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Kathleen B. Schwarz, … , Scott M. Grundy, William E. Connor

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Research Article

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KATHLEEN B. SCHWARZ, JOSEPH WITZTUM, GUSTAV SCHONFELD, SCOTT M. GRUNDY, and WILLIAM E. CONNOR, *Departments of Preventive Medicine, Medicine, and The Edward Mallinckrodt Department of Pediatrics at Washington University School of Medicine, and Division of Gastroenterology, St. Louis Children's Hospital, St. Louis, Missouri 63110; Department of Medicine, Veterans Administration Hospital, and University of California, San Diego, California 92161; Department of Medicine, University of Oregon Health Sciences Center, Portland, Oregon 97201*

ABSTRACT Cholesterol balance studies were carried out twice in a young male patient with homozygous familial hypercholesterolemia. At 13 mo, cholesterol balance in this patient averaged 31.3 mg/kg per d, and bile acid excretion was 12.0 mg/kg per d; at 3 yr, results were similar, 27.3 and 15.5 mg/kg per d for cholesterol balance and bile acids, respectively. A normal boy of 3 yr was also studied for comparison with the second study in our patient. Cholesterol balance and bile acid outputs in the normal child were 11.5 and 3.3 mg/kg per d, respectively. Thus, in comparison with the normal child, the patient with homozygous familial hypercholesterolemia had a marked increase in synthesis of cholesterol and bile acids. Although synthesis of bile acids was high in this patient, the fraction of newly synthesized cholesterol converted into bile acids (40–56%) was in the normal range; this suggests that the enhanced output of bile acids was secondary to an increased synthesis of cholesterol and not to malabsorption of bile acids, which likely would have produced a higher fractional conversion. Although our patient has been studied at a younger age than any reported in the literature, two similar children 5 and 6 yr of age were also observed to have elevated cholesterol synthesis. This finding contrasts with those in older children with the homozygous as well as heterozygous forms of this disease who appear to have normal synthesis of cholesterol and bile acids. Therefore, increased synthesis of cholesterol seems to be characteristic of early homozygous familial hypercholesterolemia, and may be a manifestation of a loss of feedback inhibition of cholesterol synthesis secondary to an absence of specific cell-surface receptors for low density lipoproteins. However, as children with this disease grow older, other mechanisms may come into play to restore cholesterol synthesis to normal levels.

INTRODUCTION

Studies in cultured skin fibroblasts from patients with homozygous familial hypercholesterolemia (HFH)[1] indicate that their cells do not normally bind and internalize low density lipoproteins (LDL) (1). Consequently, cholesterol synthesis in these cells is depressed and increased. Theoretically, overproduction of cholesterol in HFH cells in vitro might reflect increased total body synthesis. Indeed, elevated cholesterol synthesis was found in a 6-yr-old child with HFH by Bilheimer et al. (2), and in a similar 10 yr old by Lewis and Myant (3). However, overproduction of cholesterol has not been found in older children with HFH or in either children or adults with heterozygous familial hypercholesterolemia (4–7).

Accumulated evidence on cholesterol production in HFH is compatible with three possibilities. First, synthesis might be high early in life as a result of the absence of LDL receptors, but with time, production might decline to normal. Second, increased production noted in younger HFH children (2, 3) could be fortuitous and atypical of what usually occurs in HFH. And third, a relatively high synthesis may be common in normal

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[1] *Abbreviations used in this paper*: HFH, homozygous familial hypercholesterolemia; LDL, low density lipoprotein.

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young children but has gone undetected because of insufficient studies. This study has attempted to distinguish between these possibilities; to this end, cholesterol synthesis was estimated by sterol balance in a 3-yr-old child with HFH and a normal child of the same age.

METHODS

Patients. R.L. was born with several small tuberous xanthomata, and at 3 yr has multiple planar and tendon xanthomata. Plasma cholesterol has ranged from 700 to 1,100 mg/dl (LDL cholesterol, 650–950 mg/dl and high density lipoprotein cholesterol, 23–28 mg/dl); triglycerides are about 160 mg/dl. Skin fibroblasts were LDL receptor defective, as determined by Dr. Brown and Dr. Goldstein (University of Texas Health Science Center at Dallas, Tex.) and in our own laboratory. Three generations in both parental lines have hypercholesterolemia. At 3 yr, R.L. had normal growth, development, cardiac status, and weighed 15 kg. He had never been on hypolipidemic medication at the time of the first study at age 13 mo, and all medications had been stopped for 6 wk before the study at age 3 yr.

Subject A.S. was a normal 3-yr-old boy of 16.4 kg. His plasma cholesterol and triglycerides were 141 and 57 mg/dl, respectively.

Diets. R.L. was studied on the General Clinical Research Center of Washington University twice, once for a week when he was 13 mo of age, and again for 28 d at 3 yr. On the first occasion, he was given a mixed solid-food and formula diet that contained 784 kcal (23% fat); it also had 22 mg cholesterol and 111 mg β-sitosterol/d. At the next study, he received a solid food and formula diet that contained ≅31% of calories as fat in the form of lard. 53% of calories were from solid food that included canned fruit, cereal, skim milk, toast, apple sauce, egg white, lard, and potato; the remaining 47% of calories consisted of formula containing lard, dry skim milk powder, dextromaltose, and Nestle's Quik (Nestle Co., White Plains, N. Y.). Daily caloric intake averaged 1411 kcal, and cholesterol intake was 53 mg. The weight did not change throughout the study.

Subject A.S. was studied in the home of one Dr. Schwartz; he was given a diet that closely matched that of R.L. Analysis of daily portions of this diet revealed 28 mg of cholesterol.

Analytic techniques. Plasma lipids and lipoproteins were measured by standard methods (8). Cholesterol balance studies were carried out as before (9). In the first study on R.L., β-sitosterol was used alone as a flow marker; his stools were collected daily for 7 d and pooled into a single sample. In the other studies on R.L. and A.S., β-sitosterol (110 mg bid plus small amounts of dietary intake) was used to correct for neutral steroid losses, and chromic oxide (60 mg bid) was used as a fecal flow marker. Stools were collected on patient R.L. for 24 d after a 4-d equilibration period and for 7 d on A.S. after equilibration. Stools were combined into 3-d pools for R.L. and were analyzed daily for A.S. They were analyzed for neutral and acidic steroids.

RESULTS

Table I shows cholesterol balance data for R.L. and A.S. At 13 mo, cholesterol balance in R.L. averaged 31.3 mg/kg per d and bile acid outputs were 12.0 mg/kg

TABLE I
Cholesterol Balance Data on Subjects R.L. and A.S.

Subject	Sample	Dietary cholesterol intake (a)	Fecal steroid excretion						Cholesterol balance	
			Neutral steroids		Acidic steroids		Total steroids (b)		(b)–(a)	
		mg/d	*mg/d*	*mg/kg/d*	*mg/d*	*mg/kg/d*	*mg/d*	*mg/kg/d*	*mg/d*	*mg/kg/d*
R.L. (13 mo)	1*	22	192	21.8	106	12.0	298	33.9	276	31.4
R.L. (3 yr)	1‡	53	187	12.5	441	29.4	628	41.9	578	38.5
	2	53	175	11.7	131	8.7	306	20.4	253	16.9
	3	53	271	18.1	203	13.5	474	31.6	421	28.1
	4	53	317	21.1	131	8.7	448	29.9	395	26.3
	5	53	263	17.5	321	21.4	584	38.9	531	35.4
	6	53	210	14.0	360	24.0	570	38.0	517	34.5
	7	53	212	14.1	184	12.3	396	26.4	342	22.8
	8	53	206	13.7	95	6.3	301	20.1	248	16.5
		Mean±SD	230±16	15.3±1.2	233±42	15.5±3.2	463±41	30.9±3.1	410±41	27.3±3.4
A.S. (3 yr)	1§	28	158	9.6	35	2.1	193	11.7	165	10.1
	2	28	171	10.4	72	4.4	243	14.8	215	13.1
	3	28	181	11.0	47	2.9	228	13.9	200	12.2
	4	28	146	8.9	38	2.3	184	11.2	156	9.5
	5	28	142	8.7	75	4.6	217	13.2	189	11.5
	6	28	171	10.9	59	3.6	230	14.0	202	12.3
		Mean±SD	162±6	9.9±0.4	54±6	3.3±0.5	216±9	13.2±0.6	188±9	11.5±0.6

* 7 d of stool collections were combined into a single pool.
‡ Each sample number represents a 3-d stool collection, and samples are listed in the sequence collected.
§ Each sample number represents a 1-d stool collection, and they are also listed in the sequence collected.

per d. Thus, at this time, acidic steroid excretion was 40% of total steroid output. It should be noted that before this study R.L. had never received any hypolipidemic therapy. At 3 yr, his net cholesterol balance was similar to the earlier measurement and was over twice that found in A.S. (27.3 vs. 11.5 mg/kg per d). Acidic steroids were also much greater in R.L. than in A.S. (15.5 vs. 3.3 mg/kg per d), and in R.L. they constituted 56% of total steroids. If it is assumed that cholesterol balance and acidic steroid excretion closely approximate synthesis of cholesterol and bile acids, respectively, the synthesis of both were greatly increased in R.L. compared with A.S. Furthermore, because the cholesterol balance technique measures only cholesterol products that are excreted from the body, it probably underestimated total body synthesis of cholesterol in R.L. This patient obviously was not in a steady state because he was continuously depositing cholesterol in his tissues, as indicated by the rapid growth of xanthomas. The rate of tissue accumulation of cholesterol cannot be estimated with any certainty, but it might be as high as 50 mg/d. This amount would have to be added to his cholesterol balance to obtain a true figure for cholesterol synthesis, and it would make his overproduction even greater.

Because of the relatively high levels of excretion of neutral and acidic steroids, subsequent studies were undertaken to rule out any intestinal pathology that might cause malabsorption of cholesterol and bile acids. All medications were stopped for 3 mo. Subsequent plasma levels of vitamins A and E, carotene, and prothrombin time were all normal. An upper gastrointestinal series with small bowel follow through was normal, as was a 3-d determination of fecal fat.

DISCUSSION

To our knowledge, this is the youngest child with HFH in whom measurements of cholesterol production have been made. The major observation of this study was that at 13 mo of age, and again at 3 yr of age, this child with HFH had a markedly increased production of cholesterol and bile acids. To determine whether these increases were the result of his disease or merely a result of his young age, a cholesterol balance study was done in a normal 3-yr-old boy. The normal child produced significantly lower amounts of both cholesterol and bile acids than those of R.L. as shown in Table I. For purposes of comparison, Table II summarizes published data for total synthesis of cholesterol and bile acids in normal infants, children, and adults along with those previously obtained for hypercholesterolemic subjects. In most cases, measurements of cholesterol and bile acid synthesis were estimated by essentially the same techniques and with similar diets as employed in this study. Our normal 3-yr-old subject, A.S., had

TABLE II
Cholesterol Synthesis in Homozygous and Heterozygous FH Patients and in Normal Subjects

Patient (or Group)	References	*n*	Sex	Age	Cholesterol synthesis	Bile acid synthesis
					mg/kg/d ± SD	*mg/kg/d ± SD*
Homozygous FH						
R.L.*	—	1	M	3 yr	27.3	15.3
M.C.	2	1	F	6 yr	22.2	4.3
—	3	1	F	10 yr	17.1	6.3
—	16	1	F	12 yr	17.0	—
De	4	1	F	13 yr	7.0	1.7
L.B.	4	1	F	14 yr	12.3	4.2
A.C.	4	1	F	15 yr	14.5	4.3
Di		1	F	16 yr	6.6	1.6
Heterozygous FH						
Children	2, 6	5	M-F	11–18 yr	11.0±1.0	3.6±1.5
Adults	6, 10	12	M-F	49 yr (19–67 yr)‡	10.4±2.9	3.6±1.6
Normolipidemic						
A.S.*	—	1	M	3 yr	11.5	3.3
Infants	11	3	M-F	8 wk	9.3±3.4	2.8±2.5
Infants-Children	13	18	M-F	4 mo–5 yr‡	—	6.1±2.7
Adults I	§	14	M	50 yr (27–63 yr)‡	9.6±2.3	4.9±2.3
Adults II	14	6	M-F	36–44 yr‡	10.3	4.5
Adults III	10	10	M-F	23–46 yr‡	12.4	3.8

* Patients R.L. and A.S. were those of this study.
‡ Age range.
§ Unpublished data on 14 normal male subjects studied in San Diego by Dr. Grundy.

synthetic rates for both cholesterol and bile acids (on a per kilogram basis) that were similar to several groups of normal adults. Potter and Nestel (11) have also reported that three normal, 8-wk-old infants had comparable normalized rates of synthesis. Additional valuable studies for comparison have come from the laboratory of Nestel and coworkers (12).[2] These workers found essentially the same synthesis rates for bile acids in infants as in older children (2.7±1.5 [SD] mg/kg per d for infants; [n = 9] vs. 3.9±1.3 mg/kg per d for older children [n = 5, ages 5–12]). They also found total cholesterol synthesis in a normal 5-yr-old child to be 11.7 mg/kg/d (12). The finding that normal children have similar production rates for bile acids has been corroborated by Weber et al. (13). Therefore, after reviewing available data, it would appear that the HFH patient of this study had a marked increase in synthesis of cholesterol and bile acids.

Consideration might be given to the possibility that increases in synthesis of cholesterol and bile acids in R.L. are secondary to a concommitant abnormality in gastrointestinal tract causing malabsorption of cholesterol and bile acids. Particularly for bile acids, current techniques are not available to detect a mild malabsorption of bile acids in small children (15). For several reasons, however, it seems highly improbable to us that the patient had a second disorder of sterol metabolism leading to a selective malabsorption of cholesterol and bile acids. First, considering the extreme rarity of HFH, the chances of having a second and unrelated defect of sterol metabolism would seem remote. In a patient with such a gross disorder of cholesterol and lipoprotein metabolism as in HFH, there is a high probability that any observed abnormalities in sterol metabolism would be the result of this primary disease. Second, the fraction of newly synthesized cholesterol converted into bile acids was not unusually high as might be expected with malabsorption of bile acids. During two separate studies, the percentage of conversion of newly synthesized cholesterol into bile acids was 40% and 56%. In recent cholesterol balance studies on 14 normal adults determined in the same laboratory (Table II, adults I), the mean percent conversion of newly formed cholesterol into bile acids was 51%, which is in the same range as R.L. Finally, there was no other evidence of gastrointestinal dysfunction found in this child that might suggest sterol malabsorption.

Table II compares the cholesterol balance data for R.L. with those of other patients with hypercholesterolemia. Previous work indicates that cholesterol and bile acid production in older children and adults with heterozygous familial hypercholesterolemia are almost in the normal range, or sometimes, bile acid synthesis may even be reduced (2, 5, 6, 10). A somewhat different picture is found in comparison of children with HFH. One 6-yr-old child (M.C.) studied by Bilheimer et al. (2), also had a marked increase in cholesterol synthesis. Likewise, a 10-yr-old with HFH, studied by Lewis and Myant (3) and a 12-yr-HFH, studied by Miettinen (16) had a striking increase. On the other hand, four older children (13–16 yr) with the same disease were found to have only slightly increased production. In unpublished investigations on young patients with HFH, Bilheimer[3] reports that a 5-yr-old also had a marked increase in synthesis of cholesterol and bile acids, but a 6-yr-old had a normal production of both. Martin and Nestel (12) have also found a normal synthesis of cholesterol and bile acids in a 5-yr-old HFH patient. Therefore, although all children with HFH do not have an overproduction of cholesterol, at least five patients have been identified who do. In most instances, the overproducers have been younger subjects suggesting that while HFH patients can have an overproduction of cholesterol early in life, their synthetic rates may later decline towards normal.

Inappropriately high production of cholesterol in R.L. could have been caused by a deficiency or defect in cell-surface LDL receptors leading to enhanced intracellular synthesis of cholesterol (1). Obviously, sequential studies are needed to determine whether cholesterol synthesis would decrease progressively over the years in patients with HFH, but we might speculate that despite a marked reduction or absence of specific LDL receptors intracellular cholesterol content may gradually increase either by uptake from nonspecific pathways of LDL degradation or by increased intracellular synthesis. In essence, older homozygotes should have had time to accumulate more intracellular cholesterol to suppress synthesis than younger ones.

Our study does not reveal whether excess cholesterol produced in young patients with HFH is derived from the liver or peripheral tissues. Either source, or both, are possible and could reflect defective receptor-mediated uptake of LDL. A possible clinical importance of our finding is that overproduction of cholesterol might cause excessive deposition in xanthoma, arterial walls, endocardium, and heart valves that is so characteristic of HFH. If cholesterol synthesis could be retarded early in life, cholesterol accumulation in tissues might be reduced.

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[2] Nestel, P. J. Personal communication.

[3] Bilhemier, D. W. Personal communication.

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