The cholesterol turnover, synthesis, and absorption in two sisters with familial hypercholesterolemia (type IIa)

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Abstract To explore the mechanisms of the profound plasma cholesterol elevations in familial homozygous hypercholesterolemia (type IIa), cholesterol turnover, sterol balance, cholesterol absorption, and low density lipoprotein studies were carried out under controlled dietary conditions in two sisters (aged 13 and 16). Cholesterol turnover was prolonged. The half-life of the first exponential of the plasma cholesterol specific activity decay curve was double that of normal adults. The rate constants for the removal of cholesterol from pool A (K_{AA} = 0.0652) and for the excretion of cholesterol from the system (K_A = 0.0197) were less than half of normal. The production rates of cholesterol were low, only 6.30 and 6.86 mg/kg per day as measured by cholesterol turnover and sterol balance techniques, respectively. Fecal neutral steroid and bile acid excretion were 5.22 and 1.64 mg/kg per day, which is remarkably low in comparison to those of normal and heterozygous children. Cholesterol absorption was within the upper limit of the values reported for normal adults. The HDL cholesterol values were extremely low (27 mg/dl) in contrast to profoundly elevated LDL levels. The fractional catabolic rate of LDL (0.127 per day) and the rate of synthesis and catabolism of apo-LDL (15 mg/kg per day) were low in comparison to previously reported values in homozygotes. These composite data indicated that the definable metabolic defects of these two sisters with homozygous familial hypercholesterolemia were the sluggish clearance of cholesterol from the body coupled with low total body synthesis of cholesterol.

Supplementary key words familial homozygous hypercholesterolemia · familial heterozygous hypercholesterolemia · two-pool model · sterol balance · LDL turnover · HDL and LDL cholesterol · fecal neutral steroids and bile acids · hyperalimentation · cholesterol-free diet · dietary cholesterol

Familial hyperlipoproteinemia type IIa is an autosomal dominant disease manifested in the heterozygous form by elevated plasma cholesterol level (1–4) and by the development of xanthomas and coronary heart disease in the third to fifth decades of life. The homozygous form presents a much more severe clinical picture, with profound hypercholesterolemia often exceeding 800 mg/dl, tuberous and tendon xanthoma appearing within the first eight years of life, and rapidly progressive coronary heart disease frequently eventuating in death from myocardial infarction before age 20 (3, 5, 6).

Because of the association between elevated plasma cholesterol levels and the development of atherosclerosis in both man and animals, there has been increasing interest in inborn errors of lipid metabolism, of which the most common is familial hypercholesterolemia. Despite the unique and devastating manifestations of the homozygous form of hypercholesterolemia which have been noted since the early descriptions of the condition "xanthelasma multiplex" (7), the entire picture of the metabolic defect still remains unknown. Some investigators have found that increased synthesis of cholesterol or low density lipoprotein (LDL) might be the abnormality (8, 9). Other studies have suggested that the defect may be in the catabolism or excretion of either LDL (10) or of plasma cholesterol (11). At the cellular level, Goldstein and Brown (12–14) discovered a deficiency of receptors for LDL in cultured fibroblasts from patients with familial hypercholesterolemia.

The current study was designed to explore the mechanism of the profound hypercholesterolemia that occurred in two sisters with the homozygous form of familial hyperlipoproteinemia, type IIa. Cholesterol turnover, sterol balance, and cholesterol absorption

Abbreviations: VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein; FCR, fractional catabolic rate.

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studies were carried out under controlled dietary conditions of a cholesterol-free diet. In addition, the effect of total parenteral hyperalimentation was evaluated. By two measurements, isotopic cholesterol turnover and sterol balance, the predominant defect was in the excretion of cholesterol from the body. While decreased excretion of both neutral steroids and bile acids was noted, the major decrease occurred in bile excretion under the steady-state conditions of the study. The decreased fecal steroid excretion also implied reduced cholesterol biosynthesis which might logically be expected with plasma cholesterol elevation.

MATERIALS AND METHODS

Subjects

The two sisters, Di aged 16 years and De aged 13 years (**Tables 1** and **2**) were clinically diagnosed as having homozygous familial hypercholesterolemia and were admitted to the Clinical Research Center for metabolic studies. Patient Di first developed tuberous xanthoma of the left elbow and knee in late infancy. These slowly increased in size until they were surgically removed at 8 years of age. Upon microscopic examination, these lesions were found to be xanthomas. At 9 years of age, the first plasma lipid determinations showed that her plasma cholesterol was 684 mg/dl and the plasma triglyceride was 88 mg/dl. Subsequently, until the present study, she was treated with a moderately restricted diet with less than 250 mg cholesterol per day; nicotinic acid, 800 mg per day; and cholestyramine, 24 g per day. The lowest plasma cholesterol value noted during that period was 462 mg/dl, the most recent value being 634 mg/dl. Her growth has been normal and she has had normal pubescent changes. She has no history of cardiovascular disease such as angina pectoris or myocardial infarction.

On physical examination, she was found to have tendon, tuberous, and planar xanthomas. Extensive tendon xanthomas were palpated involving both Achilles tendons. Smaller tendon xanthomas, less than 1 cm in diameter, were noted in the extensor tendons at the metacarpal–phalangeal joints of both hands, particularly involving the second, third, and fourth digits of the left hand, and the metatarsal–phalangeal joints of both great toes. Tuberous xanthomas of 0.75–1.5 cm in diameter were noted about

TABLE 1. Clinical description of the two sisters

	Di	De
Age, yr	16	13
Weight, kg	43.5	39.8
Height, cm	160	154
Plasma lipids[a] (mg/dl)		
Cholesterol	684 (737)	841 (786)
Triglycerides	88 (100)	109 (97)
Xanthomas		
Tendon	Both Achilles Extensor tendons of both hands Great toes	Both Achilles Extensor tendons of both hands Great toes
Tuberous	Both elbows Back of both heels	Back of both heels
Planar	On the web between thumb and index finger of right hand	Tip of coccyx
Cardiovascular findings		
Angina pectoris	Absent	Absent
Aortic stenosis	Present	Absent
Resting and stress electrocardiogram	Normal	Normal

[a] Their first plasma lipid determination on unrestricted diet in 1967 at age 9 years (Di) and 6 years (De). Figures in parentheses are values determined before the beginning of the studies described on moderately restricted cholesterol (~250 mg/day) diet.

TABLE 2. Representative plasma and lipoprotein lipid levels of the homozygous hypercholesterolemic children and their parents

Diet	Cholesterol				Triglycerides			
	Plasma	VLDL	LDL	HDL	Plasma	VLDL	LDL	HDL
Di: 250 mg cholesterol	737	8	669	29	100	34	54	8
cholesterol-free	578	13	531	27	86	37	37	5
hyperalimentation	402	7	384	11	114	32	75	7
De: 250 mg cholesterol	786	5	727	25	97	27	66	7
cholesterol-free	644	4	588	27	64	17	48	3
hyperalimentation	418	3	404	11	83	23	55	5
Father: 100 mg cholesterol	283	9	223	49	80	40	29	9
Mother: 100 mg cholesterol	261	6	185	61	69	28	25	11

the extensor surface of the heels. One planar xanthoma, 3–4 mm in diameter, was found on the web between thumb and index finger of the right hand. She also had a grade 3/6 systolic ejection murmur localized at the right sternal border with radiation into the neck which was diagnosed as aortic stenosis. Chest x-rays were normal as were resting and stress electrocardiograms.

Patient De developed a yellowish lesion on her tongue shortly after birth. She was otherwise asymptomatic until 5 years of age. At that time large nodules were noted on the extensor surface at the base of her great toe. When her sister was diagnosed as having xanthoma, this patient's serum cholesterol was determined and found to be 841 mg/dl and the plasma triglyceride was 109 mg/dl. Over the ensuing years, both tendon and tuberous xanthomas appeared at the Achilles, the elbows, the knees, and the dorsum of the hands and feet. She also was treated with a moderately restricted cholesterol (250 mg/day) diet, nicotinic acid (1500 mg/day), and cholestyramine (20 g/day). With this combined diet and drug therapy, her plasma cholesterol was reduced only slightly. She had normal growth and development and at the time of our study had not yet entered puberty. She has had no anginal attacks or other symptomatology suggestive of coronary artery disease.

On physical examination, she was found to have prominent tendon and tuberous xanthomas. Tendon xanthomas of 1–1.5 cm in diameter were noted at the extensor tendons of the hands both at metacarpal–phalangeal joints of all fingers and at the metatarsal–phalangeal joints on the dorsum of the great toe bilaterally. There were also extensive tendon xanthomas of both Achilles tendons. Tuberous xanthomas were found on the back of the heels. Planar xanthomas were noted in the lateral creases of both popliteal spaces. There were no significant cardiac findings; her chest x-rays and resting and stress electrocardiograms were normal.

The girls' parents and nearly all aunts and uncles were screened for plasma lipid abnormalities. The parents were found to have hyperbetalipoproteinemia type IIa with plasma cholesterol levels of 286 mg/dl (father) and 261 mg/dl (mother) on a moderately restricted cholesterol diet. The LDL cholesterol concentrations on a low cholesterol diet were 223 and 185 mg/dl in the father and mother, respectively. Hypercholesterolemia occurred on both sides of the family. Adult onset diabetes was present on the maternal side. The family pedigree is shown in **Fig. 1.**

Experimental design and methods

The children were studied both as inpatients in the Clinical Research Center Metabolic Ward and as outpatients under controlled dietary conditions. Both patients had stopped all cholesterol-lowering medications at least 6 weeks before admission to the hospital, but they had continued on their prescribed low-cholesterol (less than 100 mg/day) diet. Informed consent was obtained from both parents and their parents in accordance with the policies of the Committee on Investigations Involving Human Beings of the University of Iowa College of Medicine.

There were three inpatient periods: two of two weeks each and one of 4 weeks duration. Before each inpatient period the patients had followed the very low cholesterol diet for at least 6 weeks. Two outpatient periods of 15 weeks and 6 weeks duration were also included in this study. During the first inpatient period, cholesterol absorption and sterol balance were measured and the isotopic cholesterol turnover period

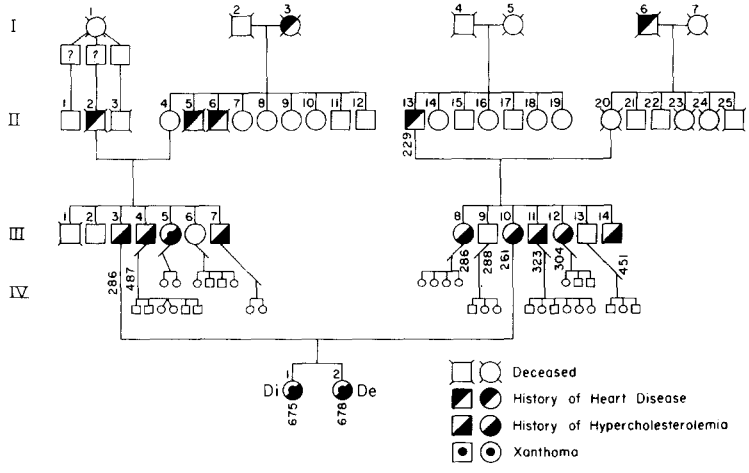

Fig. 1. Family history of Di and De. The numbers below each ○ or □ indicate the plasma cholesterol level of that individual.

was initiated. The children were followed as outpatients for the remainder of the isotopic turnover period with blood specimens collected twice weekly. The second inpatient period occurred at the termination of the turnover study 16 weeks later and included cholesterol absorption and sterol balance studies. The third inpatient period followed the second by 6 weeks and included a 2-week period of total parenteral alimentation.

Diets

During the inpatient periods of study, both patients were fed cholesterol-free formula diets in which dietary fat contributed 20% (saturated fat 6.4%, monounsaturated 9.5%, and polyunsaturated 4.3%), protein 15%, and carbohydrates 65% of the total caloric intake (about 2000 Kcal) together with 8 g of cellulose and vitamin and mineral supplements to meet all nutritional requirements as previously published (15, 16). The diet was fed six times each day divided into three primary meals containing 22% of the total caloric intake each, and three snacks, each providing 11% of calories. During the outpatient periods, a mixed-food diet very similar in composition was prescribed. The cholesterol content of the mixed-food diet was greater than the formula diet, but was kept to a maximum intake not to exceed 100 mg cholesterol per day.

Cholesterol turnover

Each child was given a single dose of 50 μCi of [1,2-H^3]cholesterol (Amersham/Searle, Arlington Heights, IL). This isotope was checked for purity by thin-layer and gas-liquid chromatography. The isotope was dissolved in 5 ml of ethanol and sterilized by passage through a Millipore filter of 0.2 μm pore size (Millipore Corporation, Bedford, MA). This ethanolic solution was suspended in 500 ml of 0.9% sodium chloride solution immediately before use. This saline suspension was infused intravenously over a period of 30–45 min. The actual amount of isotope injected was measured precisely for each child by analyzing the residual radioactivity in the infusion set and syringes used in the procedure.

Venous blood samples were collected in the fasting state every morning for the first 5 days and then twice weekly for the next 16 weeks. Plasma was separated by centrifugation at 4°C and stored frozen at −20°C for subsequent determination of plasma total cholesterol, triglycerides, and radioactivity. Plasma total cholesterol and triglycerides were measured fluorometrically with the Technicon Auto Analyzer (AAII, Technicon Instrument Corporation, Tarrytown, NY) (17). The radioactivity was measured on another aliquot of the extract using 10 ml of scintillation solution (4 g of 2,5,5-diphenyloxazole and 0.1 g of 1,4,bis 2-(5-phenyloxazole)-benzene per liter of toluene). A Packard Tri-Carb liquid scintillation spectrometer (Model 3380, Packard Instrument Company, Incorporated, Downers Grove, IL) equipped with external standardization was used for the measurement of radioactivity.

Plasma [^{3}H]cholesterol specific radioactivity was plotted semilogarithmically against time and the decay curves were resolved into the sum of two exponential functions. The parameters of the cholesterol turnover were calculated based on the kinetics of the two-pool model (18).

Computer analysis of the cholesterol turnover curves was carried out as follows. The logarithm of the serum cholesterol specific activity as a function of time after injection of labeled cholesterol was fitted to the logarithm of the sum of two exponentials using an integration procedure described by Snedecor and Cochran (19). This procedure is analogous to the graphical "peeling" procedure using semilog plot and it provided identical results if the data were analyzed using the weighted least-squares method described by Dell et al. (20).

Sterol balance studies

Stool samples were collected daily and frozen. For analysis, the samples were thawed and a 7-day pool was weighed and homogenized with an equal volume of water. The fecal neutral and acid sterols were recovered and their total mass was determined by the methods of Miettinen, Ahrens, and Grundy (21, 22) and as previously published from this laboratory (15). Samples of all diets were taken and sterol excretion was corrected for sterol loss by the determination of the differences between plant sterol ingestion and excretion as has been suggested in previous publications (23, 24).

Cholesterol absorption

Cholesterol absorption was determined according to the method described by Connor and Lin (25). Each child was given a test meal containing 500 mg of cholesterol and 150 mg of β-sitosterol, including 5 μCi of [4-^{14}C]cholesterol and 2.5 μCi of [22,23-^{3}H]β-sitosterol dissolved in peanut oil. This was prepared as "scrambled eggs" and served in conjunction with the usual formula breakfast. For calculations of the exact intake of radioactive cholesterol, the residue cholesterol and radioactivity left in the eating utensils of each subject were measured. Stools were collected for the next 7 days after the test meal and analyzed as described above for sterol balance. The radioactivity contained in the neutral sterol fraction was measured

by a Packard Tri-Carb liquid scintillation spectrometer equipped with external standardization (Packard Instrument Company, Incorporated, Downers Grove, IL). Cholesterol absorption was calculated by subtracting the amount of unabsorbed radiolabeled cholesterol in the stools from the total amount ingested in the test breakfast and expressed as percent absorbed.

Total parenteral alimentation

The central venous nutrient solution consisted of protein hydrolysate (Amigen Solution, Baxter Laboratories, Morton Grove, IL), dextrose, and electrolytes. This was prepared to provide one calorie per ml. All necessary vitamins and minerals were added to the first liter of solution each day, and the total continuous infusion for each child was adjusted to provide 2000 calories per 24 hr. The fluid was infused through an indwelling catheter inserted into the superior vena cava near the right atrium via the subclavian vein. During this period these patients had no oral intake.

Low density lipoprotein turnover[4]

Both patients were studied using ^{125}I-labeled low density lipoprotein (LDL, between density 1.025 and 1.050 g/ml). The detailed methodology of isolation of LDL by preparative ultracentrifugation (26) and radioiodination of LDL protein by modification (27) of the iodine monochloride method (28) have been published previously (10, 29). About 1.5 mg of LDL protein containing 50 μCi of ^{125}I radioactivity was injected intravenously into each patient in a volume not exceeding 2 ml. Daily fasting blood samples (5–7 ml) were obtained using EDTA as anticoagulant for the next 14 days. Twenty-four urine collections were made in bottles containing small amounts of preservative. Total daily urine volume was recorded and aliquots were assayed for radioactivity. Plasma and urine samples were assayed for radioactivity in a Packard Autogamma scintillation spectrometer (Packard Instruments Co., Inc., Downers Grove, Ill.).

The kinetic parameters for LDL turnover were calculated using methods originally described by Mathews (30) and adapted to LDL turnover studies by Langer, Strober, and Levy (10) and Bilheimer et al. (29).

Plasma lipids

The plasma total cholesterol and triglycerides were measured with the Technicon Auto Analyzer II (17). The plasma lipoproteins were separated by ultracentrifugation and heparin–manganese chloride precipitation (17). The cholesterol and triglyceride contents of each fraction were then determined. The plasma [^{3}H]cholesterol radioactivity was determined on another aliquot of the isopropanol extract obtained above using 10 ml of the scintillation solution described above.

[4] In cooperation with Drs. Harold Tarsvik and Robert Lees.

RESULTS

Plasma lipids, lipoproteins, and body weights

During the time period of these studies (175 days), the plasma cholesterol values remained stable in the range of 564–680 mg/dl (mean 618 ± 40 mg/dl) for Di and 609–706 mg/dl (mean 660 ± 28 mg/dl) for De during the study periods, excluding the period of parenteral alimentation. During these same time periods, the plasma triglyceride ranged from 71 to 134 mg/dl (mean 98 ± 19 mg/dl) for Di and from 67 to 119 mg/dl (mean 82 ± 15 mg/dl) for De. The plasma cholesterol levels of these girls for the time previous to this study, the baseline period, and the different study periods are summarized in **Figs. 2** and **3** and Table 2. To be noted is the hypocholesterolemic effect of the very low cholesterol diet.

The majority of the plasma cholesterol (91–92%) was in the LDL fraction (Table 2). The cholesterol in the HDL fraction was very low. It was 25-29 mg/dl and only comprised 3–5% of the total plasma cholesterol (see Discussion for comparison with normals).

Both children gained slightly in weight and height. This amounted to a total gain of, for Di, 1.3 kg and 1 cm, and for De, 1.7 kg and 1.5 cm.

Cholesterol absorption

Cholesterol absorption was measured at the beginning and at the end of the cholesterol turnover study period in both patients (**Table 3**). For Di, the percent of absorption was 69.8 for the first test and 43.0 for the second test with a mean of 56.4%. The [22,23-^{3}H]-β-sitosterol recovery was 102 and 98.6%, respectively. The isotopic β-sitosterol was incorporated in the test meal, with isotopic cholesterol, as a nonabsorbable marker. Its recovery was included in the calculation of cholesterol absorption to correct the possible loss from sample collection and bacterial degradation (23, 24). For De, the percent of absorption was 62.8 and 55.4 for the two tests with a mean of 59.1%. The recovery of [22,23-H^3]β-sitosterol was 86.2 and 73.9%. The overall range for both patients was 43.0–69.8% with a mean absorption of 57.8%. The results showed that cholesterol absorption in the two sisters with clinical

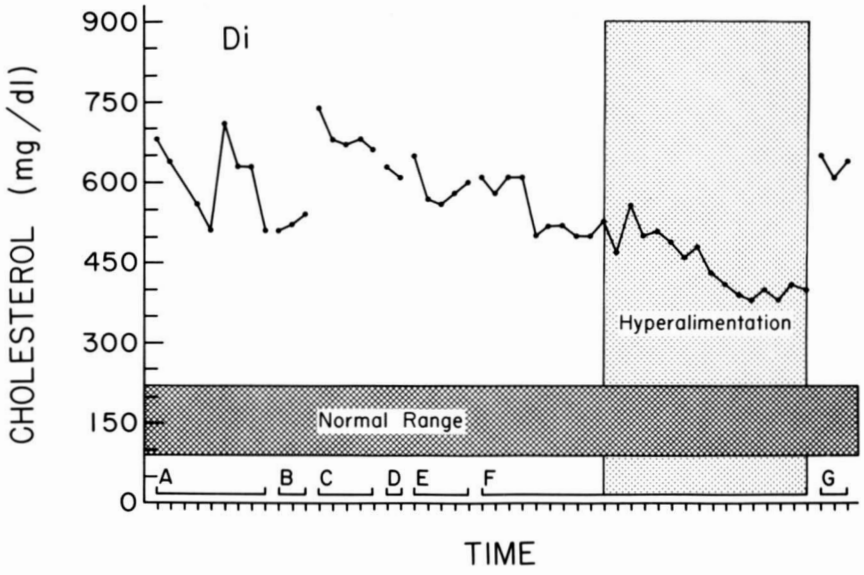

Fig. 2. *A*, July 1967 to Dec. 1973 (irregular time intervals). *B*, First seen UI hospitals (Dec, 1973 Jan, and Mar, 1974). *C*, First hospital period (samples 2×/ week). *D*, Samples start and end of outpatient period (cholesterol turnover). *E*, Second hospital period (Samples 2×/ week). *F*, Third hospital period (daily samples. *G*, Rebound from hyperalimentation (outpatient period with monthly samples).

homozygous familial hypercholesterolemia was no different from normal adults or heterozygous type II adult patients (25).

Cholesterol turnover

The plasma cholesterol turnover was measured by analysis of the plasma cholesterol specific activity decay curve after pulse-labeling according to the two-pool model (18). The curves derived for these patients are shown in **Figs. 4** and **5** and the derived data in **Table 4.** These data were also subjected to computer analysis and similar results were obtained. The comparison data obtained from the cholesterol turnover curves of normal and heterozygous hypercholesterolemic patients were determined in previous studies (11).

The most striking abnormality of the cholesterol turnover data (Table 4) was the sluggish removal of cholesterol from the plasma. The rate constant for total removal of cholesterol from pool A (K_{AA}) was 0.0652 in the homozygous children and the rate constant for the excretion of cholesterol pool A and the

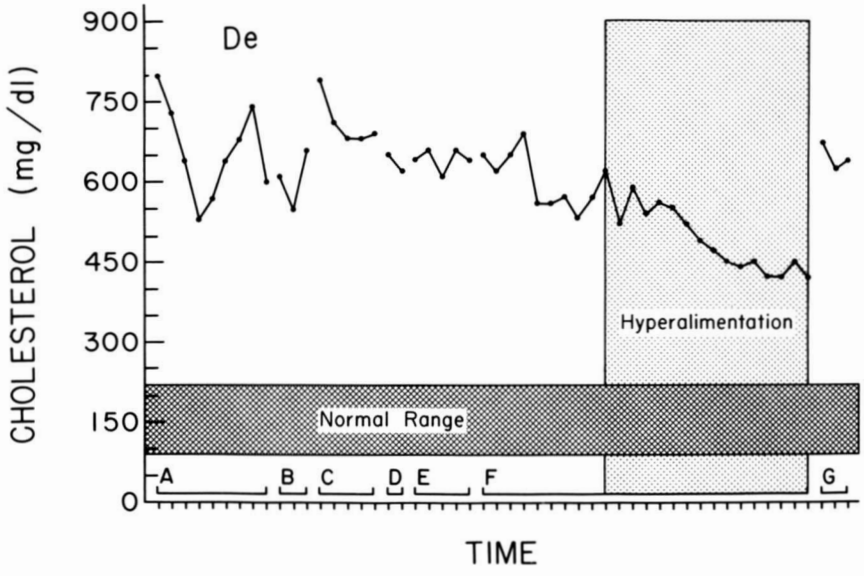

Fig. 3. Periods identical to Fig. 2.

TABLE 3. Intestinal absorption of cholesterol in the two sisters

Patient	Time Period		Cholesterol Absorption
			%
Di	First inpatient period		69.8
	Second inpatient period		43.0
		Mean	56.4
De	First inpatient period		62.8
	Second inpatient period		55.4
		Mean	59.1

system (K_A) was 0.0197, much lower than for normals and heterozygotes. Thus, the fractional turnover rate for cholesterol in pool A was lower by 32% in the homozygous sisters than in normal adults and lower by 46% than in the heterozygous adults.

The half-life of the first exponential, $t^A_{1/2}$, was extremely prolonged, 8.2 and 8.7 days in Di and De, respectively. The size of the rapidly exchangeable pool, M_A, was similar in both sisters, about 380 mg/kg body weight, of which about 72% was present in the plasma pool and only 28% was present in the tissues comprising the rapidly exchangeable pool, M_A (tissues such as liver, bile, and intestine). The other significant feature of the cholesterol turnover data was the very low values obtained for the pool B or the slowly exchangeable pool. In both sisters, the pool B size was smaller than the pool A size and constituted only about 47% of the total exchangeable pool $M_A + M_B$. The production rate of cholesterol in the pool A was also low, only 6.5 and 6.1 mg/kg per day in patients Di and De, respectively.

Sterol balance

Three sterol balance studies were carried out in each of the two patients: at the beginning (time 1) and at the end (time 2) of the cholesterol turnover period, and before the period of total parenteral alimentation (time 3). The results are tabulated in **Table 5.** The mean endogenous neutral sterol excretion was 225 mg/day for Di and 219 mg/day for De; the mean bile acid excretion was 71 mg/day for Di and 69 mg/day for De, resulting in a mean total sterol excretion of 296 mg/day for Di and 288 mg/day for De. Expressed in terms of body mass, the daily excretion rates for Di and De, respectively, were 5.05 and 5.36 mg of neutral steroid/kg body weight per day, and 1.60 and 1.68 mg of bile acids/kg body weight per day. The mean total daily steroid excretion of these two sisters was 6.86 mg/kg per day. This figure is in close agreement with the mean production rate (6.3 mg/kg per day) obtained by kinetic method of cholesterol turnover curves. The fecal neutral steroid results presented in the table have been corrected for β-sitosterol recovery (23). The recovery of β-sitosterol was 108, 87.4, and 97.1% for Di in the three balance studies. It was 90.6, 76.1, and 107% for De.

The percentage distribution of the two major fecal bile acids was 46.0 and 41.8 for deoxycholic and lithocholic acid, respectively, for Di and 48.0 and 41.9 for these two bile acids for De.

The sterol balance results in these two homozygous familial hypercholesterolemic sisters are in great contrast to the sterol balance data obtained in 5 normal and 11 heterozygous children (Table 5). As is clearly evident, the total fecal steroid excretion in the homozygotes which represents total body cholesterol biosynthesis is profoundly lower than in the normal and heterozygous children: 6.86 mg/kg per day vs. 14.19 and 11.92. Especially notable were the low bile acid excretions in the homozygotes: 1.64 mg/kg per day vs. 4.21 and 4.08, or only about 40%.

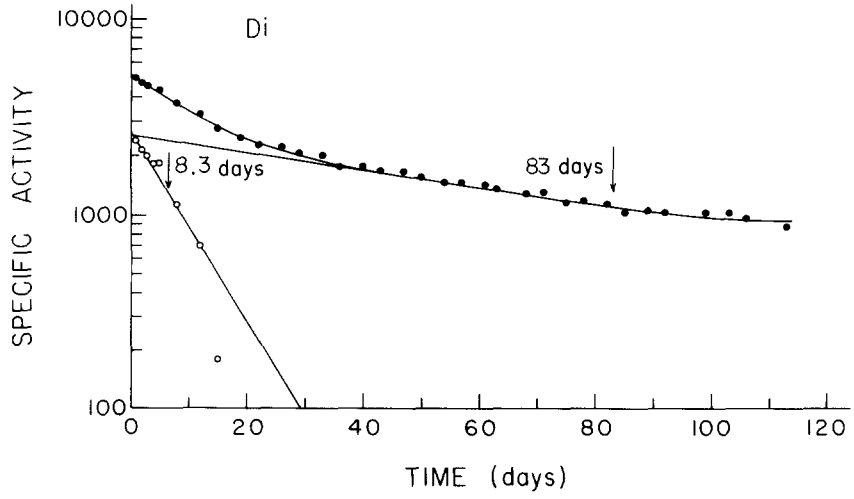

Fig. 4. [^{3}H]Cholesterol turnover curve for Di.

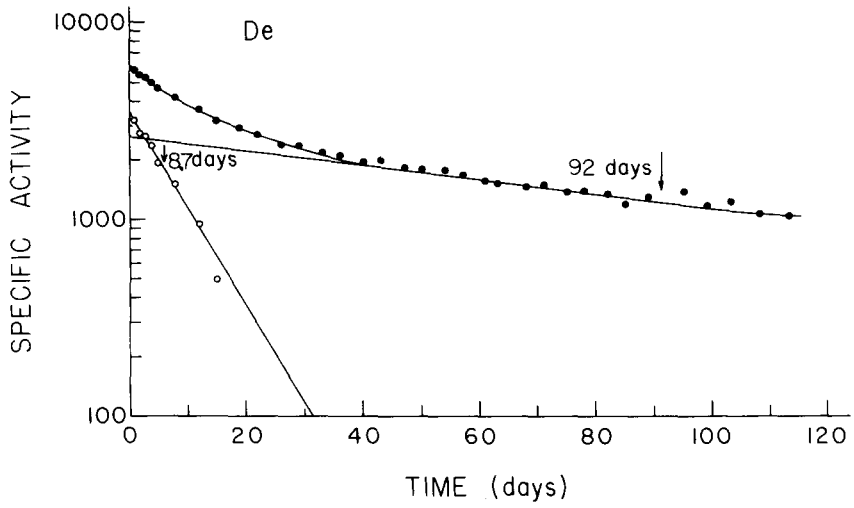

Fig. 5. [^{3}H]Cholesterol turnover curve for De.

Total parenteral alimentation

During the 2-week hyperalimentation period, the plasma cholesterol level fell in both girls. For Di the fall was from a baseline of 507 ± 10 mg/dl to 392 ± 16 mg/dl, and for De from a baseline of 556 ± 13 mg/dl to 436 ± 15 mg/dl. There was one stool produced by Di and two by De during these 2 weeks. The total fecal steroid excretion of these two girls in this period was 9 mg/day for Di and 37 mg/day for De, clearly a tremendous reduction from their usual fecal steroid output. This shutback in steroid output occurred during the time when plasma cholesterol levels were falling. HDL cholesterol fell to very low levels, 11 mg/dl.

DISCUSSION

These results suggest that one significant metabolic defect in these two patients with homozygous hypercholesterolemia was a decreased rate of removal of cholesterol from the rapid exchangeable pool of the body. Evidence for impaired cholesterol removal from the body was derived from several sources. The data of our turnover study indicated that the half-life of the first exponential of the cholesterol turnover curve $t^A_{1/2}$, was prolonged to over 8 days which is double that of normal adults (11). The fractional turnover rate for cholesterol in pool A ($-k_{AA}$) for both sisters was lower by 32% than that of the normal adults, and was lower by 46% than that of the adult heterozygotes.

This defect in cholesterol removal from the plasma was supported by the sterol balance data which suggested a failure in the excretion of cholesterol from the body. The total neutral sterol excretion in these homozygous type II patients was significantly lower than in normal children and in heterozygous type II children. Especially low and unique was the bile acid excretion in the homozygotes, 1.64 mg/kg per day, only 40% of the bile acid excretion in our normal and heterozygous children. Values obtained from the available literature (31) for neutral steroid and bile acid excretion in other normal children were 15.4–17.9 and 3.0–3.8 mg/kg per day, respectively, again very different from our homozygotes.

TABLE 4. Cholesterol turnover in the two sisters with clinical homozygous familial hypercholesterolemia

	Di	De
Half-life of first exponential, $t^A_{1/2}$, days	8.2	8.7
Half-life of second exponential, $t^B_{1/2}$, days	83	92
Pool size A, M_A, mg/kg	380	374
Plasma pool size,[a] M_{AP}, mg/kg	275	274
Pool size of tissues in pool A,[b] M_{AX}, mg/kg	105	100
Pool size B,[c] M_B, mg/kg	324	348
Total exchangeable pool, $M_A + M_B$, mg/kg	704	722
Production rate in pool A, PR_A, mg/day/kg	6.5	6.1
Rate constants, per day, (for explanation, see below)		
K_{AA}	−0.0519	−0.0506
K_{AB}	0.0347	0.0343
K_A	0.0173	0.0163
K_{BB}	−0.0407	−0.0369
K_{BA}	0.0407	0.0369

[a] Calculated by multiplying plasma cholesterol concentration (mg/ml) by plasma volume (estimated as 45 ml/kg body weight (From Edelman, I. S., and J. Liebman. 1959. Anatomy of body water electrolytes. *Am. J. Med.* **27:** 256–277)).

[b] Obtained as the difference between M_A and M_{AP}.

[c] Minimum estimate, assuming no synthesis of cholesterol in pool B, and no direct excretion from pool B. K_{AA} rate constant for total removal of cholesterol from pool A; K_{AB} rate constant for transfer of cholesterol from pool A to pool B; K_A rate constant for excretion of cholesterol from pool A and the whole system; K_{BB} rate constant for total removal of cholesterol from pool B: K_{BA} rate constant for transfer of cholesterol from pool B to pool A.

TABLE 5. Sterol balance in the two sisters with clinical homozygous familial hypercholesterolemia, normal children, and heterozygote children

	Endogenous Neutral Steroids		Bile Acids		Total	
	mg/day	*mg/kg/day*	*mg/day*	*mg/kg/day*	*mg/day*	*mg/kg/day*
Homozygotes						
Di: First inpatient period	262	6.02	86	1.98	348	8.00
Second inpatient period	206	4.61	44	0.98	250	5.59
Third inpatient period	209	4.61	84	1.85	284	6.46
De: First inpatient period	223	5.60	70	1.76	293	7.36
Second inpatient period	180	4.38	60	1.46	240	5.84
Third inpatient period	255	6.10	77	1.84	332	7.94
Mean ± SD	223 ± 31	5.22 ± 0.79	70 ± 16	1.64 ± 0.36	291 ± 43	6.86 ± 1.05
Normal children (5)[a]	224 ± 33	10.28 ± 3.10	99 ± 50	4.21 ± 1.32	324 ± 79	14.49 ± 3.82
Heterozygote children (11)	301 ± 94	7.84 ± 2.19	146 ± 52	4.08 ± 1.71	447 ± 108	11.92 ± 3.20

[a] Numbers in parentheses are total number of subjects.

A second metabolic defeat was a low calculated production rate of cholesterol in these two homozygotes, 6.30 mg/kg per day, as derived from cholesterol turnover data. This is only one-fourth that of normal adults and one-fifth that of adult heterozygotes (11). The biosynthetic rate of cholesterol obtained from three repeated sterol balance studies in these two homozygous patients was 6.85 mg/kg per day, a figure similar to the derived production rate of 6.30. In view of the profoundly elevated plasma cholesterol levels and the existence of partially intact LDL receptor in the cell membrane of these patients, it is likely that the low synthetic rate observed resulted from feedback inhibition of cholesterol synthesis.

It should be borne in mind while analyzing these data that the cholesterol production rate measured in these metabolic studies represents the biosynthetic rate of cholesterol under steady-state conditions. In this state, the production rate equaled the excretion rate and the plasma cholesterol remained relatively constant. However, before reaching this steady state, the initial elevation of plasma cholesterol must have resulted from the imbalance of production and excretion. The interplay of cholesterol absorption, production, and excretion must play the key roles in maintaining the homeostasis of total body cholesterol in vivo; therefore, the metabolic defect causing elevated plasma cholesterol may not rest on a single metabolic parameter but rather upon the interrelationship of these parameters. The production rate observed in the steady state represents the rate of biosynthesis; but it may also represent the maximal excretion rate which in turn dictates the biosynthetic rate under steady-state conditions. Consistent with this view is the observation that total body cholesterol synthesis in homozygotes can be markedly increased by treatment that tends to lower the expanded body pool of cholesterol by increasing its excretion, such as ileal bypass procedure (32) and cholestyramine administration (33).

The effects of varying restrictions in dietary cholesterol intake upon plasma cholesterol levels would also be consistent with a defect in cholesterol excretion. Thus, increasing amounts of dietary cholesterol could produce an increasing stress on the excretory pathways. By reducing the dietary load with a defect in excretion, the new steady state would be achieved at a lower plasma cholesterol level. This is illustrated in our patients by the plasma cholesterol changes that followed the severe reduction of dietary cholesterol (Figs. 2 and 3, Table 2). At the time of initial diagnosis, the plasma cholesterol levels were 664 ± 28 mg/dl for Di and 766 ± 54 mg/dl for De. The best levels attained with intensive drug therapy were 519 ± 13 mg/dl for Di and 604 ± 47 mg/dl for De. With a cholesterol-free diet only, the lowest plasma cholesterol levels achieved were 507 ± 10 mg/dl for Di and 556 ± 13 mg/dl for De. These levels were lower than those achieved with a 200–250 mg cholesterol intake, even with drug therapy. We suggest that the severe restriction of dietary cholesterol to below 100 mg/day may be a very important component in the therapy of homozygous hypercholesterolemia.

Starzl et al. (34) have suggested that the route of nutrient or intestinal flow to the liver via the portal vein may be a significant factor in the regulation of the cholesterol metabolism in the steady state. In their study, a profound drop in the plasma cholesterol level was noted both during total parenteral alimentation and after the portacaval shunt procedure. We observed a similar fall in the plasma cholesterol levels in these children during total parenteral alimentation. This decline was noted within a few days after the initiation of the parenteral alimentation regimen. However, our data do not suggest an explanation as to the etiology

of this plasma cholesterol lowering. We would emphasize the virtual shutdown of cholesterol excretion that occurred with the intravenous feeding (there being virtually no feces). Whether this also represents a decrease of cholesterol synthesis is only speculative but is not an unreasonable explanation.

The defect of cholesterol removal observed in these two homozygous patients could be attributed to several possibilities. First, it could be due to the defect of excretory mechanism. Following hepatic cellular uptake of plasma cholesterol, excretion into bile occurs either directly as cholesterol or by enzymatic conversion of cholesterol into bile acid. A defect in any of the enzymes responsible for the conversion of cholesterol to bile acids would decrease the rate of degradation of cholesterol and, unless compensated by increased hepatic excretion of cholesterol, would result in a greater level of intracellular cholesterol. This unusually low bile acid excretion observed seems to point to this possibility. Since we did not find any difference in the ratio of deoxycholic to lithocholic acid in these children, the defect, if any, would most likely be in the first steps of bile acid formation. Low excretion of bile acids was also observed in a family with hypercholesterolemia by Miettinen et al. (35). Another possibility for the low bile acid excretion might be a defect of clearance of cholesterol from the plasma compartment. From the turnover data, the sluggish removal of plasma cholesterol is graphically shown in Figs. 4 and 5 by the markedly decreased slope of the first exponential of the turnover curve. This produces a definitely flatter curve. The rate constant of cholesterol transfer from pool A to a more slowly exchangeable B (K_{AB}) was lower in comparison with that of normals and heterozygotes. Furthermore, when the cholesterol in plasma (M_{AP}) was calculated in these two patients, it represented 72.2% of the mass of pool A. Comparably, it only represents 26.5% and 32.5% in normal subjects and heterozygous type II patients, respectively, estimated by the same method (15). This suggested that, even within rapidly exchanging pool A, there was a tendency for cholesterol to be retained in the plasma compartment in these two homozygous sisters.

Recently, it has been proposed that HDL is the primary vehicle for transferring cholesterol back to the liver after its removal from LDL by the peripheral tissues (36). It is interesting to note that the HDL cholesterol level was extremely low in these two homozygous patients, only 27 mg/dl in the cholesterol-free diet period. The HDL cholesterol/LDL cholesterol ratio was 0.05. The reported HDL cholesterol level of normal girls at the same age was 54 ± 18 mg/dl (37). The HDL cholesterol/LDL cholesterol ratio was 0.52. The extremely low level of HDL in these patients may be one cause of difficulty in transporting cholesterol from the plasma to the liver for excretion and, consequently, of the slow clearance of cholesterol from the plasma compartment.

The minimum estimate of pool B (the slowly exchangeable pool) size in the two sisters was only 47% of the total body exchangeable pool of cholesterol (Table 3). This was extremely low compared to normal adults in whom 71% of the total body exchangeable cholesterol was found within the pool B and about 60% in the adult heterozygotes (11). The calculation of the size of pool B is dependent upon equilibration between plasma cholesterol and the tissue cholesterol radioactivities. Therefore, one possible explanation for the extremely low pool B size obtained from the kinetic analysis in the two sisters reported herein could be the nonequilibration of plasma cholesterol radioactivity with that of the tissue cholesterol radioactivity within the 16-week period of study. This possible suggestion comes out of the fact that, in the patient De, the xanthoma cholesterol specific activity, 21 days after intravenous administration of radioactive cholesterol, was only 11% of the plasma cholesterol specific activity (38), whereas in an adult severely hypercholesterolemic woman, B.J., the xanthoma cholesterol specific activity 28 days after intravenous injection of radioactive cholesterol, was 82% of the plasma cholesterol specific activity (38). Since the uptake of cholesterol from plasma by tissues could occur either by exchange or by net transfer or by both processes, the small amount of radioactivity found in the tendon xanthoma biopsy of De, 21 days after injection of radioactive cholesterol, may represent only that amount of plasma radioactivity that could only enter the tissue by exchange. In other words, little transfer of plasma cholesterol to the tissues was occurring. This finds some support from the fact that the rate constant for the transfer of cholesterol from pool A to pool B, K_{AB} (Table 3), was lower in the two sisters compared to that in the normal adults or in the adult heterozygous patients (11).

Because of the rare nature of this disease and the premature death of familial hypercholesterolemic homozygotes, studies of total cholesterol production conducted in such patients are few in number. From the limited studies, the reported results are conflicting (37). Of the five patients studied either by sterol balance (29, 32, 39) or by isotopic method (40), two showed overproduction of cholesterol with synthetic rates that were twofold greater than controls (29, 39), and the other three showed cholesterol production rates that were at the upper limits of the normal range (32, 40). The result of the present study showed the

other end of the spectrum in that the cholesterol production rates in our two patients were lower than in normal subjects.

Because of the similarity of the techniques used by both groups, it is of interest to compare our results with those reported recently by Bilheimer et al. (29) in a metabolic study of a 6-year-old girl with homozygous familial hypercholesterolemia. Their results indicated that the defect of cholesterol metabolism in the 6-year-old girl was the overproduction of both cholesterol and apo-LDL. From sterol balance studies, her total cholesterol and bile acid synthesis rates were determined to be 22.2 and 4.3 mg/kg per day, respectively. The LDL turnover study indicated that the fractional catabolic rate (FCR) of apo-LDL was 0.142 ± 0.009 and the rate of synthesis and catabolism was 41.6 mg/kg per day. Finally, her cultured skin fibroblasts revealed a complete deficiency of cell-surface LDL receptors.

In comparison, the metabolic defects in our two patients were found to be low excretion and production of cholesterol. The total cholesterol and bile acids synthesis rate of the two homozygous girls in the present study were only 6.88 and 1.64 mg/kg per day, respectively. An LDL turnover study of one of the two sisters indicated that the apo-LDL FCR was 0.127 ± 0.024 and the daily turnover was 15 mg/kg per day. Cultured skin fibroblasts from these two patients indicated that each had 40–60% the normal number of cell-surface LDL receptors as determined by the LDL-dependent incorporation of [^{14}C]oleate into cellular cholesteryl esters, i.e., were LDL receptor defective (41).

The divergent results obtained from homozygous hypercholesterolemic patients arrived at with similar methods suggest the possibility that clinically definable homozygous patients may be composed of at least two subgroups. One group has enhanced total body synthesis of cholesterol and LDL with absent cell membrane receptors for LDL, i.e., the patient reported by Bilheimer et al. (29). The second group has reduced total body synthesis of cholesterol and LDL with defective cell membrane receptors for LDL (i.e., our patients). Both groups of homozygotes excreted cholesterol poorly with regard to their very elevated plasma cholesterol levels. In the second group, however, there was internal recognition of the hypercholesterolemic state and inhibition of cholesterol biosynthesis. This was apparent in our patients under conditions of cholesterol-free diet which should stimulate maximal endogenous synthesis of cholesterol. These patients under these conditions still had low endogenous cholesterol synthesis.

The biochemical heterogeneities observed in a clinically definable homozygous familial hypercholesterolemia type II disease offer a new challenge to the workers in the field in terms of redefining this disease, understanding the variety of metabolic defects, and developing appropriate treatments.

We gratefully acknowledge permission to use the tissue culture cell receptor studies of the skin biopsies of these patients which were performed by Dr. Joseph Goldstein and Dr. Michael Brown, Southwestern University Medical School, Dallas, Texas. Drs. L. Torsvik, Robert Lees, and Robert Brooks provided us with the LDL turnover data on these patients. Dr. Douglas Egan of the Department of Pathology, Louisiana State University Medical School, assisted in carrying out the computer analyses of the cholesterol turnover curves. This research was supported in part by U.S. Public Health Service Research Grants HL-14230 (SCOR) and HL-19130, and Clinical Research Center Grants FR-59 and FR-334.

Manuscript received 14 December 1976 and in revised form 19 June 1978; accepted 28 August 1978.

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