Sterol balance in the Smith-Lemli-Opitz syndrome: reduction in whole body cholesterol synthesis and normal bile acid production

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Abstract **The Smith-Lemli-Opitz syndrome (SLOS) is a multiple malformation/mental retardation syndrome caused by a deficiency of the enzyme 7-dehydrocholesterol Δ^7-reductase. This enzyme converts 7-dehydrocholesterol (7-DHC) to cholesterol in the last step in cholesterol biosynthesis. The pathology of this condition may result from two different factors: the deficiency of cholesterol itself and/or the accumulation of precursor sterols such as 7-DHC. Although cholesterol synthesis is defective in cultured SLOS cells, to date there has been no evidence of decreased whole body cholesterol synthesis in SLOS and only incomplete information on the synthesis of 7-DHC and bile acids. In this first report of the sterol balance in SLOS, we measured the synthesis of cholesterol, other sterols, and bile acids in eight SLOS subjects and six normal children. The diets were very low in cholesterol content and precisely controlled. Cholesterol synthesis in SLOS subjects was significantly reduced when compared with control subjects (8.6 vs. 19.6 mg/kg per day, respectively, $P < 0.002$). Cholesterol precursors 7-DHC, 8-DHC, and 19-nor-cholestatrienol were synthesized in SLOS subjects (7-DHC synthesis was 1.66 ± 1.15 mg/kg per day), but not in control subjects. Total sterol synthesis was also reduced in SLOS subjects (12 vs. 20 mg/kg per day, $P < 0.022$). Bile acid synthesis in SLOS subjects (3.5 mg/kg per day) did not differ significantly from control subjects (4.6 mg/kg per day) and was within the range reported previously in normals. Normal primary and secondary bile acids were identified. This study provides direct evidence that whole body cholesterol synthesis is reduced in patients with SLOS and that the synthesis of 7-DHC and other cholesterol precursors is profoundly increased. It is also the first reported measure of daily bile acid synthesis in SLOS and provides evidence that bile acid supplementation is not likely to be necessary for treatment. These sterol balance studies provide basic information about the biochemical defect in SLOS and strengthen the rationale for the use of dietary cholesterol in its treatment.**—Steiner, R. D., L. M. Linck, D. P. Flavell, D. S. Lin, and W. E. Connor. **Sterol balance in the Smith-Lemli-Opitz syndrome: reduction in whole body cholesterol synthesis and normal bile acid production.** ***J. Lipid Res.*** **2000.** 41: **1437–1447.**

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The Smith-Lemli-Opitz syndrome (SLOS) (1) is an autosomal recessive disorder. It is characterized by microcephaly, cleft palate, mental retardation, growth retardation, dysmorphic facies, limb abnormalities (especially syndactyly of the toes), genital disorders, endocrine malfunction, cataracts, and heart and kidney malformations (1–8). Affected individuals may have virtually all these features or may be quite mildly affected with only growth impairment, subtle dysmorphic facial features, toe syndactyly, and learning disability. Heterozygotes (carriers) have no discernible phenotype. No proven therapy is available. SLOS is estimated to occur in 1 in 20,000 births, yielding an estimated carrier frequency of 1 to 2%, making it one of the most common autosomal recessive disorders (4, 8). Tint and colleagues found reduced cholesterol and elevated 7-dehydrocholesterol (7-DHC) levels in the plasma and tissues of these patients and postulated that SLOS resulted from a defect in cholesterol and bile acid biosynthesis (9–12). Besides 7-DHC, other sterol derivatives (e.g., 8-dehydrocholesterol) have been found in the plasma of these patients (13). Deficiency of the human sterol Δ^7-reductase enzyme (7-dehydrocholesterol Δ^7-reductase; EC 1.3.1.21) was subsequently shown in hepatocytes and fibroblasts from affected individuals (14, 15). This enzyme converts 7-DHC to cholesterol in the final step of cholesterol biosynthesis. With our collaborator, F. D. Porter, and

Abbreviations: 7-DHC, 7-dehydrocholestrerol; 8-DHC, 8-dehydrocholesterol; GLC, gas–liquid chromatography; HMG-CoA reductase, 3-hydroxy-3-methylglutaryl-CoA reductase; 19-nor-cholestatrienol, 19-nor-5,7,9,(10)-cholestatrienol; SLOS, Smith-Lemli-Opitz syndrome; TLC, thin-layer chromatography; TMS, trimethylsilyl ether.

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colleagues, we and others have found that mutations in the 7-dehydrocholesterol Δ^7-reductase gene (*DHCR7*) cause SLOS (16–18).

The clinical manifestations of SLOS may result from cholesterol deficiency or from the toxicity of precursor sterols, particularly 7-DHC (normally absent or detected in only trace quantities in plasma). Cells use cholesterol for membrane synthesis and as a precursor for steroid hormones and bile acids. Cholesterol is also needed for autoprocessing (activation) of Sonic hedgehog (Shh), an important protein in the early limb patterning and craniofacial development in the human embryo (19–21). Abnormal Shh activation and/or signaling may explain the malformations in SLOS (22).

7-DHC likely does play a role in the pathogenesis of SLOS. 7-DHC impairs learning in a rat model of SLOS, where rats are treated with an inhibitor of 7-dehydrocholesterol Δ^7-reductase, BM 15.766. The learning impairment was prevented by administration of supplemental cholesterol, which lowered the 7-DHC content (but did not raise cholesterol content) in rat brain (23). AY 9944 is another inhibitor of the 7-dehydrocholesterol Δ^7-reductase enzyme. Oxidized derivatives of 7-DHC induce growth retardation and embryotoxicity in cultured AY 9944-treated rat embryos. Cholesterol supplementation ameliorated the growth retardation and morphologic abnormalities normally caused by the inhibitor in these embryos. Supplementation with 7-DHC, on the other hand, did not restore growth and, in fact, impaired the beneficial effects of cholesterol added simultaneously. Photooxidation of the 7-DHC-supplemented culture medium enhanced the embryotoxicity of 7-DHC (24).

Supplemental dietary cholesterol may be beneficial for SLOS patients because they appear to have a cholesterol deficiency syndrome (25–28). In addition, production of precursors of cholesterol synthesis that might contribute to the SLOS phenotype might also be inhibited by cholesterol supplementation due to inhibition of 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase. Basic information about the synthesis of cholesterol, precursor sterols, and bile acids and methods for evaluating the effects of cholesterol supplementation on SLOS subjects are needed to understand more fully the biochemical defects before truly rational and effective therapy can be devised.

As a prelude to development of a rational therapy for SLOS, we sought in this initial investigation to measure whole body cholesterol and sterol precursor synthesis as well as bile acid synthesis under steady state conditions in subjects with SLOS and normal age-matched control subjects. Such measurements can best be accomplished by the sterol balance technique. We hypothesized that cholesterol and bile acid synthesis in SLOS subjects would be markedly reduced compared with control subjects because of the enzymatic block in cholesterol synthesis, but that total sterol synthesis would be normal. We also hypothesized that synthesis of cholesterol precursors could be measured by the sterol balance technique. Data on cholesterol and bile acid synthesis in SLOS subjects will serve as a basis for optimizing the dose of cholesterol (and possibly bile acids) needed for potential therapy. On the basis of our current study, we report that whole body cholesterol synthesis is reduced in SLOS, that total sterol synthesis is reduced in SLOS subjects, and that bile acid synthesis in SLOS does not differ significantly from that in control subjects.

MATERIALS AND METHODS

Subject characteristics

These studies were approved by the Oregon Health Sciences University (OHSU, Portland, OR) Investigational Review Board and for all subjects studied the parents gave informed consent. Eight subjects with SLOS were enrolled. Their ages, gender, weight, and cholesterol, 7-DHC, 8-DHC, 19-nor-cholestatrienol, and total sterol levels after consuming very low cholesterol diets are listed in **Table 1**. Low plasma cholesterol levels and the characteristic accumulation of 7-DHC and other cholesterol precursors in plasma were observed. The mean plasma cholesterol level was 84 mg/dl and means of the abnormal sterols were 10.1, 6.9, and 1.4 mg/dl for 7-DHC, 8-DHC, and 19-nor-cholestatrienol, respectively. Three of the SLOS subjects had cholesterol levels greater than 100 mg/dl, suggesting that the diagnosis might have been missed if biochemical testing included only cholesterol level rather than sterol profile. All the subjects have SLOS type I; none would be classified as the more severe SLOS type II. Not all subjects had severity scores calculated, but the least severely affected patient has a severity score of 11. Control subjects ranged in age from 1 to 5 years. Control subjects 3–6 were studied as inpatients and are siblings of four SLOS subjects. The mean age of control subjects was 2.8 years, and the mean weight was 16 kg (range, 11.1–26.7 kg). We attempted to use age-matched control subjects but were unsuccessful in studying control subjects as young (6 weeks) as the youngest SLOS subject or as old (13 years) as the oldest SLOS subject. Stools in young children are often too runny to use in sterol balance studies and older children are often unwilling to collect their stools.

Control of dietary cholesterol intake

SLOS subjects and control subjects 3–6 were admitted to the OHSU General Clinical Research Center (GCRC) for 1-week periods. Instructions were given for an essentially cholesterol-free diet to be fed at home for three or more weeks prior to admission to the GCRC. This was easily accomplished in most cases because many of the infants were receiving exclusively infant formula containing cholesterol concentrations of only 10.5–35.7 mg/1,000 ml. During each admission a very low cholesterol (essentially cholesterol-free) diet was fed. The study diet was fed for at least 3 weeks total to allow for stabilization and steady state conditions. The subjects were studied in the GCRC under metabolic ward conditions.

GCRC dieticians and cooks prepared the specialized diets and nurses collected patient samples at baseline and during the study periods. The food intake for infants consisted of commercially available infant formula feedings plus pureed cereals, fruits, and vegetables in which protein contributed 15–20%, fat 20–30%, and carbohydrate 45–55% of the total calorie intake and cholesterol content was low. The diets were provided with precise cholesterol content known and controlled. Older children were fed mixed general foods with the same caloric distribution (29). The dietary prescriptions met the daily recommended allowances of the National Research Council. Some SLOS subjects were tube fed because of sucking and swallowing difficulties, but the same principles were applied to the diets of those individuals. The mothers of the children were given diet

TABLE 1. Characteristics and plasma sterol levels of SLOS subjects under conditions of very low cholesterol diets

Subject	Sex	Age	Weight	Cholesterol	7-DHC	8-DHC	19-nor[a]	Total
			kg	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*	*mg/dl*
1	M	6 wk	3.85	75.5	9.40	4.50	1.2	90.6
2	M	7 mo	5.37	88.3	13.2	12.9	2.1	116.5
3	M	1 yr	5.54	52.8	11.0	7.90	1.9	73.6
4	F	2.25 yr	9.4	101.6	2.30	2.50	0.3	106.7
5	F	2.5 yr	11.4	59.5	11.6	9.70	1.5	82.3
6	F	2.75 yr	8.90	87.6	10.5	3.60	0.8	102.5
7	F	3.5 yr	11.3	100.9	9.40	2.30	0.8	113.4
8	M	13 yr	61.8	104.5	13.6	11.8	2.6	132.5
Means ± SD				83.8 ± 19.6	10.1 ± 3.5	6.9 ± 4.2	1.4 ± 0.8	102.3 ± 19.3

[a] 19-nor-Cholestatrienol.

instruction and were asked to keep intake records. For inpatient studies, food and formula were weighed prior to being served to the subjects and refused food and formula were returned to the metabolic kitchen to be reweighed to determine actual intake. Control subjects 1 and 2 were unrelated healthy infants and children (the SLOS subjects were all infants and children). These control subjects were studied as outpatients, using published guidelines (30, 31) with our modifications below. Control subjects 3–6 were siblings of SLOS subjects and were studied as inpatients. Diet instruction was provided by a registered dietitian; both oral and written instructions were provided. The dietitian was in frequent contact, every 1–3 days, with the parents during the study, either by phone or actual meetings. A record was kept by the parent of the subjects' dietary intake. The nutrient content of the diet was calculated from manufacturer information and by using the Food Processor Plus Nutrient Analysis Program (version 7.02; ESHA Research, Salem, OR).

Sample collection

We have developed a system for performing sterol balance in infants and children without having to use a metabolic frame. We have been able to scrape frozen stool from cloth diapers and measure sterols and bile acids in pooled stool samples. Toilet-trained subjects collected all stools and the stools were individually placed in labeled plastic bags and the samples frozen for later analysis. Where stool and urine were mixed, or when stool could not be scraped off the diapers accurately, the samples were discarded, and analysis of sterol balance was not accomplished. For outpatients, refrigerators were distributed for stool collections, and stools were brought in at the end of a 1-week period of collection. These stools were then processed exactly as the stools of the SLOS subjects.

Some stool may be left on the diapers after scraping. This would lead to an underestimate of cholesterol, sterol, and bile acid synthesis. We tried to minimize this error by not including sterol balance studies where stools were too runny to allow easy scraping off of diapers. We have also performed a series of experiments determining how much stool (and therefore sterols) is left behind when scraping diapers by extracting stool remaining in diapers after scraping and quantitating. The amount left behind was minuscule and not enough to extract for analysis.

Sterol balance technique

Synthesis of cholesterol and other sterols was analyzed by the sterol balance technique in SLOS subjects and control subjects. In this study, we analyzed the fecal sterol excretion of eight SLOS subjects and six normal control subjects by the methods initially developed by Ahrens, Grundy, and Miettinen (32, 33) and modified by us (34–37). The sterol balance technique is based on the concept that in the metabolic steady state, the input of sterol into the body (the intake of dietary cholesterol and the endogenous synthesis of cholesterol) is balanced by the output (fecal excretion of neutral sterols and bile acids). Therefore, whole body sterol synthesis can be estimated by subtracting intake from excretion (38). In SLOS subjects, the sterol balance technique also provides an estimation of the synthesis of 7-DHC and other cholesterol precursor sterols by measurement of these individual sterols in stool samples. All these patients and control subjects were consuming a very low cholesterol diet. Seven-day stools of these subjects were pooled. Stools were homogenized with equal amounts of water. An aliquot was taken and frozen immediately. For analysis of fecal sterols, 0.5–1.0 g of fecal homogenate was weighed out and traces of [4-^{14}C]cholesterol and [24-^{14}C]deoxycholic acid were added to monitor the recovery. After mild saponification, the fecal neutral sterols were extracted from bile acids. The lipid extracts of fecal neutral sterols were then subjected to thin-layer chromatography (silica gel H TLC plate with solvent ether–heptane 55:45) to separate sterols from stanols plus stanones (bacterially modified products). Sterols and stanols, plus stanone, were extracted from the TLC plate. Trimethylsilyl ether (TMS) derivatives of these compounds were subjected to analysis by gas–liquid chromatography (GLC). Because of the complex nature of the fecal sterols of these infants, to obtain complete resolution of these compounds it is necessary to analyze these samples by GLC with nonpolar as well as polar columns. Therefore, the samples were first analyzed with a GLC equipped with a hydrogen flame ionization detector (3B gas chromatograph; Perkin-Elmer, Norwalk, CT) and containing a nonpolar 30-m SE-30 capillary column with 0.25-mm i.d. and 0.25-μm film thickness. The samples were also analyzed with a Perkin-Elmer gas chromatograph (model 8500) equipped with a polar 25-m CP-wax-57 capillary column (Chrompack-Varian, Walnut Creek, CA) with 0.32-mm i.d. and 0.25-μm film thickness. The temperatures of the columns were 260°C for the SE-30 column and 200°C for the CP-wax column. Helium was used as the carrier gas, and cholestane as an internal standard.

After removing neutral sterols, the aqueous layer containing the bile acids was put in a pressure cooker at 15 psi to cleave conjugated bile acids. The free bile acids were then extracted with chloroform–methanol and methylated with diazomethane. Methyl esters of bile acids were further purified by TLC. The region including and between cholic acid and lithocholic acid on the plate was scraped. Bile acids were extracted from the plate and TMS derivatives made. The samples were run on the GLC equipped with the SE-30 columns. Bile acids were separated and quantified.

Considering the possible loss of aalyzed compounds in these lengthy procedures, we added [4-^{14}C]cholesterol and [24-^{14}C]deoxycholic acid to the fecal homogenate to monitor the loss of neutral sterols and bile acids. The recovery of labeled

cholesterol was 78.6 ± 9.9% for SLOS subjects and 79.7 ± 8.8% for control subjects. Recovery for bile acids was 93 ± 7.1% for SLOS subjects and 90.8 ± 8.5% for control subjects. We used the percent recovery to correct for loss of sample in the TLC step in the calculations of sterol and bile acid synthesis.

From the daily excretion of fecal steroids (neutral sterols and bile acids) and the cholesterol intake, we measured the daily total sterol synthesis of these subjects by the balance technique (intake−total excretion). Because identification of the bile acids made from the noncholesterol compounds is problematic, precise calculation of the synthesis is not yet possible. Therefore, we assumed that the quantity of bile acid from these compounds was proportional to the quantity of their precursor neutral sterols in the stool. With this assumption, using the total bile acid excretion, we estimated the production of bile acid from these sterols. This estimate will yield an upper limit estimate of individual sterol and bile acid synthesis from precursor sterols because bile acids may not be made in great quantities from precursor sterols because dehydrocholesterols cannot likely be converted to bile acids via the 7α-hydroxylase pathway. The first step in bile acid synthesis is 7α hydroxylation. It is likely that the double bond at the 7-carbon of 7-DHC blocks this reaction. Some 7-DHC and other precursors may be converted to bile acids via the 27-hydroxylation pathway but the amount is likely to be small (39). The estimates for sterol synthesis are likely to be close to the actual synthesis because the majority of these compounds are found in stool in the neutral sterol fraction rather than as bile acids, so that the contribution of bile acids to the overall equation was much smaller than the contribution of neutral sterols.

From the sum of neutral sterol excretion (measured) and bile acid excretion (calculated) the synthesis of these noncholesterol sterols was calculated. The synthesis of cholesterol was the difference between total sterol synthesis (by balance technique) and the sum of the synthesis of the other noncholesterol sterols (7-DHC, etc.).

For determination of plasma sterols, plasma was saponified with alcohol−KOH, plasma sterols were extracted with hexane, and TMS derivatives were made. Plasma sterols were measured by GLC as described for fecal sterol measurements (40, 41).

Statistical analysis

Data are reported as means ± SD. *P* values were calculated by the Student's *t*-test. Calculations were made with SPSS for Windows version 8.0 (SPSS).

RESULTS

Sterol balance

The results of sterol balance of eight SLOS subjects and six normal control subjects are presented in **Table 2**. Cholesterol intake was calculated by dietary records and varied from 0 to 2.96 (mean, 1.26) mg/kg per day in SLOS subjects and from 0.62 to 2.3 (mean, 1.29) mg/kg per day for control subjects. The goal was for all the diets to be essentially cholesterol free. Most were not completely cholesterol free, but could be characterized as very low cholesterol diets. Total excretion of sterols (endogenous plus exogenous sources) equals neutral sterols plus bile acids. Total sterol synthesis equals total excretion minus cholesterol intake. The results of cholesterol synthesis, total sterol synthesis, and bile acid synthesis in SLOS subjects and control subjects are presented in **Fig. 1**.

The total sterol synthesis (cholesterol plus other precursor sterols such as 7-DHC, etc.) was lower in SLOS subjects versus control subjects (12.0 vs. 20.2 mg/kg per day in normal control subjects, $P < 0.022$). This difference

TABLE 2. Total sterol synthesis measured by the sterol balance technique under conditions of very low cholesterol diets

		Fecal Excretion			
Subject	Cholesterol Intake	Neutral[a,b]	Bile Acids[b]	Total[b]	Total Sterol Synthesis[c]
	mg/kg/day		*mg/kg/day*		
1	1.53	15.00	4.39	19.40	17.87
2	0.00	7.30	3.37	10.70	10.67
3	1.24	11.60	4.59	16.20	14.95
4	1.28	6.28	4.05	10.33	9.05
5	2.96	12.60	1.62	14.21	11.25
6	0.57	9.22	2.67	11.89	11.32
7	2.11	11.10	4.25	15.33	13.22
8	0.40	5.18	2.92	8.10	7.70
Mean ± SD	1.26 ± 0.96	9.78 ± 3.38	3.48 ± 1.03	13.27 ± 3.69	12.00 ± 3.27
Control					
1	2.25	9.11	2.90	12.01	9.76
2	0.62	14.23	5.71	19.94	19.32
3	1.86	12.84	1.43	14.27	12.41
4	1.41	28.09	4.59	32.68	31.27
5	0.62	18.55	7.16	25.71	25.09
6	1.00	18.21	6.07	24.28	23.28
Mean ± SD	1.29 ± 0.67	16.84 ± 6.54	4.64 ± 2.14	21.48 ± 7.68	20.19 ± 8.08
SLOS vs. Control[d]	ns	$P < 0.022$	$P < 0.187$	$P < 0.021$	$P < 0.022$

[a] Includes cholesterol and its bacterially altered products, and 7-DHC, 8-DHC, and 19-nor-cholestatrienol.
[b] Total excretion (endogenous plus exogenous sources) equals neutral sterols plus bile acids.
[c] Total sterol synthesis is total fecal steroid excretion acid excretion minus intake.
[d] *t*-test, ns, not significant.

Fig. 1. Whole body synthesis of cholesterol, total sterols, and bile acids by sterol balance in eight SLOS subjects versus six control subjects. All subjects consumed a very low cholesterol diet. Data represent means ± SD. Presented here is a summary of data shown in Tables 2, 4, and 5. Significant differences between synthesis rates of SLOS subjects and control subjects are marked by an asterisk (*). Cholesterol synthesis in SLOS was reduced compared with control subjects ($P < 0.002$). 7-DHC is not present in the stool sterols of normal subjects. Total sterol synthesis was also reduced compared with control subjects ($P < 0.022$). Bile acid synthesis did not differ significantly between SLOS and control subjects ($P < 0.2$).

was mainly due to lower excretion of neutral sterols in SLOS subjects (9.8 vs. 16.8 mg/kg per day, $P < 0.022$). Although the bile acid synthesis was also lower in SLOS subjects (3.48 vs. 4.64 mg/kg per day in normal control subjects), this difference was not statistically significant ($P < 0.2$).

Fecal neutral sterol excretion

Eleven different sterols were identified and quantified in the stools of SLOS subjects (**Table 3**). In the stools from normal subjects, only eight sterols were identified. Among the three extra sterols in SLOS, 7-DHC is in the cholesterol synthetic pathway (9). 8-DHC is formed from 7-DHC through isomerism (13). While the origin of 19-nor-cholestatrienol is unknown, it is likely a conversion product of 7- or 8-DHC (42). Cholestanol may be present in the diet and is also a degradation product of cholesterol; the five plant sterols (campestanol, campesterol, stigmasterol, sitosterol, and sitostanol) are from the diet (43–45). Lathosterol is an intermediate in cholesterol synthesis (9).

Sterols are subject to bacterial modification in the gut to form stanols and stanones. Bacterially modified products of sterols were found in all the subjects and control subjects except the youngest subjects (1.5 years or younger). This may be due to the lack of bacterial colonization of the gut in infancy. The bacterially modified products from cholesterol (coprostanol and coprostanone) contributed 7–91% of total neutral sterols in SLOS and 3–91% in control subjects. Stanols and stanones of plant sterols were found in both SLOS and control subjects. Bacterially modified products of 7-DHC, 8-DHC, and 19-nor-cholestatrienol were found in only two SLOS pa-

TABLE 3. Individual fecal neutral sterols

Subjects	Cholesterol	7-DHC	8-DHC	19-nor-Cholestatrienol	Lathosterol	Cholestanol	Campesterol	Campestanol	Stigmasterol	Sitosterol	Sitostanol
						mg/kg/day					
1	12.13	1.58	0.75	0.34	0.16	0.05	2.94	0.13	1.17	10.31	1.27
2	5.23	1.23	0.56	0.22	0.02	0.04	0.54		0.15	0.84	0.02
3	8.71	1.68	0.4	0.6	0.07	0.14	0.78		0.72	2.87	0.2
4	5.6	0.25	0.07	0.35			0.47		0.24	1.81	
5	7.8	2.72	0.93	0.71	0.23	0.2	0.98		0.85	4.06	0.41
6	7.72	0.76	0.4	0.16	0.11	0.1	1.18	0.1	0.7	4.93	0.6
7	9.49	0.4	0.95	0.03	0.05	0.13	0.51	0.06	0.32	1.74	0.29
8	3.66	0.4	0.95	0.03	0.05	0.13	0.51	0.06	0.32	1.74	0.29
Mean ± SD	7.54 ± 2.69[a]	1.31 ± 0.85	0.61 ± 0.13	0.32 ± 0.24	0.09 ± 0.08	0.11 ± 0.07	1.13 ± 0.83	0.07 ± 0.08	0.63 ± 0.36	4.57 ± 3.69	0.47 ± 0.45
Controls											
1	8.89				0.09	0.13	0.59		0.36	1.77	0.13
2	14.23						1.1		0.36	3.65	
3	12.56				0.04	0.19	0.51		0.23	1.76	0.31
4	26.72				0.22	1.01	2.31	0.34	1.2	9.9	1.38
5	17.6				0.64	0.32	2.25	0.24	0.63	7.77	0.69
6	17.98				0.08	0.15	1.51		0.86	6.99	0.69
Mean ± SD	16.33 ± 6.1[a]				0.18 ± 0.24	0.3 ± 0.36	1.38 ± 0.79	0.08 ± 0.14	0.61 ± 0.37	5.31 ± 3.4	0.53 ± 0.5

[a] SLOS versus control, $P < 0.003$.

tients (patients 4 and 8). These contributed about 60% of total 7-DHC and 8-DHC in both patients. Bacterially modified products of 19-nor-cholestatrienol constituted 13 and 95% of the total sterols in these two patients, respectively.

Cholesterol excretion was lower in SLOS subjects (7.5 vs. 16.3 mg/kg per day in normal control subjects, $P < 0.003$). The excretion of lathosterol and cholestanol was 0.09 and 0.11 mg/kg per day, respectively, for SLOS subjects and 0.18 and 0.30 mg/kg per day for normal control subjects. The plant sterols were found in the stools of both SLOS and most control subjects. The excretion of campesterol, campestanol, stigmasterol, sitosterol, and sitostanol was 1.13, 0.07, 0.63, 4.57, and 0.47 mg/kg per day for SLOS subjects, and 1.38, 0.08, 0.61, 5.31, and 0.53 mg/kg per day for normal control subjects, roughly similar.

Synthesis of cholesterol, 7-DHC, and other sterols

The synthesis of individual sterols in SLOS subjects and control subjects is reported in **Table 4**. SLOS subjects synthesized cholesterol plus significant quantities of cholesterol precursors and 19-nor-cholestatrienol.

No cholesterol precursors or isomers were detected in control subjects. Cholesterol synthesis was significantly lower in SLOS subjects than in control subjects (8.56 ± 2.4 vs. 19.6 ± 7.6 mg/kg per day, $P < 0.002$) (Table 4). The reduction in cholesterol synthesis in SLOS subjects compared with control subjects was statistically significant.

7-DHC was easily resolved from cholesterol in the excreted neutral sterol fraction. 7-DHC was the predominant cholesterol precursor synthesized by SLOS subjects. Because 7-DHC is not found in the diet, 7-DHC synthesis was calculated as 7-DHC excretion in the neutral sterol fraction plus calculated bile acids derived from 7-DHC. 7-DHC synthesis then equaled 1.66 ± 1.15 mg/kg per day. The calculation for the synthesis of 8-DHC and 19-nor-cholestatrienol was performed similarly. The calculated synthesis of these compounds, for reasons stated earlier, represents upper limit estimates. Contols did not synthesize measurable quantities of 7-DHC, 8-DHC, or 19-nor-cholestatrienol.

Cholesterol synthesis correlated strongly with body weight in the SLOS subjects ($r = 0.99$, $P < 0.001$) and in control subjects ($r = 0.88$, $P < 0.02$). Plasma 7-DHC and 8-DHC levels did not show a significant correlation with their synthesis ($r = 0.58$, $P < 0.13$ and $r = 0.46$, $P < 0.25$, respectively). In the SLOS subjects, 7-DHC and 8-DHC synthesis also correlated with weight ($r = 0.72$, $P < 0.05$ and $r = 0.99$, $P < 0.001$, respectively), but those correlations were less significant when subject 8 was not included in the calculations ($r = 0.25$ and $r = 0.66$, respectively). Cholesterol synthesis was not significantly correlated with plasma cholesterol levels in the SLOS subjects ($r = 0.53$, $P < 0.25$). We were unable to look for the same correlation in control subjects because we did not draw blood from the control children.

Fecal bile acid excretion and synthesis

We have identified and quantified six common bile acids in the stools of SLOS subjects and control subjects: two primary bile acids (cholic and chenodeoxycholic acids) and the four secondary bile acids (lithocholic, deoxycholic, ursocholic, and ursodeoxycholic acids) (**Table 5**). There is a wide age range in the SLOS subjects. To observe the effects of age on fecal bile acid composition in these patients, we grouped them in three age groups (Table 5). Both primary and secondary bile acids were identified in stools from SLOS subjects and control subjects. The younger group excreted more primary bile acids and the older groups more secondary bile acids, reflecting higher bacterial activity in the gut of the older subjects. Quantitatively, bile acid synthesis in SLOS did not differ significantly from control subjects (3.5 vs. 4.6 mg/kg per day, $P < 0.2$). Even when comparing the 2- to 4-year-old SLOS group with age-matched control subjects there was

TABLE 4. Synthesis of individual sterols by SLOS subjects and controls

Subject	Cholesterol	7-DHC	8-DHC	19-nor-Cholestatrienol	Lathosterol	Cholestanol
			mg/kg/day			
1	11.90	3.33	1.18	1.05	0.31	0.10
2	7.65	1.80	0.82	0.32	0.03	0.05
3	10.60	2.34	0.55	0.84	0.10	0.52
4	7.95	0.40	0.13	0.57	0.00	0.00
5	5.68	3.04	1.01	0.80	0.26	0.46
6	8.97	1.23	0.52	0.37	0.14	0.09
7	10.48	0.54	1.12	0.17	0.10	0.81
8	5.27	0.62	1.48	0.04	0.08	0.21
Mean ± SD	8.56 ± 2.37[a]	1.66 ± 1.15	0.85 ± 0.43	0.52 ± 0.35	0.13 ± 0.11	0.28 ± 0.29
Control						
1	9.48				0.11	0.17
2	19.32				0.00	0.00
3	12.14				0.04	0.23
4	29.83				0.26	1.18
5	23.76				0.89	0.44
6	22.98				0.1	0.2
Mean ± SD	19.59 ± 7.64[a]				0.23 ± 0.33	0.37 ± 0.42

[a] SLOS versus control, $P < 0.002$ by t-test.

TABLE 5. Fecal bile acids of SLOS subjects and control infants

Bile Acid	6 wk–1 yr (n = 3)	2–4 yr (n = 4)	13 yr old (n = 1)	All SLOS (n = 8)	Control (n = 6)
			mg/kg/day		
Primary bile acids	2.65 ± 1.19	0.16 ± 0.22	0.16	1.09 ± 1.44	0.36 ± 0.37
Chenodeoxycholic	0.89 ± 0.46	0.05 ± 0.09	0.10	0.37 ± 0.50	0.13 ± 0.13
Cholic	1.76 ± 0.74	0.11 ± 0.13	0.06	0.73 ± 0.94	0.23 ± 0.26
Secondary bile acids	0.69 ± 1.19	1.74 ± 1.11	1.92	1.37 ± 1.12	2.76 ± 1.52
Lithocholic	—	0.69 ± 0.51	0.71	0.44 ± 0.49	0.95 ± 0.54
Ursocholic	0.53 ± 0.93	0.09 ± 0.06	0.02	0.25 ± 0.55	0.28 ± 0.18
Deoxycholic	—	0.96 ± 0.72	1.19	0.63 ± 0.71	1.34 ± 0.93
Ursodeoxycholic	0.15 ± 0.27	—	—	0.06 ± 0.16	0.28 ± 0.20
Others	0.78 ± 0.57	1.26 ± 0.26	0.84	1.03 ± 0.43	1.53 ± 0.66
Total	4.12 ± 0.65	3.15 ± 1.24	2.92	3.48 ± 1.03	4.64 ± 2.14
(SLOS vs. control)					NS

Values are expressed as means ± SD; NS, not significant.

no significant difference in bile acid synthesis between groups. Qualitatively, bile acids also did not differ in SLOS subjects versus control subjects, although we were unable to identify a significant portion of bile acids in both control subjects and in SLOS subjects and it is possible that those bile acids could have differed in the two groups. It is interesting to note that infants younger than 1 year old failed to form the bacterially modified secondary bile acids, suggesting that infants do not have the gut bacterial flora needed to modify either sterols or bile acids.

DISCUSSION

Our data have demonstrated, for the first time, the effects of the cellular deficiency of 7-dehydrocholesterol Δ^7-reductase on whole body cholesterol and total sterol synthesis in SLOS. Deficiency of this final enzyme in cholesterol synthesis in SLOS subjects led to decreased whole body cholesterol synthesis compared with both historical control subjects and normal control subjects in the present study. The magnitude of the reduction in cholesterol synthesis is enlightening. We found a 56% reduction in cholesterol synthesis in SLOS subjects (Table 4). Even so, substantial quantities of cholesterol were synthesized, suggesting that the enzymatic block in cholesterol synthesis was clearly incomplete in the subjects studied. 7-DHC and its derivatives accumulated in the SLOS subjects but not in control subjects. Because 7-DHC conversion to cholesterol was partially blocked by the enzyme deficiency, this led to substrate accumulation. These data provide proof of the concept that the synthesis of the cholesterol precursors can be measured by the sterol balance technique. This will be important with future therapeutic trials, where the effects of supplemental cholesterol and drugs that inhibit cholesterol synthesis on 7-DHC synthesis can be measured.

In addition to decreased whole body cholesterol synthesis, total sterol synthesis was also decreased in SLOS subjects compared with control subjects. A decrease in total sterol synthesis in SLOS subjects was not necessarily expected because total sterol synthesis would include the synthesis of cholesterol and of 7- and 8-DHC and other sterols and bile acids. The synthesis of cholesterol plus other sterols in SLOS might be expected to be similar to cholesterol synthesis in control subjects. There are several possible explanations for the decreased total sterol synthesis compared with the synthesis in control subjects. First, shunting provides an alternative explanation. The mevalonate shunt links isoprenoid metabolism with mitochondrial acetyl-CoA metabolism. Plasma 3-methylglutaconic acid, an intermediate in the leucine metabolic pathway, is increased in SLOS subjects, suggesting that shunting occurs (46). It was suggested that in SLOS subjects the overflow of isoprenoid precursors is shunted to 3-methylglutaconic acid as a result of the enzyme block downstream of isoprenoid synthesis. Alternatively, the cholesterol synthetic pathway could be inhibited through feedback from 7-DHC. Cholesterol inhibits HMG-CoA reductase. If 7-DHC is a more potent inhibitor of HMG-CoA reductase than cholesterol, this sterol could feed back on the proximal enzyme inhibiting synthesis of all sterols. There is some precedence for inhibition of HMG-CoA reductase by sterol derivatives other than cholesterol. An oxygenated lanosterol compound, 3β-hydroxy-lanost-8-en-32-al, is a potent regulator of HMG-CoA reductase at the level of translation (47, 48). More importantly, Honda and colleagues showed that 7-DHC does indeed inhibit HMG-CoA reductase in human cells in vitro (49). 7-DHC was noted to have a greater inhibitory effect on HMG-CoA reductase than cholesterol.

For the first time, analysis of bile acids and measures of their synthesis were obtained in SLOS subjects in the sterol balance study. Only fragmentary data about fecal bile acid excretion in SLOS has been previously reported (9). In that first report, bile acids excreted by three SLOS subjects on uncontrolled diets were analyzed after collection of only single stools and not total collection as a part of a sterol balance study. The fecal bile acid and sterol excretion were most variable. Although 7-DHC was detected in the stools of all three patients, it was the predominant fecal neutral sterol in one patient and a minor sterol in another. One patient excreted only neutral sterols and no detectable bile acids. Another patient had a bile acid-to-

neutral sterol ratio of 1 to 2; the third patient had a bile acid-to-neutral sterol ratio of 1 to 1. When the patient with no detectable bile acids was given supplemental cholesterol, primary bile acids were detected in feces (26). In view of these divergent results and the paucity of data on bile acid excretion in the literature, further study of bile acid excretion was warranted. This is especially important given the possibility that bile acid deficiency contributes to the SLOS phenotype due to deficiency of cholesterol substrate for enzymatic synthesis of bile acids in the liver. Bile acid deficiency could lead to impaired cholesterol absorption, which could confound therapy.

In our study, all eight of the SLOS subjects synthesized primary bile acids (cholic and chenodeoxycholic acids). Secondary bile acids (lithocholic and deoxycholic) were also found in the stools of the SLOS subjects in normal quantities (Table 2). Unidentified bile acids were found in both control subjects and SLOS subjects. Some of the unidentified bile acids in SLOS subjects likely include bile acids derived from 7- and 8-DHC. Honda et al. (39) showed that abnormal bile acids seen in SLOS are mainly synthesized by an alternative pathway via mitochondrial sterol 27-hydroxylase. In the younger subjects of the current study almost all the bile acids were primary bile acids, while older subjects produced more secondary bile acids, as expected from increasing bacterial colonization of the gut with aging. The total bile acid synthesis in SLOS subjects on cholesterol-free diets was somewhat reduced compared with control subjects, but this reduction was not statistically significant. These data again emphasize that at least some cholesterol is synthesized in even moderately severely affected SLOS patients and that this cholesterol may then be used for bile acid synthesis. The quantity of bile acids synthesized in SLOS subjects in this study is similar to bile acid synthesis reported in children in previous studies. The range of bile acid synthesis in infants and children in the literature is 1.6–16.0 mg/kg per day, with most reports showing synthesis in the 3- to 5-mg/kg per day range (50–58). Five control children studied previously in the Connor laboratory showed mean bile acid synthesis on a cholesterol-free diet of 4.2 mg/kg per day (58).

In embarking on this study to evaluate sterol and bile acid synthesis in SLOS subjects who are predominantly infants and children, we noted a paucity of data in the literature on cholesterol and bile acid synthesis in this age group. Bile acid excretion may differ between adults and children owing to the different intestinal length, stool transit time, and quantity and quality of intestinal flora. Huang et al. (59) determined the bile acid and neutral sterol excretion in five infants less than 18 months of age, five children 4 years of age, and in nine adults. The subjects were all healthy and ate a normal Western-type mixed diet during the study. Progressive changes in both bile acid and neutral sterol profiles were observed with maturation. Therefore, it is not appropriate to extrapolate sterol balance results from normal adults to children. Cholesterol synthesis may also be expected to differ between children and adults because of the different needs for cholesterol at different ages. We, therefore, needed to perform sterol balance on normal infants and children in order to have an appropriate control group.

Most published reports on cholesterol and bile acid synthesis in infants and children have either included subjects with hypercholesterolemia, or older children, or have included small subject numbers, or were not done with subjects on cholesterol-free diets, or have not been formal balance studies. Nestel et al. (31) reported cholesterol synthesis in 10 normal infants, ages 3–16 months. Four infants were 3–5 months old and 6 infants were 11 to 16 months old. Cholesterol synthesis on a very low cholesterol diet ranged from 6 to 31 mg/kg per day. Estimates of daily cholesterol synthesis in infants and children have ranged from 0 to 40 mg/kg per day in other published reports (32, 50–58). Diets varied in these studies. Whole body cholesterol synthesis varies a great deal depending on cholesterol intake. The whole body cholesterol synthetic rate in the control subjects (19.6 mg/kg per day) in the present study was similar to that reported previously.

Cholesterol synthesis correlated strongly with body weight in the SLOS subjects and in control subjects. In the SLOS subjects, 7-DHC and 8-DHC synthesis also showed positive correlations with weight. Sterol synthesis must adapt to the needs of the individual. As more cells and tissues are synthesized and as cell and tissue turnover increases with growth, cholesterol synthesis normally must increase to meet the demand. It is interesting that these SLOS subjects increased cholesterol synthesis as they grew, in the same manner as normal control subjects. One might have speculated that, because of a block in cholesterol synthesis, SLOS subjects might not have been capable of increasing cholesterol synthesis with growth. It is unclear, though, whether the increase in cholesterol synthesis with growth of the SLOS subjects was sufficient to meet demand. There was no significant correlation between colesterol synthesis and plasma cholesterol levels in the SLOS subjects, or for plasma 7-DHC and 8-DHC and their respective synthesis. The lack of a correlation is not altogether unexpected because the plasma levels do not always reflect synthesis. One possible reason for the lack of a correlation is that the clearance rate of sterols from the plasma may differ in these subjects.

The Smith-Lemli-Opitz syndrome is usually a devastating disorder with birth defects and mental retardation. The elucidation of SLOS as a cholesterol synthetic defect has paved the way for development of therapy. To date, there is no proven treatment. Cholesterol supplementation is a logical therapeutic approach. Bile acids synthesized from cholesterol are needed for cholesterol absorption, so it is not unreasonable to expect that bile acid supplementation might also be needed for treatment of SLOS. Exactly how cholesterol should be administered, and the optimal amount and form, and whether bile acids are needed are not known. Therapeutic trials with supplemental cholesterol with and without added bile acids are now underway in SLOS (26–28). The hypothesis is that supplemental cholesterol will raise cholesterol levels and lower cholesterol precursor levels in SLOS patients. Anec-

dotal reports of improvement in growth, of older children learning to walk, and of adults speaking for the first time all attest to the possible efficacy of cholesterol supplementation in SLOS (60). The results of these sterol balance studies are relevant in considering therapeutic approaches to SLOS.

This study was designed to lay the groundwork for development of therapy for SLOS by further delineating the effects of 7-dehydrocholesterol Δ^7-reductase deficiency on the whole body production of cholesterol, the precursor sterols, and bile acids. As a first step, we have adapted the sterol balance technique to study subjects with SLOS. The resulting data support the use of dietary cholesterol in the therapy of this genetic disease and indicate a method for estimation of the optimal amount of dietary cholesterol. The best estimate for dosage of cholesterol needed in SLOS is the difference between whole body cholesterol synthesis in control subjects and SLOS subjects, because this provides an estimate of the whole body cholesterol deficit. Cholesterol absorption in children has seldom been measured. There are only data in the literature on cholesterol absorption in two children. Cholesterol absorption in a child with α_1-antitrypsin deficiency and hypercholesterolemia averaged 32% compared with 45% in adults studied in the same laboratory (58). Cholesterol absorption in a 5- to 6-year-old with abetalipoproteinemia averaged 30.7% (57). The difference in mean cholesterol synthesis in the current study is 11 mg/kg per day. Assuming 33% absorption of cholesterol, the dose estimate for a 10-kg, 1-year-old infant would be approximately 330 mg/day. The average egg yolk contains roughly 213 mg of cholesterol, so this translates to 1.5 egg yolks/day. We have found that ½ to 2 egg yolks/day in infants more than doubled the plasma cholesterol and lowered 7-DHC by two-thirds (61). The bile acids synthesized by the SLOS subjects in the present study should be sufficient for adequate cholesterol absorption given the similarity in bile acid quantity and quality between control subjects and SLOS subjects. That result, with the data showing that egg yolk cholesterol without supplemental bile acids raises plasma cholesterol levels (61), leads us to conclude that bile acid supplementation is not needed at least in mildly or moderately severely affected individuals.

These initial studies of the sterol balance in SLOS pave the way for future studies. The effects of cholesterol supplementation on sterol balance can be measured to aid in the determination of therapeutic efficacy. Cholesterol absorption can theoretically be measured with the sterol balance technique, and those studies are planned to help determine the optimal form cholesterol supplementation should take: crystalline cholesterol in oil, cholesterol in aqueous suspension, butterfat, egg yolk, or another form. SLOS subjects synthesize sizable amounts of potentially toxic cholesterol precursors and other sterols and these sterols accumulate in plasma.

In this group of mild to moderately affected infants and children the mean plasma cholesterol level was 84 mg/dl as compared with cholesterol levels in normal boys and girls ages 3 and 4 years of 141 ± 6.4 (mean ± SD, in mg/dl) (S. L. Connor and W. E. Connor, unpublished data). In plasma, the ratio of noncholesterol sterols to cholesterol in SLOS subjects was 22% (18.4/83.8). In stool, the ratio was 45% (1.22/2.69). While noncholesterol sterols (7-DHC, 8-DHC, etc.) constituted 11.4% of total sterols in the plasma, they represented 25% of the whole body sterol synthesis. The fecal sterols represent what is happening in the whole body rather than just in the plasma, so that the ratio of 45% is more representative of the situation in the whole body. Whole body sterol synthesis may be more reflective of the tissue concentrations of 7-DHC and other sterols than plasma levels. Both the highly elevated ratios of plasma and stool 7-DHC and its derivatives to cholesterol, and their whole body synthesis, would suggest a need to suppress synthesis of these noncholesterol sterols, which may be toxic. To maximally decrease production of these abundant precursor sterols, HMG-CoA reductase inhibitors may be needed in concert with cholesterol supplementation (62).

The results of this study provide the first opportunity to observe the effects of a disorder in cholesterol synthesis on whole body sterol and bile acid synthesis. The studies described herein can also provide insight into the effect of reduced cholesterol substrate on bile acid synthesis. Our current understanding of cholesterol metabolism was greatly advanced by elucidation of cholesterol regulation in patients with familial hypercholesterolemia. Similarly, SLOS presents a unique model disorder that can expand our understanding of cholesterol metabolism and ultimately improve our treatment of these patients but also patients with other defects in cholesterol metabolism.

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REFERENCES

1. Smith, D., L. Lemli, and J. Opitz. 1964. A newly recognized syndrome of multiple congenital anomalies. *J. Pediatr.* **64:** 210–217.
2. Curry, C. J., J. C. Carey, J. S. Holland, D. Chopra, R. Fineman, M. Golabi, S. Sherman, R. A. Pagon, J. Allanson, S. Shulman, M. Barr, V. McGravey, C. Dabiri, N. Schimke, E. Ives, and B. Hall. 1987.

Smith-Lemli-Opitz syndrome-type II: multiple congenital anomalies with male pseudohermaphroditism and frequent early lethality. *Am. J. Med. Genet.* **26:** 45–57.
3. Pober, B. 1990. Smith-Lemli-Opitz syndrome. *In* Birth Defects Encyclopedia. M. Buyse, editor. Blackwell Scientific, Dover, MA. 1570–1572.
4. Opitz, J. M., V. B. Penchaszadeh, M. C. Holt, and L. M. Spano. 1987. Smith-Lemli-Opitz (RSH) syndrome bibliography. *Am. J. Med. Genet.* **28:** 745–750.
5. Chasalow, F. I., S. L. Blethen, and K. Taysi. 1985. Possible abnormalities of steroid secretion in children with Smith-Lemli-Opitz syndrome and their parents. *Steroids.* **46:** 827–843.
6. Natowicz, M. R., and J. E. Evans. 1994. Abnormal bile acids in the Smith-Lemli-Opitz syndrome. *Am. J. Med. Genet.* **50:** 364–367.
7. Cunniff, C., L. E. Kratz, A. Moser, M. R. Natowicz, and R. I. Kelley. 1997. Clinical and biochemical spectrum of patients with RSH/Smith-Lemli-Opitz syndrome and abnormal cholesterol metabolism. *Am. J. Med. Genet.* **68:** 263–269.
8. Ryan, A. K., K. Bartlett, P. Clayton, S. Eaton, L. Mills, D. Donnai, R. M. Winter, and J. Burn. 1998. Smith-Lemli-Opitz syndrome: a variable clinical and biochemical phenotype. *J. Med. Genet.* **35:** 558–565.
9. Tint, G. S., M. Irons, E. R. Elias, A. K. Batta, R. Frieden, T. S. Chen, and G. Salen. 1994. Defective cholesterol biosynthesis associated with the Smith-Lemli-Opitz syndrome. *N. Engl. J. Med.* **330:** 107–113.
10. Irons, M., E. R. Elias, G. Salen, G. S. Tint, and A. K. Batta. 1993. Defective cholesterol biosynthesis in Smith-Lemli-Opitz syndrome [letter]. *Lancet.* **341:** 1414.
11. Tint, G. S. 1993. Cholesterol defect in Smith-Lemli-Opitz syndrome [letter]. *Am. J. Med. Genet.* **47:** 573–574.
12. Tint, G. S., M. Seller, R. Hughes-Benzie, A. K. Batta, S. Shefer, D. Genest, M. Irons, E. Elias, and G. Salen. 1995. Markedly increased tissue concentrations of 7-dehydrocholesterol combined with low levels of cholesterol are characteristic of the Smith-Lemli-Opitz syndrome. *J. Lipid Res.* **36:** 89–95.
13. Batta, A. K., G. S. Tint, S. Shefer, D. Abuelo, and G. Salen. 1995. Identification of 8-dehydrocholesterol (cholesta-5,8-dien-3 beta-ol) in patients with Smith-Lemli-Opitz syndrome. *J. Lipid Res.* **36:** 705–713.
14. Honda, A., G. S. Tint, G. Salen, A. K. Batta, T. S. Chen, and S. Shefer. 1995. Defective conversion of 7-dehydrocholesterol to cholesterol in cultured skin fibroblasts from Smith-Lemli-Opitz syndrome homozygotes. *J. Lipid Res.* **36:** 1595–1601.
15. Shefer, S., G. Salen, A. K. Batta, A. Honda, G. S. Tint, M. Irons, E. R. Elias, T. C. Chen, and M. F. Holick. 1995. Markedly inhibited 7-dehydrocholesterol-delta 7-reductase activity in liver microsomes from Smith-Lemli-Opitz homozygotes. *J. Clin. Invest.* **96:** 1779–1785.
16. Fitzky, B. U., M. Witsch-Baumgartner, M. Erdel, J. N. Lee, Y. K. Paik, H. Glossmann, G. Utermann, and F. F. Moebius. 1998. Mutations in the Delta7-sterol reductase gene in patients with the Smith-Lemli-Opitz syndrome. *Proc. Natl. Acad. Sci. USA.* **95:** 8181–8186.
17. Wassif, C. A., C. Maslen, S. Kachilele-Linjewile, D. Lin, L. M. Linck, W. E. Connor, R. D. Steiner, and F. D. Porter. 1998. Mutations in the human sterol delta7-reductase gene at 11q12–13 cause Smith-Lemli-Opitz syndrome. *Am. J. Hum. Genet.* **63:** 55–62.
18. Waterham, H. R., F. A. Wijburg, R. C. Hennekam, P. Vreken, B. T. Poll-The, L. Dorland, M. Duran, P. E. Jira, J. A. Smeitink, R. A. Wevers, and R. J. Wanders. 1998. Smith-Lemli-Opitz syndrome is caused by mutations in the 7- dehydrocholesterol reductase gene. *Am. J. Hum. Genet.* **63:** 329–338.
19. Porter, J. A., D. P. von Kessler, S. C. Ekker, K. E. Young, J. J. Lee, K. Moses, and P. A. Beachy. 1995. The product of hedgehog autoproteolytic cleavage active in local and long-range signalling. *Nature.* **374:** 363–366.
20. Porter, J., K. Porter, and P. Young. 1996. Cholesterol modification of hedgehog signaling proteins in animal development. *Science.* **274:** 255–259.
21. Porter, J. A., S. C. Ekker, W. J. Park, D. P. von Kessler, K. E. Young, C. H. Chen, Y. Ma, A. S. Woods, R. J. Cotter, E. V. Koonin, and P. A. Beachy. 1996. Hedgehog patterning activity: role of a lipophilic modification mediated by the carboxy-terminal autoprocessing domain. *Cell.* **86:** 21–34.
22. Cooper, M. K., J. A. Porter, K. E. Young, and P. A. Beachy. 1998. Teratogen-mediated inhibition of target tissue response to Shh signaling. *Science.* **280:** 1603–1607.
23. Xu, G. R., R. J. Servatius, S. Shefer, G. S. Tint, W. T. O'Brien, A. K. Batta, and G. Salen. 1998. Relationship between abnormal cholesterol synthesis and retarded learning in rats. *Metabolism.* **47:** 878–882.
24. Gaoua, W., F. Chevy, C. Roux, and C. Wolf. 1999. Oxidized derivatives of 7-dehydrocholesterol induce growth retardation in cultured rat embryos: a model for antenatal growth retardation in the Smith-Lemli-Opitz syndrome. *J. Lipid Res.* **40:** 456–463.
25. Connor, W. E. 1995. A cholesterol deficiency syndrome in humans [editorial; comment]. *J. Clin. Invest.* **95:** 2.
26. Irons, M., E. R. Elias, G. S. Tint, G. Salen, R. Frieden, T. M. Buie, and M. Ampola. 1994. Abnormal cholesterol metabolism in the Smith-Lemli-Opitz syndrome: report of clinical and biochemical findings in four patients and treatment in one patient. *Am. J. Med. Genet.* **50:** 347–352.
27. Nwokoro, N. A., and J. J. Mulvihill. 1997. Cholesterol and bile acid replacement therapy in children and with Smith-Lemli-Opitz (Slo/Rsh) syndrome. *Am. J. Med. Genet.* **68:** 315–321.
28. Elias, E. R., M. B. Irons, A. D. Hurley, G. S. Tint, and G. Salen. 1997. Clinical effects of cholesterol supplementation in six patients with the Smith-Lemli-Opitz syndrome (SLOS). *Am. J. Med. Genet.* **68:** 305–310.
29. Connor, W., D. Stone, and R. Hodges. 1964. The interrelated effects of dietary cholesterol and fat upon human serum lipid levels. *J. Clin. Invest.* **43:** 1691–1696.
30. Cressey, D. E., P. M. Kris-Etherton, and J. H. Zavoral. 1978. Experimental note: sterol balance study conducted in the home. *J. Am. Diet. Assoc.* **73:** 543–545.
31. Nestel, P. J., A. Poyser, and T. J. Boulton. 1979. Changes in cholesterol metabolism in infants in response to dietary cholesterol and fat. *Am. J. Clin. Nutr.* **32:** 2177–2182.
32. Grundy, S., E. Ahrens, Jr., and T. Miettinen. 1965. Quantitative isolation and gas-liquid chromatographic analysis of total dietary and fecal neutral sterols. *J. Lipid Res.* **6:** 397–410.
33. Miettinen, T., E. Ahrens, Jr., and S. Grundy. 1965. Quantitative isolation and gas-liquid chromatographic analysis of total dietary and fecal neutral sterols. *J. Lipid Res.* **6:** 411–424.
34. Connor, W. E., D. T. Witiak, D. B. Stone, and M. L. Armstrong. 1969. Cholesterol balance and fecal neutral steroid and bile acid excretion in normal men fed dietary fats of different fatty acid composition. *J. Clin. Invest.* **48:** 1363–1375.
35. Connor, W. E., and D. S. Lin. 1974. The intestinal absorption of dietary cholesterol by hypercholesterolemic (type II) and normocholesterolemic humans. *J. Clin. Invest.* **53:** 1062–1070.
36. Lin, D. S., and W. E. Connor. 1980. The long term effects of dietary cholesterol upon the plasma lipids, lipoproteins, cholesterol absorption, and the sterol balance in man: the demonstration of feedback inhibition of cholesterol biosynthesis and increased bile acid excretion. *J. Lipid Res.* **21:** 1042–1052.
37. McMurry, M. P., W. E. Connor, D. S. Lin, M. T. Cerqueira, and S. L. Connor. 1985. The absorption of cholesterol and the sterol balance in the Tarahumara Indians of Mexico fed cholesterol-free and high cholesterol diets. *Am. J. Clin. Nutr.* **41:** 1289–1298.
38. Grundy, S. M., and E. H. Ahrens, Jr. 1969. Measurements of cholesterol turnover, synthesis, and absorption in man, carried out by isotope kinetic and sterol balance methods. *J. Lipid Res.* **10:** 91–107.
39. Honda, A., G. Salen, S. Shefer, A. K. Batta, M. Honda, G. Xu, G. S. Tint, Y. Matsuzaki, J. Shoda, and N. Tanaka. 1999. Bile acid synthesis in the Smith-Lemli-Opitz syndrome: effects of dehydrocholesterols on cholesterol 7alpha-hydroxylase and 27-hydroxylase activities in rat liver. *J. Lipid Res.* **40:** 1520–1528.
40. Connor, W. E., and D. S. Lin. 1981. Absorption and transport of shellfish sterols in human subjects. *Gastroenterology.* **81:** 276–284.
41. Gregg, R. E., W. E. Connor, D. S. Lin, and H. B. Brewer, Jr. 1986. Abnormal metabolism of shellfish sterols in a patient with sitosterolemia and xanthomatosis. *J. Clin. Invest.* **77:** 1864–1872.
42. Batta, A. K., G. Salen, G. S. Tint, and S. Shefer. 1995. Identification of 19-nor-5,7,9(10)-cholestatrien-3 beta-ol in patients with Smith-Lemli-Opitz syndrome. *J. Lipid Res.* **36:** 2413–2418.
43. Miettinen, T. A., R. S. Tilvis, and Y. A. Kesaniemi. 1989. Serum cholestanol and plant sterol levels in relation to cholesterol metabolism in middle-aged men. *Metabolism.* **38:** 136–140.
44. Salen, G., A. K. Batta, G. S. Tint, S. Shefer, and G. C. Ness. 1994. Inverse relationship between plasma cholestanol concentrations and bile acid synthesis in sitosterolemia. *J. Lipid Res.* **35:** 1878–1887.
45. Bhattacharyya, A. K., and W. E. Connor. 1974. Beta-sitosterolemia

and xanthomatosis. A newly described lipid storage disease in two sisters. *J. Clin. Invest.* **53:** 1033–1043.
46. Kelley, R. I., and L. Kratz. 1995. 3-Methylglutaconic acidemia in Smith-Lemli-Opitz syndrome. *Pediatr. Res.* **37:** 671–674.
47. Leonard, D. A., M. A. Kotarski, J. E. Tessiatore, M. F. Favata, and J. M. Trzaskos. 1994. Post-transcriptional regulation of 3-hydroxy-3-methylglutaryl coenzyme A reductase by 3 beta-hydroxy-lanost-8-en-32-al, an intermediate in the conversion of lanosterol to cholesterol. *Arch. Biochem. Biophys.* **310:** 152–157.
48. Trzaskos, J. M. 1995. Oxylanosterols as modifiers of cholesterol biosynthesis. *Prog. Lipid Res.* **34:** 99–116.
49. Honda, M., G. S. Tint, A. Honda, L. B. Nguyen, T. S. Chen, and S. Shefer. 1998. 7-Dehydrocholesterol down-regulates cholesterol biosynthesis in cultured Smith-Lemli-Opitz syndrome skin fibroblasts. *J. Lipid Res.* **39:** 647–657.
50. Bilheimer, D. W., J. L. Goldstein, S. M. Grundy, and M. S. Brown. 1975. Reduction in cholesterol and low density lipoprotein synthesis after portacaval shunt surgery in a patient with homozygous familial hypercholesterolemia. *J. Clin. Invest.* **56:** 1420–1430.
51. Martin, G. M., and P. Nestel. 1979. Changes in cholesterol metabolism with dietary cholesterol in children with familial hypercholesterolaemia. *Clin. Sci.* **56:** 377–380.
52. Heubi, J. E., S. Burstein, M. A. Sperling, D. Gregg, M. T. Subbiah, and D. E. Matthews. 1983. The role of human growth hormone in the regulation of cholesterol and bile acid metabolism. *J. Clin. Endocrinol.* **57:** 885–891.
53. Levy, R. A., R. E. Ostlund, Jr., A. C. Goldberg, and S. M. Grundy. 1986. Long-term changes in cholesterol biosynthesis and the effect of plasmapheresis therapy in a hypercholesterolemia homozygote. *Metabolism.* **35:** 415–418.
54. Schwarz, K. B., J. Witztum, G. Schonfeld, S. M. Grundy, and W. E. Connor. 1979. Elevated cholesterol and bile acid synthesis in a young patient with homozygous familial hypercholesterolemia. *J. Clin. Invest.* **64:** 756–760.
55. Carter, G. A., W. E. Connor, A. K. Bhattacharyya, and D. S. Lin. 1979. The cholesterol turnover, synthesis, and absorption in two sisters with familial hypercholesterolemia (type IIa). *J. Lipid Res.* **20:** 66–77.
56. Zavoral, J. H., D. C. Laine, L. K. Bale, D. L. Wellik, R. D. Ellefson, K. Kuba, W. Krivit, and B. A. Kottke. 1982. Cholesterol excretion studies in familial hypercholesterolemic children and their normolipidemic siblings. *Am. J. Clin. Nutr.* **35:** 1360–1367.
57. Illingworth, D. R., W. E. Connor, D. S. Lin, and J. Diliberti. 1980. Lipid metabolism in abetalipoproteinemia: a study of cholesterol absorption and sterol balance in two patients. *Gastroenterology.* **78:** 68–75.
58. Diliberti, J. H., M. P. McMurry, W. E. Connor, and P. Alaupovic. 1984. Hypercholesterolemia associated with alpha-1 antitrypsin deficiency and hepatitis: lipoprotein and apoprotein determinations, sterol balance and treatment. *Am. J. Med. Sci.* **288:** 81–85.
59. Huang, C. T., J. T. Rodriguez, W. E. Woodward, and B. L. Nichols. 1976. Comparison of patterns of fecal bile acid and neutral sterol between children and adults. *Am. J. Clin. Nutr.* **29:** 1196–1203.
60. Kelley, R. I. 1998. RSH/Smith-Lemli-Opitz syndrome: mutations and metabolic morphogenesis. *Am. J. Hum. Genet.* **63:** 322–326.
61. Linck, L. M., D. S. Lin, D. Flavell, W. E. Connor, and R. D. Steiner. 2000. Cholesterol supplementation with egg yolk increases plasma cholesterol and decreases plasma 7-dehydrocholesterol in Smith-Lemli-Opitz syndrome. *Am. J. Med. Genet.* In press.
62. Jira, P., R. Wevers, J. Dejong, E. Rubiogozalbo, and J. Smeitink. 1997. New treatment strategy for Smith-Lemli-Opitz syndrome. *Lancet.* **349:** 1222.