vitro to embryo cultures (12), but did not cause any damage to the development of embryos when given to AY9944-treated pregnant rats with high levels of plasma 7-DHC (11).

Dietary cholesterol has been shown to regulate HMG-CoA reductase activity by feedback inhibition (43–44). Egg yolk supplemented high cholesterol diets not only increased plasma cholesterol levels but also decreased urinary mevalonate excretion in our experiments. The results of the current study indicate that cholesterol induced feedback regulation of HMG-CoA reductase is indeed functional in SLOS subjects. In these patients, there was a significant correlation ($R = 0.948$, $P = 0.001$) between baseline urinary mevalonate excretion in a given individual and the reduction in urinary mevalonate excretions in response to cholesterol supplementation (**Fig. 4**). This indicates that dietary cholesterol induced a greater change in urinary mevalonate in SLOS patients with high baseline urinary mevalonate. A similar correlation was found in patients with homozygous familial hypercholesterolemia in response to treatment with atorvastatin, a HMG-CoA reductase inhibitor (45).

The long-term feeding of a high cholesterol diet to SLOS subjects not only increased plasma cholesterol lev-

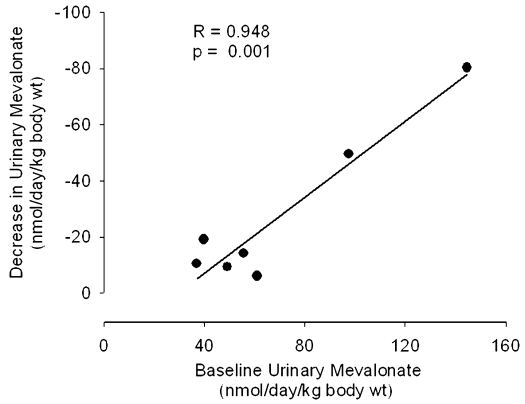

Fig. 4. Correlation between baseline urinary mevalonate excretion and its decrease with high cholesterol diet in seven patients with SLOS. Both baseline and the changes in urinary mevalonate excretion are expressed as nmol/day/kg body weight.

els but also reduced 7-DHC. This is probably attributable to the long-standing inhibition of HMG-CoA reductase as indicated by reduced urinary mevalonate excretion. This observed reduction in total plasma 7-DHC and its metabolites is similar to that reported in two patients with SLOS treated with simvastatin for 14 and 23 months (29). Simvastatin, a competitive inhibitor of HMG-CoA reductase activity, decreases urinary mevalonate excretion (46).

Sterol balance studies in our laboratory have shown that in children with SLOS (n = 8) fed a very low cholesterol diet, the rate of cholesterol biosynthesis was reduced and the synthesis of 7-DHC and 8-DHC was profoundly increased (47). The total sterol synthesis was reduced to 60% of controls. The observed reduction in sterol synthesis in these subjects probably resulted from considerable diversion of the synthesized mevalonate away from sterol synthesis to production of other isoprenoid intermediates and to the mitchondrial mevalonate shunt pathway (Fig. 1). The mevalonate shunt links isoprenoid metabolism with mitochondrial acetyl-CoA metabolism through an intermediate 3-methylglutaconic acid. This intermediate is excreted in urine when shunt pathway is stimulated and overloaded (21,48). The studies of Kelly and Kratz have shown that increased mevalonate shunt activity does occur in SLOS patients, as they excrete significant amounts of 3-methylglutaconic acid (49). Other reports have shown that it is not unusual for the mevalonate shunt pathway to divert as much as 30–45% of mevalonate away from sterol synthesis (50–53). Being female, sex hormones, fasting, and experimental diabetes increased the shunt activity by 30–35% (50–53). In SLOS, shunting of mevalonate and its isoprenoid derivatives may be increased by the accumulation of both non-sterol and sterol products of mevalonate (7-DHC and its metabolites). Shunting of mevalonate and its down stream products may be protective as these sterols and non-sterol mevalonate products enhance HMG-CoA reductase degradation (54). Under normal metabolic conditions, urinary mevalonate excretion is a measure of cholesterol and sterol synthesis. However, in SLOS, urinary mevalonate does not represent total sterol synthesis. The apparent discrepancy between the current data and reported data is due to the fact that urinary mevalonate excretion in SLOS does not represent the total sterol synthesis because of diversion to the shunt pathway. The enzymes involved in the shunt pathway are perhaps activated by the accumulation of 7-DHC and its metabolites. Studies are planned to elucidate the role of Popjak's mevalonate shunt pathway in SLOS.

In summary, our studies indicate that mevalonate production is the same in SLOS patients as in normals, thus indicating the integrity of the HMG-CoA reductase enzyme system and that the early stages of cholesterol biosynthesis proceed normally despite the presence of 7-DHC. A new and interesting finding is that normal feedback inhibition of HMG-CoA reductase occurred in SLOS patients by high amounts of dietary cholesterol. We postulated a role for mevalonate shunt pathway in SLOS that needs to be investigated.

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