Reduction in Cholesterol and Low Density Lipoprotein Synthesis after Portacaval Shunt Surgery in a Patient with Homozygous Familial Hypercholesterolemia

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ABSTRACT The turnover of ^{125}I-labeled low density lipoprotein (LDL) and the total body balance of cholesterol were studied in a 6-yr-old girl with the homozygous form of familial hypercholesterolemia (FH) before and after the surgical creation of an end-to-side portacaval shunt. The results were compared with those of similar studies simultaneously performed in untreated patients with the heterozygous form of FH and with the results of earlier studies performed on normolipidemic subjects.

Before shunt surgery, the rate of synthesis of LDL in the FH homozygote (mg/kg per day) was fourfold higher than in normolipidemic subjects and twofold higher than in her heterozygous mother. The fractional catabolic rate for LDL in the homozygote was decreased to 33% of normal control values. The rate of cholesterol synthesis, estimated by chemical sterol balance, was higher in the FH homozygote than in two FH heterozygotes of similar age studied simultaneously. When considered in relation to the markedly elevated level of plasma cholesterol, the observed rate of cholesterol synthesis in the FH homozygote was inappropriately elevated. Bile acid production was normal in all three children.

5 mo after shunt surgery, the rate of LDL synthesis in the homozygote had declined by 48% as compared with the preoperative value, and this caused a 39% drop in the plasma LDL cholesterol level despite a 17% reduction in the fractional catabolic rate of the lipoprotein. The rate of cholesterol synthesis fell by 62% as compared with the preoperative value.

The findings of an inappropriately elevated rate of production of both cholesterol and LDL as well as a reduced fractional catabolic rate for the lipoprotein in the untreated FH homozygote are consistent with results of studies in cultured fibroblasts indicating that the primary genetic defect in FH involves a deficiency in a cell-surface receptor for LDL that regulates both cholesterol synthesis and LDL degradation. Although the mechanism for the decline in production of cholesterol and LDL after portacaval shunt surgery is unknown, it was observed that these changes were associated with marked increases in the plasma concentrations of bile acids and glucagon.

INTRODUCTION

Recent studies of patients with the homozygous form of familial hypercholesterolemia (FH)[1] have provided new insight into the mechanism underlying the elevation of plasma low density lipoprotein (LDL) in this disorder. Three observations have been especially informative. First, studies of cultured skin fibroblasts from FH homozygotes have suggested that the primary genetic defect in this disorder involves a deficiency in a cell-surface receptor for LDL (1-4). In normal cells,

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[1] *Abbreviations used in this paper:* FH, familial hypercholesterolemia; LDL, low density lipoprotein; HDL, high density lipoprotein; FCR, fractional catabolic rate; GLI, glucagon-like immunoreactivity.

binding of LDL to this receptor facilitates the cellular uptake and degradation of the lipoprotein (1, 2). The subsequent hydrolysis of the protein and cholesteryl ester components of LDL within the cell results in the liberation of free cholesterol which, in turn, suppresses cellular cholesterol synthesis (5). Cells from FH homozygotes, which lack the LDL receptor, have a deficient ability to take up LDL (1-3) and, as a result, they overproduce cholesterol (6, 7) and show a diminished rate of degradation of the lipoprotein, even in the presence of high extracellular levels of LDL (1, 2).

A second important observation has been made recently by Myant and co-workers, who have measured the turnover of ^{125}I-LDL in the plasma of FH homozygotes and have demonstrated that these subjects manifest both an excessive production of LDL and a reduced fractional catabolic rate for the lipoprotein (8, 9). As suggested by Simons et al. (9), these in vivo data can be interpreted as consequences of the LDL receptor defect demonstrated in cultured cells. Finally, Starzl and co-workers have succeeded in lowering plasma LDL levels in one FH homozygote by the surgical creation of a portacaval shunt (10). This latter observation is important because it provides the opportunity to identify the changes in LDL and cholesterol metabolism that bring about a reduction in plasma LDL levels in FH homozygotes who are resistant to more conventional forms of therapy.

In the present study, simultaneous measurements were made of plasma ^{125}I-LDL turnover and total body cholesterol balance in an FH homozygote before and after the creation of a portacaval shunt. The findings before portacaval shunt surgery confirm the previous observations of overproduction of LDL in FH homozygotes (8, 9) and clearly demonstrate an inappropriately high rate of cholesterol production. After the portacaval shunt, a 39% reduction occurred in plasma LDL levels and was associated with a 48% reduction in the rate of LDL synthesis and a 62% reduction in the total body rate of cholesterol synthesis.

METHODS

Patients. Four subjects were studied while hospitalized in a metabolic ward. Each subject consumed an isocaloric, low cholesterol, partial solid food diet designed to maintain stable weight and to facilitate cholesterol balance studies. All diets were formulated with a lard base as previously described (11) and contained 18–20% of the total calories as protein, 35–38% as fat, and 43–45% as carbohydrate. The clinical data and caloric intake for each patient are summarized in Table I.

M. C., a 6-yr-old Caucasian girl with the receptor-negative form of homozygous FH (12), was born with xanthomatous deposits on both heels. Severe hypercholesterolemia was first documented at age 5. Because of an aortic systolic ejection murmur coupled with anginal chest pain and syncopal episodes, the patient underwent cardiac catherization with coronary angiography at age 5. The findings included aortic stenosis with a 40-mm Hg gradient across the aortic valve and diffuse coronary atherosclerosis with complete occlusion of the lateral branch of the circumflex artery. The aortic root was also involved with atheroma formation. Treatment with cholestyramine (12 g/day) failed to lower her plasma cholesterol level significantly, and her cardiac symptoms progressed. In 1974 she was referred to the University of Texas Health Science Center at Dallas, where family studies revealed that both of her parents and two of her three siblings had clinical features consistent with heterozygosity for the FH gene. Her family pedigree has been published elsewhere (13). Biochemical analysis of M. C.'s cultured skin fibroblasts revealed a complete deficiency of cell-surface LDL receptors. Cells from each of her parents manifested a 40–60% reduction in their receptors (3). M. C. underwent a combined LDL turnover–cholesterol balance study in July and August of 1974, 1 mo after discontinuing all medications. In October 1974, an end-to-side portacaval shunt was performed by Dr. Thomas E. Starzl at the University of Colorado, Denver, Colo. After surgery, her dietary treatment consisted of a low cholesterol, high polyunsaturated fat diet, and in December 1974, the administration of propranolol (20 mg/day) was begun to suppress the frequency of paroxysmal episodes of atrial tachycardia associated with angina. In February 1975,

TABLE I

*Clinical Data on Patients during ^{125}I-LDL Turnover and Cholesterol Balance Studies**

Patient	Age	Sex	Weight	Height	Cholesterol intake	Calorie intake	Plasma cholesterol			Plasma triglyceride
							Total	LDL	HDL	
	yr		*kg*	*cm*	*mg/day*	*cal/day*	*mg/dl*			*mg/dl*
M. C. (before shunt)	7	F	20.7±0.1	121.9	109	1,185	997±47	801±28	89±7	112±16
M. C. (after shunt)	7	F	21.9±0.2	125.7	109	1,185	577±22	492±23	59±9	67±8
J. C.	30	F	59.6±0.3	157.5	175	2,058	333±9	199±22	85±9	97±23
L. C.	11	F	31.2±0.2	144.8	145	1,803	278±17	209±19	59±5	66±9
Je. Ch.	12	M	40.9±0.3	157.5	193	2,227	303±17	251±12	38±3	57±7

* All values are expressed as mean ±1 SD of observations made during the course of these studies.

she was readmitted to the University of Texas Health Science Center at Dallas for another combined LDL turnover–cholesterol balance study during which the propranolol therapy was continued.

J. C., the 30-yr-old mother of M. C., had an elevated plasma cholesterol level (Table I), but had no cutaneous or tendinous xanthomas and no clinical signs of coronary heart disease. Biochemical analysis of her cultured skin fibroblasts showed a 55% reduction in cell-surface LDL receptors, a finding consistent with the clinical and genetic diagnosis of heterozygous FH (3). Before her hospitalization for the LDL turnover study, she had taken no medications and was on an unrestricted diet.

L. C., the 11-yr-old sister of M. C., had an asymptomatic elevation in plasma cholesterol level. In view of the family history, she is presumed to have the heterozygous form of FH. She had no cutaneous or tendinous xanthomas. Her fibroblasts were not studied.

Je. Ch., a 12-yr-old Caucasian boy from a different family, had an asymptomatic elevation in plasma cholesterol level and no cutaneous or tendinous xanthomas. His maternal grandmother, mother, one uncle, one aunt, and two of his four siblings each exhibited hypercholesterolemia, and each had at least one of the following clinical findings: xanthelasma, tendinous xanthoma, or premature coronary heart disease. Biochemical analysis of the cultured skin fibroblasts of Je. Ch. and of his mother showed that each had a 40–50% reduction in cell-surface LDL receptors, a finding consistent with the clinical diagnosis of heterozygous FH (3).

Lipoprotein turnover studies. 10 days to 2 wk after admission to the metabolic ward, plasma from each subject was obtained by plasmapheresis using standard citrate phosphate-dextrose anticoagulant and then dialyzed for 24 h against 12 liters of solution containing 0.15 M NaCl, 0.1% EDTA, pH 7.4 (*d*-1.006 g/ml). The density of the plasma was adjusted to 1.019 by addition of solid KBr (14), and centrifuged in a Beckman 60 Ti rotor (Beckman Instruments, Inc., Palo Alto, Calif.) at 60,000 rpm for 11 h at 4°C (1.68×10^8 avg. *g*-min) (15). The infranatant fraction was adjusted to 1.063 by another addition of solid KBr, and this material was centrifuged in a Beckman 60 Ti rotor at 60,000 rpm for 14 h (2.14×10^8 avg. *g*-min) or in a Beckman 65 rotor at 65,000 rpm for 14 h at 4°C (2.26×10^8 avg. *g*-min). The supernatant fraction was isolated by tube slicing and the density was readjusted to 1.063 by addition of KBr using the density of a salt blank tube similarly processed to assess the degree of density alteration caused by the centrifugation. The 1.063 top material was then layered with 1.063 salt solution and centrifuged in a Beckman 65 rotor at 65,000 rpm for 16 h at 4°C (2.58×10^8 avg. *g*-min). The LDL, isolated by tube slicing, was resuspended, dialyzed for 2 h against 4 liters of solution containing 0.15 M NaCl and 0.1% EDTA, pH 7.4, and an aliquot was taken for protein determination by a modification of the method of Lowry et al. (16). The LDL was then centrifuged in a Sorvall RC2-B centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.) at 15,000 rpm for 30 min at 4°C to remove small amounts of particulate matter. Immunoelectrophoresis using antibodies to human whole serum and to human beta lipoprotein (Hyland Labs., Costa Mesa, Calif.) indicated that the LDL preparations contained no other plasma proteins or lipoproteins.

LDL protein was radioiodinated by a modification (17) of the iodine monochloride method (18), assuming an LDL protein molecular weight of 100,000. The reactants were added in such a way as to assure a ratio of iodine atoms to moles of protein of less than 1. Unbound iodine was removed by dialysis against a total of 16 liters of solution containing 0.15 M NaCl and 0.1% EDTA, pH 7.4 for 8 h. Iodination efficiency ranged between 21 and 30%. The degree of lipid labeling of the LDL, determined by chloroform: methanol extraction (2:1) of ^{125}I-LDL, varied between 3.6 and 7.7%. Greater than 97% of the radioactivity in the ^{125}I-LDL was precipitated with TCA and the ^{125}I-LDL was indistinguishable from native LDL electrophoretically (19), immunologically (20), and biologically as determined by its ability to suppress 3-hydroxy-3-methylglutaryl CoA reductase activity in human fibroblasts (1, 21). The ^{125}I-LDL was mixed with 30 mg/ml of human serum albumin, sterilized by Millipore filtration (0.45 μm) (Millipore Corp., Bedford, Mass.), and pyrogen tested before use (22).

Each lipoprotein turnover study was initiated by injecting autologous ^{125}I-LDL through a running intravenous line kept open with normal saline. From 0.2 to 1.2 mg of LDL protein was injected in a volume not exceeding 2 ml. M. C. received 15 μCi in each of her studies and J. C. received 50 μCi. Plasma volume was calculated by the isotope dilution technique using the 10-min sample (23). Daily fasting a.m. blood samples (5–7 ml) were obtained using EDTA anticoagulant. Every 3rd or 4th day, 20 ml (M. C.) or 35 ml (J. C.) of blood was obtained for quantitative lipoprotein analysis. 24-h urine collections were made in bottles containing small amounts of preservative to minimize volatilization of ^{125}I (24). Total daily urine volumes were recorded and aliquots were assayed for radioactivity. Plasma and urine samples were assayed for radioactivity in a Packard model 5285 Autogamma Scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.).

To show that all the ^{125}I radioactivity in plasma was associated with LDL, plasma samples from days 0, 2, 4, and 8 of the first study in M. C. and from days 0, 1, and 3 of the study in J. C. were subjected to column chromatography using Bio-Gel A-50m (Bio-Rad Laboratories, Richmond, Calif.). The gel was packed in glass columns measuring 1.5×30 cm with a bed height of 27 cm. 1-ml fractions were eluted from the column at 4°C in a buffer containing 0.05 M Tris-chloride, 0.1 M sodium chloride, and 0.02% sodium azide, pH 8.6. Authentic ^{125}I-very low density lipoprotein eluted with a peak in fractions 22 and 23; authentic ^{125}I LDL eluted as a symmetrical peak in fractions 33 and 34. When 1 or 2 ml of whole plasma, obtained from each patient at the above time points, was applied to the column, more than 99% of the total plasma ^{125}I radioactivity eluted in a symmetrical peak that corresponded with the authentic LDL, suggesting that virtually all of the plasma ^{125}I radioactivity remained in LDL throughout the course of these studies.

The kinetic parameters for LDL turnover were calculated using methods originally described by Matthews (25) and by Nosslin (26) and recently adapted to LDL turnover studies by Langer et al. (23). Curve fitting was performed using the SAAM 25 Computer Program to derive the two exponential components of the plasma die-away curve (27). These methods yielded results for the absolute rates of synthesis and degradation, the percent of the apo-LDL pool in the intravascular space, and the fractional catabolic rate (FCR) (defined as the fraction of the intravascular pool of LDL catabolized per day). The FCR was independently measured by relating the daily urinary excretion rate of ^{125}I radioactivity to the ^{125}I radioactivity in plasma (U/P ratio) (28). The rate of synthesis for apo-LDL was calculated using the following formula and is expressed as the milligrams of apo-LDL synthesized per day (23): SR = (FCR) (PV) (apo-LDL concentration), where SR = rate

of synthesis, FCR = fractional catabolic rate, and PV = plasma volume. The total rate of synthesis was also expressed in terms of body weight as the milligrams of apo-LDL synthesized per kilogram body weight per day. The apo-LDL concentration was calculated from the measured value for LDL cholesterol and the measured ratio of protein to cholesterol in each patient's LDL. The ratio of protein to cholesterol for LDL in M. C. was 0.71 (first study) and 0.66 (second study); in J. C. this ratio was 0.69. During the ^{125}I-LDL turnover study, each patient received 1 g of potassium iodide by mouth daily in divided doses (23).

Measurement of plasma cholesterol and triglyceride levels. Cholesterol was determined by a modification (29) of the method of Zak (30). Triglyceride determinations were performed using a commercially available enzyme method (Boehringer Mannheim Corp., New York, catalog no. 15747). Moni-Trol I Serum (American Hospital Supply Corp., Evanston, Ill.) was used for standardization.

Measurement of plasma lipoproteins. Lipoprotein concentrations in plasma were estimated by standard ultracentrifugal techniques combined with heparin-manganese precipitation to estimate the content of high density lipoprotein cholesterol (31). Completeness of precipitation was assessed by performing agarose gel electrophoresis of the supernate from the heparin-manganese precipitation, and no contamination with LDL was found. Since LDL was being studied between salt densities 1.019 and 1.063, the 1.019 density was used instead of 1.006 to perform the lipoprotein measurements.

Cholesterol balance studies. Chemical cholesterol balance was performed according to previously described methods using β-sitosterol and chromium oxide as internal standards (11, 32). Serial 4-day stool collections were performed while the patient was consuming the lard base diet. The stools were frozen until the study was completed, each collection was then homogenized, and aliquots were taken for sterol analyses, which were performed at the University of California, San Diego, Calif.

Blood chemistries. Routine blood chemistry determinations were performed using standard techniques in the metabolic laboratory associated with the Clinical Research Center at the University of Texas Health Science Center.

Arginine infusion test. An intravenous arginine infusion test was performed as previously described (33) and plasma specimens were assayed for insulin, glucagon, glucagon-like immunoreactivity (GLI), and glucose by Dr. Roger Unger.

Plasma bile acids. 10 ml of blood was collected from patient M. C. after an overnight fast and 2 h postprandially. The plasma samples were stored at −20°C and assayed for bile acid levels by Dr. Norman Javitt (34).

RESULTS

Evidence for a steady metabolic state. Several lines of evidence suggest that each patient maintained a relatively steady metabolic state during each study. First, caloric intake was constant as reflected by the absence of any significant weight change (Table I). Second, plasma lipids in each patient showed little variation throughout the study (Table I and Fig. 1). Third, the fecal steroid excretion for each subject was essentially constant during each study (Table II).

Effect of portacaval shunt on plasma lipids in M. C. Before portacaval shunt surgery, M. C. maintained a

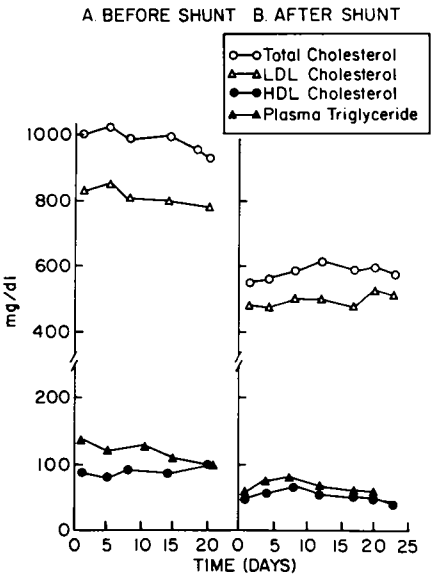

FIGURE 1 Plasma lipids and lipoproteins in patient M. C. before (A) and after (B) portacaval shunt surgery. The values represent those obtained at regular intervals during each of the two ^{125}I-LDL turnover studies. Zero time in each case represents initiation of the study. The interval between the first and second study was 5 mo.

total plasma cholesterol level that was consistently above 900 mg/dl over a 1-yr period of observation. 5 mo after portacaval shunt surgery, she manifested a 42% reduction in total plasma cholesterol, a 39% reduction in LDL cholesterol, a 33% reduction in HDL cholesterol and a 40% reduction in plasma triglyceride (Fig. 1). The fall in plasma lipids occurred gradually during the 4 mo after surgery, after which the lipid levels remained constant at the values shown in Fig. 1B.

Chemical cholesterol balance. (Tables II and III). Before portacaval shunt surgery, the total fecal steroid excretion in M. C. exceeded the sterol intake by 459 mg/day (Table II). Since there was no reason to suspect that M. C. was losing body cholesterol, this figure represents a minimal estimate for the amount of total cholesterol synthesized per day. When related to body weight, the rate of cholesterol synthesis in M. C. was 22.2 mg/kg per day, a value that was more than twofold higher than that observed in L. C. and Je. Ch., the two FH heterozygous children who were studied at the same time and by the same balance method (Table III). Moreover, the rate of cholesterol synthesis in M. C. was more than twofold higher than the mean rate determined for 15 adult FH heterozygotes (10.6, range 6.0-15.5 mg/kg per day) using the same balance technique.[2] These values for daily rates of cholesterol synthesis in FH heterozygotes are similar to those that

[2] Grundy, S. M. Unpublished observations.

TABLE II

Cholesterol Balance Data

Patient	Sample number*	Dietary cholesterol intake (*a*)	Fecal steroid excretion			Steroid balance (*b*)-(*a*)
			Neutral	Acidic	Total (*b*)	
			mg/day			
M. C.	1	109	456	68	524	415
(before shunt)	2	109	503	105	608	499
	3	109	492	114	606	497
	4	109	459	92	551	442
	5	109	469	76	545	436
	6	109	496	75	571	462
		Mean ±SD	479±20	88±18	568±34	459±34
M. C.	1	109	269	38	307	198
(after shunt)	2	109	270	32	302	193
	3	109	264	37	301	192
	4	109	269	30	299	183
	5	109	249	29	278	169
		Mean ±SD	263±8	33±4	297±11	187±11
L. C.	1	145	369	121	490	345
	2	145	391	101	492	347
	3	145	338	82	420	275
	4	145	358	41	399	254
	5	145	353	127	480	335
		Mean ±SD	363±20	94±35	456±44	311±44
Je. Ch.	1	193	492	238	730	537
	2	193	470	284	754	561
	3	193	439	93	532	339
	4	193	381	294	675	482
	5	193	319	206	525	332
	6	193	329	213	542	349
	7	193	317	367	684	491
	8	193	339	302	641	448
		Mean ±SD	368±72	250±82	635±91	442±91

* Each sample number represents a 4-day stool collection, and samples are listed in the sequence collected.

have been determined for healthy subjects in many laboratories using a variety of techniques (35-37).

The significance of the cholesterol overproduction in M. C. was further emphasized by the finding that 5 mo after portacaval shunt surgery, at a time when the plasma cholesterol level had fallen by about 40% (Fig. 1), cholesterol balance had dropped by 59% (from 459 to 187 mg/day) (Table II). The calculated daily rate of cholesterol synthesis in M. C. after portacaval shunt surgery was 8.5 mg/kg per day, a value similar to those of the two FH heterozygous children, L. C. and Je. Ch. (Table III). In calculating the rate of cholesterol synthesis from the measured sterol balance, it is assumed that the patient is neither accumulating cholesterol excessively nor losing cholesterol from body stores; i.e., the total body content of cholesterol is constant during the period of study and therefore the daily amount of fecal steroid excreted equals the daily amount of cholesterol synthesized. If the reduction in fecal steroid excretion in M. C. after portacaval shunt surgery was due solely to a preferential retention of cholesterol in the body without a change in the rate of cholesterol synthesis, she would have been accumulating cholesterol at the rate of 272 mg/day (459-187, Table II), an enormous value. This was unlikely in view of two observations: (*a*) her plasma cholesterol level was lower after surgery and (*b*) her cutaneous xanthomas were observed to be regressing in the interval after surgery. If anything, M. C. may have been in a net negative cholesterol balance during the course of the second study, causing the calculated cholesterol synthesis rate, after shunt surgery, to be a slight overestimation of her true production rate.

The total fecal bile acid excretion in M. C. before

TABLE III

Cholesterol and Bile Acid Synthesis Rates as Determined by Cholesterol Balance Studies

Patient	Synthesis rates*	
	Total cholesterol	Bile acids
	mg/kg/day	
M. C. (before shunt)	22.2	4.3
M. C. (after shunt)	8.5	1.5
L. C.	10.0	3.0
Je. Ch.	10.8	6.1

* The synthesis rate of total cholesterol was determined by dividing the mean total steroid balance (Table II) by the body weight (Table I). The synthesis rate of bile acids was determined by dividing the mean bile acid excretion (Table II) by the body weight (Table I). The values for total cholesterol include values for both neutral sterols and bile acids.

portacaval shunt surgery was 4.3 mg/kg per day, a value similar to that obtained in the two FH heterozygous children, L. C. and Je. Ch. (Table III), and also similar to reported values in healthy subjects (35-37). After portacaval shunt surgery, there was a striking reduction in M. C.'s bile acid production (Table III).

LDL turnover. (Table IV and Fig. 2). The LDL turnover studies were performed simultaneously with the chemical cholesterol balance studies. Before shunt surgery, the plasma apo-LDL level in M. C. was 4.2-fold higher than that of her mother, J. C. (Table IV). In addition, the FCR in M. C., calculated either from the plasma ^{125}I-LDL decay curve or from the renal clearance (U/P ratio), was about one-half that of J. C. However, in view of the marked increase in the plasma apo-LDL pool in M. C. as compared with that in J. C., the absolute rate of catabolism of LDL corrected for

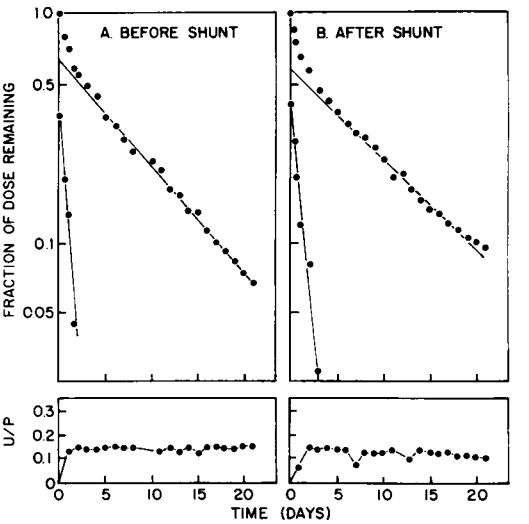

FIGURE 2 Plasma die-away curve and urine/plasma (U/P) ratios in M. C. after intravenous injection of ^{125}I-LDL before (A) and after (B) portacaval shunt surgery. The fraction of the injected dose of ^{125}I-LDL remaining in plasma is plotted semilogarithmically against time. The U/P ratio for each study is plotted below its corresponding plasma die-away curve.

body weight (i.e., the milligrams of apo-LDL degraded per kilogram per day) was 2.4-fold higher in M. C. as compared with J. C. In previous studies of ^{125}I-LDL turnover using the same techniques, we have observed a mean absolute catabolic rate for LDL of 10.0 mg/kg per day (range, 8.0-13.6) in 11 normolipidemic subjects having a mean age of 22 yr (range, 19-26).[3] Since in the steady state the absolute rate of LDL synthesis is equal to the absolute catabolic rate, the data indicate that M. C. produced 2.4 times as much LDL as did J. C.

[3] Bilheimer, D. W. Unpublished observations.

TABLE IV

Kinetic Parameters for ^{125}I-LDL Turnover Studies

Patient	Plasma volume	Plasma apo-LDL	$t_{\frac{1}{2}}$ for exponential decay*		FCR‡		Rate of synthesis and catabolism of apo-LDL		Distribution of apo-LDL§
			(*a*)	(*b*)	(*c*)	(*d*)			
	ml	*mg/dl*	*days*	*days*			*mg/day*	*mg/kg/day*	%
M. C. (before shunt)	989	569	0.63	6.5	0.153	0.142±0.009	861	41.6	72.0
M. C. (after shunt)	1,149	325	1.1	8.0	0.127	0.123±0.013	474	21.6	72.8
J. C.	2,286	137	1.0	3.3	0.334	0.322±0.022	1,046	17.6	77.5

* Calculated from the first (*a*) and second (*b*) exponential of the plasma die-away curve. The second exponential represents an estimate of the half-life of apo-LDL.

‡ Fraction of the intravascular apo-LDL pool metabolized each day, calculated either from the plasma die-away curve (*c*) or from the U/P ratio (*d*). The difference in the U/P ratios in M. C. before and after shunt was statistically significantly different ($P < 0.001$) (see Footnote 4).

§ Percent of total body apo-LDL contained in intravascular space.

TABLE V
Plasma Bile Acids in M.C. before and after Portacaval Shunt

Serum bile acids	Before shunt		After shunt	
	Fasting	2 h post-prandial	Fasting	2 h post-prandial
	μg/ml		μg/ml	
Deoxycholic acid	ND*	ND	0.1	0.6
Chenodeoxycholic acid	0.5	ND	0.9	7.4
Cholic acid	1.3	ND	1.7	9.6
Total serum bile acid	1.8	<1	2.7	17.6

* ND, none detected.

and about 4 times as much as normolipidemic control subjects.

After shunt surgery, the plasma level of apo-LDL in M. C. declined by 43%. The FCR decreased 17%[4] after surgery, and her absolute catabolic and synthetic rates for LDL declined by 45% (Table IV and Figs. 1 and 2).

Liver function studies. Liver function studies were followed monthly after portacaval shunt surgery in M. C. Total serum protein and albumin levels remained normal. Serum bilirubin rose transiently to 1.6 mg/dl 2 mo after surgery but subsequently returned to normal. Although the serum alkaline phosphatase, serum aspartate aminotransferase, and serum alanine aminotransferase have each been slightly abnormal on several occasions, there was no consistent trend toward a worsening of liver function.

Plasma bile acids. The fasting total plasma bile acid levels in M. C. before portacaval shunt surgery were within the normal range (38) and fell slightly 2 h postprandially (Table V). After shunt surgery, M. C.'s fasting levels of bile acids were slightly elevated (2.7 μg/ml) and rose postprandially to 17.6 μg/ml, a value that is higher than that previously reported for subjects with chronic cirrhosis (38). This elevation is presumably due to the postprandial shunting of bile acid-rich portal blood into the systemic circulation.

Plasma levels of glucagon, GLI, and insulin after arginine infusion. (Table VI). Before portacaval shunt surgery, the fasting plasma levels of glucose, glucagon, GLI, and insulin were normal, and both insulin and glucagon rose normally in response to the infusion of arginine. GLI showed no significant rise. After shunt surgery, there was no change in the response to arginine with regard to peripheral blood levels of glucose, insulin, and GLI. However, glucagon levels, which were elevated even in the fasting state, rose to extremely high values after arginine infusion.

DISCUSSION

The major finding of the current study was the demonstration of an inappropriately high total body production rate for both cholesterol and LDL in an untreated FH homozygote (M. C.), and a reduction in both parameters after portacaval shunt surgery. Our observations with regard to LDL turnover before the shunt surgery in M. C. were similar to those previously reported by Myant and co-workers (8, 9).[5] These latter workers found a mean rate of LDL synthesis of 33.9 mg/kg per day in four homozygotes, ranging in age up to 22 yrs, as compared with a mean rate of synthesis of 18.9 mg/kg per day in four control subjects. In the current study M. C. was found to produce LDL at a rate of 41.6 mg/kg per day. Her heterozygous mother produced 17.6 mg/kg per day. When measured by our techniques, LDL production in control subjects averaged 10.0 mg/kg per day. Thus, despite the variations in control data between the different laboratories, it can still be concluded that overproduction of LDL appears to be a consistent finding in homozygotes at least up to age 22.

Interpretation of the observed rate of cholesterol synthesis in M. C. is not quite so clear-cut because of the relative paucity of control data in children of comparable age. In an attempt to obviate this problem in the current study, cholesterol synthesis was measured simultaneously in M. C. (age 6, 21 kg body weight) and in two children with the heterozygous form of FH, L. C. (age 11, 31 kg) and Je. Ch. (age 12, 41 kg). In the two heterozygous children, cholesterol production, when expressed on a milligrams per kilogram body weight basis, was 10.0 and 10.8 mg/kg per day, values that are similar to the production rates previously observed in adults with heterozygous FH (10.6 mg/kg per day). Thus, when the rate of cholesterol synthesis is compared in adults and children with weights as low as 31 kg, the expression of the data in terms of body weight gives a consistent value. When adjusted for body weight, M. C.'s rate of cholesterol synthesis before shunt surgery (22.2 mg/kg per day) was twofold higher than the values obtained in the two heterozygous

[4] The U/P ratio data in M. C. before and after shunt surgery were subjected to statistical analysis using the approximate t test. This particular test was used when it was found that the variances of the before shunt values ($n = 19$) were not equal to the variances of the after-shunt values ($n = 20$) ($P < 0.05$). The approximate t value was 4.327 indicating that the U/P ratios before and after surgery were significantly different ($P < 0.001$).

[5] Torsvik, Fischer, Feldman, and Lees have recently reported interesting data on the turnover of plasma ^{125}I-LDL in three patients with severe FH before and after intravenous hyperalimentation (1975. *Lancet.* 1: 601–604.). The three subjects that were studied were presumably FH homozygotes. The authors, however, did not make a comparison of their LDL production rates with those of normal subjects.

TABLE VI

Influence of Arginine Infusion on Glucagon, GLI, and Insulin in M. C. before and after Portacaval Shunt Surgery

	Before shunt				After shunt			
Time	Glucose	Glucagon	GLI	Insulin	Glucose	Glucagon	GLI	Insulin
min	*mg/dl*	*pg/ml*	*ng/ml*	*μU/ml*	*mg/dl*	*pg/ml*	*ng/ml*	*μU/ml*
−20	68	48	1.8	9	56	720	1.8	7.4
0	72	52	1.8	6	54	550	1.1	10
5	81	180	1.8	33	—	—	—	—
10	84	200	1.8	32	65	>2,000	2.1	23
20	93	270	1.9	44	65	>2,000	2.0	48
30	95	290	1.9	52	67	>2,000	1.9	58
40	98	240	1.9	48	69	>2,000	1.6	54

children, in adult heterozygotes, and in normal adults. Since M. C.'s body weight was 10 kg less than that of the highest control subjects, the possibility still exists that the expression of cholesterol synthesis in terms of body weight in M. C. may give a somewhat falsely elevated value. However, even when one makes no correction for body weight, neutral sterol excretion in M. C. was still 30% higher than in her 11-yr-old sister (L. C.) despite the fact that M. C.'s plasma cholesterol level was 997 mg/dl as compared with a value of 278 mg/dl in L. C. Even if one considers the cholesterol production rate in M. C. to be only "high normal," it is clearly inappropriate in the face of her markedly expanded body and plasma cholesterol pools.

Previous studies of total body cholesterol synthesis in FH homozygotes have been limited. In one study of two FH homozygotes, Grundy et al. calculated the endogenous fecal steroid excretion from measurements of the appearance of radiolabeled plasma cholesterol in the stool (35). In the two patients studied by these workers, cholesterol production, although somewhat high, could not be clearly differentiated from that in normal subjects (35). While the reason(s) for the difference in the results of the former study and the current one is not completely clear, one factor may be related to the older age of the FH homozygotes previously studied (ages 14 and 15 [35]) as compared to M. C. (age 6). In a study of a 10-yr-old FH homozygote, Lewis and Myant observed an elevated total endogenous fecal steroid excretion of 17.1 mg/kg per day, a value higher than that observed in any of their five normolipidemic control subjects (range, 6.6–12.6 mg/kg per day) (36, 39) and not unlike that observed in M. C. (22.2 mg/kg per day).

The inappropriately high rate of production of cholesterol and LDL in M. C. is presumably related to the genetic deficiency in cell-surface LDL receptors that has been demonstrated in cultured fibroblasts (1-4). In normal fibroblasts, binding of LDL to this receptor initiates a sequence of events that results in the cellular uptake and degradation of the lipoprotein. After the LDL is bound to the receptor, the surface-bound LDL becomes incorporated into endocytotic vesicles, the interiorized endocytotic vesicles fuse with lysosomes, the cholesteryl ester and protein components of LDL are hydrolyzed by lysosomal enzymes to degradative products, including free cholesterol and amino acids, and the liberated free cholesterol is transferred from lysosomes to cellular membranes (1-6, 40). The resultant accumulation of cholesterol within the cell has at least two important effects on cellular cholesterol metabolism: (*a*) it reduces cholesterol synthesis by suppressing the activity of 3-hydroxy-3-methylglutaryl-CoA reductase, the rate-limiting enzyme in the cholesterol biosynthetic pathway, and (*b*) it enhances the activity of fatty acyl-CoA: cholesteryl acyltransferase which facilitates the storage of incoming free cholesterol in the form of cholesteryl esters (40, 41). It has been postulated that this LDL receptor mechanism may function in the body to allow plasma LDL to transfer cholesterol derived from either intestinal absorption or endogenous hepatic synthesis to nonhepatic cells (40). Since fibroblasts from FH homozygotes (including M. C.) lack functional LDL receptors, these mutant cells are deficient in their ability to take up cholesterol from plasma LDL, to degrade the lipoprotein, and to suppress cholesterol synthesis (1-7).

When applied to cholesterol metabolism in intact subjects, the LDL receptor model predicts overproduction of body cholesterol in FH homozygotes, provided that no compensatory metabolic changes occur when the total body cholesterol content rises to extremely high levels (4). Although our findings of an inappropriately high rate of cholesterol synthesis in M. C. are consistent

with the predictions of the fibroblast model, the current data do not establish whether cholesterol is being produced in the liver, nonhepatic tissues, or both. High rates of sterol production in FH would presumably occur in those body tissues that normally utilize the LDL receptor to derive cholesterol from plasma LDL.

In fibroblasts from FH homozygotes, cholesterol synthesis can be suppressed even in the absence of the LDL receptor when the intracellular cholesterol content is artificially elevated by the addition to the culture medium of nonlipoprotein-bound cholesterol (21). That such nonreceptor-mediated suppression may be operative in FH homozygotes in vivo is indicated by two observations: (*a*) the relative degree of cholesterol overproduction in vivo (i.e., 2-fold) is much less than the 40-fold overproduction of cholesterol observed in FH fibroblasts in the presence of high extracellular LDL levels (6), and (*b*) total body cholesterol synthesis in FH homozygotes can be markedly increased by treatments that tend to lower the expanded body pool of cholesterol, such as ileal bypass procedures (35) and cholestyramine administration (42). The presence in FH homozygotes of a state of partial suppression of cholesterol synthesis possibly related to the expansion of the total body cholesterol pool might explain the variable results of studies of cholesterol synthesis in these patients. In particular, older homozygotes who have had more time to accumulate cellular cholesterol, may show lower rates of sterol synthesis in vivo than do younger homozygotes.

The precise mechanism by which a cellular deficiency of LDL receptors results in an overproduction of LDL is not known. However, one of at least two processes might be involved. First, the expansion of body cholesterol pools due to the failure of receptor-mediated feedback suppression of cholesterol synthesis might stimulate the hepatic production of apolipoprotein B. In this regard, it is of interest that the administration of excessive dietary cholesterol to normal and hypercholesterolemic subjects leads to an elevation in the plasma level of LDL, which is likely related to increased lipoprotein synthesis (43). Alternatively, if the LDL receptor functions in the liver to regulate LDL synthesis, its absence in FH homozygotes would lead directly to LDL overproduction.

In M. C., as well as in the four FH homozygotes studied by Myant and co-workers, (8, 9), the FCR for LDL was reduced, a finding consistent with a role for the LDL receptor in mediating LDL catabolism. However, in view of their extremely high plasma LDL levels, the absolute catabolic rate for LDL in these FH homozygotes was increased. The simplest explanation for these observations is that when the plasma LDL level rises as a result of the combination of overproduction and underutilization of LDL, a secondary process for the catabolism of LDL that does not involve the LDL receptor may become operative. This secondary process is less efficient than the LDL receptor-mediated process because to establish a steady state it requires a higher level of plasma LDL to achieve an absolute catabolic rate equivalent to the synthesis rate.

After portacaval shunt surgery, the LDL synthesis rate in M. C. decreased by about 48% and this led to a 39% decrease in the plasma concentration of LDL. The relative decrease in plasma LDL was slightly less than the relative decrease in the LDL rate of synthesis because the FCR also decreased slightly (17%).

The fall in the rate of synthesis of LDL in M. C. after portacaval shunt was associated with a fall in total body cholesterol synthesis. Whether the drop in cholesterol synthesis primarily dictated the drop in LDL synthesis (or vice versa) or whether these two events were independently affected by the shunt surgery is not known. It was possible to demonstrate, however, that the shunt-related changes in cholesterol and LDL metabolism in M. C. were associated with profound changes in the plasma levels of both bile acids and glucagon. Whether the elevation in either of these two substances is causally related to the reduction in lipid and lipoprotein synthesis is currently under investigation. It is also possible that the decreased rates of cholesterol and LDL synthesis are due to hepatic malfunction that is secondary to a reduced supply of nutrients from portal blood.

Bile acid synthesis in M. C. declined to a level below normal after shunt surgery. Whether this drop in bile acid production was due simply to the portal bypass itself or whether it reflects a possible requirement in FH homozygotes for elevated plasma LDL levels to drive bile acid synthesis normally is not known.

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