Apolipoprotein B metabolism in homozygous familial hypercholesterolemia

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Abstract This report describes the metabolism of apolipoprotein B-containing lipoproteins in seven familial hypercholesterolemic (FH) homozygotes and compares the results to the values obtained from five healthy control subjects. The concentration, composition, and metabolism of large, triglyceride-rich very low density lipoproteins ($VLDL_1$, S_f 60–400) were the same in the control and FH groups, indicating that this component of the VLDL delipidation cascade was unaffected by the absence of receptors. In contrast, familial hypercholesterolemic small $VLDL_2$ (S_f 20–60) was enriched with cholesterol and depleted in triglyceride. Moreover, its plasma concentration was elevated as a result of an increase in its synthesis and a defect in the removal of a remnant population within this density interval. The latter accounted for up to 50% of the total mass of the fraction. Onward transfer of apolipoprotein B (apoB) from small VLDL through intermediate density lipoprotein (IDL) to low density lipoprotein (LDL) was retarded, suggesting that receptors were involved in this supposedly lipase-mediated event. IDL and LDL concentrations increased up to fourfold above normal in the plasma of the FH patients due partly to the delay in maturation and partly to defective direct catabolism. We conclude that the LDL receptor plays multiple and important roles in the metabolism and transformation of apoB-containing particles in the S_f 0–400 flotation interval. —**James, R. W., B. Martin, D. Pometta, J. C. Fruchart, P. Duriez, P. Puchois, J. P. Farriaux, A. Tacquet, T. Demant, R. J. Clegg, A. Munro, M. F. Oliver, C. J. Packard, and J. Shepherd.** Apolipoprotein B metabolism in homozygous familial hypercholesterolemia. *J. Lipid Res.* 1989. **30:** 159–169.

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Familial hypercholesterolemia (FH) is a common metabolic disorder caused by defective expression of the gene that codes for low density lipoprotein (LDL) receptors on cell membranes. Affected individuals accumulate the lipoprotein in their plasma where it predisposes to tissue sterol deposition (xanthomatosis) and premature vascular disease. The trait shows autosomal codominant inheritance and therefore occurs in heterozygous and homozygous forms. Within these two subdivisions there is a broad spectrum of clinical presentations which is thought to result from multiple potential mutations affecting the structure of the receptor protein. FH is therefore a clinically useful umbrella term for a group of conditions, all of which reflect a defect in LDL receptor activity (1).

Early clues to the etiology of the disease came from a series of metabolic studies that showed that its characteristic feature, hypercholesterolemia, arises primarily from delayed clearance of LDL from the circulation (2, 3). However, in some patients, oversynthesis of LDL apoprotein (apoLDL) also seems to contribute to the expanded plasma LDL pool (3). This intriguing finding led to more detailed investigations designed to locate the source of LDL overproduction. In normal subjects, most if not all apoLDL comes (4) from the lipolysis of very low density lipoprotein (VLDL). During this process the VLDL apolipoprotein B (apoB) moiety is conserved and constitutes a marker of the fate of the lipoprotein particle. Soutar, Myant, and Thompson (5) used this information to trace the flux of B protein from VLDL to LDL in FH, and concluded that such individuals synthesized insufficient VLDL to account for the mass of LDL in their circulation. It followed that direct secretion of LDL, possibly by the liver, might be a feature of FH homozygosity. The discovery of a spontaneous mutation (6) in the LDL receptor protein in rabbits (the Watanabe Heritable Hyperlipemic rabbit) provided a means of addressing this question. In contrast to the human studies, analysis of apolipoprotein B transit from VLDL to LDL in the animal model sug-

Abbreviations: S_f, negative sedimentation coefficient at d 1.063 $kg \cdot l^{-1}$ and 26°C; $VLDL_1$, very low density lipoproteins, S_f 60–400; $VLDL_2$, very low density lipoproteins, S_f 20–60; IDL, intermediate density lipoproteins, S_f 12–20; LDL, low density lipoproteins, S_f 0–12; HDL, high density lipoproteins; apoB, apolipoprotein B; FH, familial hypercholesterolemia.

TABLE 1. Plasma lipids and lipoproteins in control and in homozygous FH subjects

Subject	Sex	Age	Body Weight	Plasma Triglyceride	Plasma Cholesterol	Cholesterol in VLDL	Cholesterol in LDL	Cholesterol in HDL	Current Therapy
		yr	*kg*				*mmol · l⁻¹*		
N_1	M	36	71	1.71	4.96	0.79	3.14	1.01	
N_2	F	56	51	0.94	3.76	0.33	1.66	1.78	
N_3	F	28	77	0.55	3.71	0.30	1.95	1.46	
N_4	M	45	79	2.01	6.19	0.99	3.96	1.23	
N_5	M	29	85	2.00	6.55	1.08	4.37	1.10	
FH_1	M	15	53	3.54	14.15	3.43	8.70	0.69	Plasmapheresis
FH_2	M	21	50	2.87	14.10	1.47	11.98	0.65	Plasmapheresis
FH_3	M	14	59	2.25	12.30	0.7	10.80	0.90	Cholestyramine
FH_4	M	10	40	1.95	14.63	0.83	12.95	0.85	Cholestyramine
FH_5	M	44	70	1.93	16.75	0.92	15.43	0.83	
FH_6	F	15	50	0.85	10.90	(0.1)[a]	10.00	0.73	Portacaval shunt/plasmapheresis
FH_7	M	25	66	0.80	11.50	(0.1)[a]	10.20	1.20	Portacaval shunt/plasmapheresis

[a]Assessment of VLDL cholesterol in subjects FH_6 and FH_7 is at the limit of detection.

gested (7) that there was no requirement for direct LDL secretion, a proposal supported (8) by perfusion experiments that showed that WHHL rabbit livers elaborate VLDL but not LDL. However, the experimental animal did demonstrate that defective receptor activity resulted in perturbations in both VLDL and LDL metabolism; and Soutar, Myant, and Thompson (9) have also reported that intermediate density lipoprotein (IDL) metabolism is altered in the human deficiency state. Thus, receptor deficiency has wider consequences for apoB metabolism than was first appreciated. The present study shows that the LDL receptor has multiple roles in VLDL, IDL, and LDL apoB metabolism.

METHODS

Subjects

Five normolipemic and seven homozygous FH individuals participated in the study. Their plasma lipid and lipoprotein profiles are presented in **Table 1.** The normal subjects were recruited from the general population of Glasgow via a coronary screening program. All were healthy individuals receiving no drug therapy. Specifically, none of the participants showed evidence of hepatic, renal, or endocrine dysfunction on the basis of biochemical and hematological screening.

Subject FH_1 was born of an Italian father and Swiss mother. Both parents were heterozygous for FH. One brother died at the age of 14 of myocardial infarction and another, although alive, is severely hypercholesterolemic. FH_1 originally presented with xanthomata of the Achilles tendons and of the extensor tendons of hands and knees. Arcus senilis was evident and there were cholesterol deposits in his natal cleft. Triple bypass grafting had been performed 3 years prior to the study because of coronary insufficiency. His fibroblasts exhibited less than 15% of normal LDL receptor activity as determined by standard procedures (10).

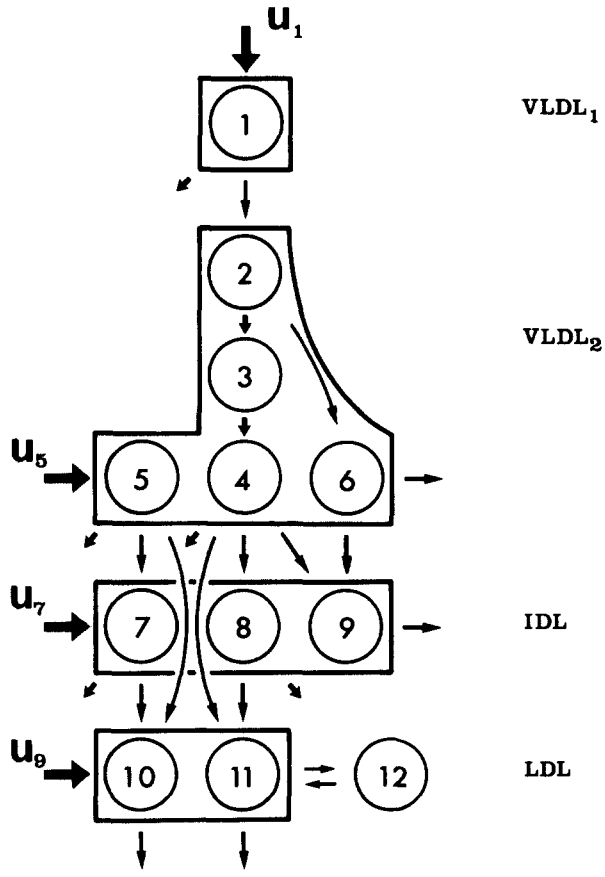

Fig. 1. Multicompartmental model describing the kinetics of $VLDL_1$, $VLDL_2$, IDL, and LDL. The parameters U_1, U_5, U_7, and U_9 represent de novo input of apoB. Exchange between compartments 11 and 12 was fixed at $k_{12,11}$ = 0.05 pools · d^{-1} and $k_{11,12}$ = 0.13 pools · d^{-1}.

TABLE 2. Compositions of apolipoprotein B-containing subfractions in control and in homozygous FH subjects

Subfraction	Subject[a]	FC	CE	TG	PL	Protein
		g/100 g				
$VLDL_1$	A	5.1 ± 2.5	14.9 ± 3.5	53.6 ± 3.9	14.9 ± 1.6	12.2 ± 4.4
$VLDL_1$	B	9.4 ± 0.4	21.8 ± 0.9	35.2 ± 2.7	19.3 ± 1.4	14.3 ± 0.8
$VLDL_1$	C	1.7 ± 2.3	16.0 ± 4.3	56.2 ± 4.8	17.0 ± 1.4	9.1 ± 2.4
$VLDL_2$	A	9.6 ± 3.2	34.5 ± 2.6	19.2 ± 2.8	19.6 ± 1.3	17.2 ± 3.2
$VLDL_2$	B	9.5 ± 0.1	34.0 ± 4.2	22.8 ± 3.6	18.6 ± 0.6	15.3 ± 0.1
$VLDL_2$	C	8.1 ± 1.4	21.1 ± 5.9	35.1 ± 4.0	21.4 ± 2.4	14.4 ± 1.6
IDL	A	10.5 ± 3.1	38.2 ± 2.0	6.5 ± 1.3	20.2 ± 2.2	24.5 ± 4.5
IDL	B	10.0 ± 0.6	42.6 ± 2.1	4.8 ± 1.4	21.3 ± 2.3	21.4 ± 0.6
IDL	C	11.2 ± 2.3	33.4 ± 4.8	23.6 ± 1.4	23.9 ± 1.3	19.1 ± 2.3
LDL	A	8.0 ± 2.8	37.5 ± 4.2	5.3 ± 1.5	19.0 ± 1.9	30.2 ± 3.2
LDL	B	8.6 ± 0.1	43.9 ± 1.8	2.2 ± 0.6	11.9 ± 1.4	22.5 ± 0.1
LDL	C	13.5 ± 1.5	34.8 ± 2.2	5.1 ± 0.2	23.0 ± 1.6	23.6 ± 1.6

[a]A, homozygous FH (n = 5): B, homozygous FH + portacaval shunt (n = 2); C, controls (n = 5).

Subjects FH_3 and FH_4 are brothers of Scottish origin. Both parents had plasma LDL cholesterol concentrations in excess of the reference values for their community, and the boys had widespread tendon xanthomata and subcutaneous cholesterol deposits in their gluteal fold. Their fibroblast LDL receptor activity was less than 30% of normal. Signs and symptoms of cardiovascular dysfunction were absent.

The clinical and biochemical features of subjects FH_6 and FH_7 are presented in earlier reports (11, 12). FH_6 is Moroccan and FH_7 French. Cell culture studies performed in the laboratory of Goldstein and Brown (10) showed that their fibroblasts expressed virtually no normal LDL receptor binding activity. Both patients had undergone portacaval shunt surgery in 1975. Their routine liver function tests remain normal.

FH_5 is a 44-year-old Englishman with widespread tendon xanthomata and severe left carotid artery stenosis. He is refractory to all lipid-lowering drug therapy including sequestrant resins. His three children are hypercholesterolemic. Fibroblast and lymphocyte assays (13) failed to reveal any detectable receptor activity.

FH_2, a Ugandan subject of Indian extraction, was found to be severely hypercholesterolemic in childhood. He exhibited widespread tendon xanthomata and had coronary artery bypass surgery for occlusive disease approximately 6 years ago. His lymphocytes expressed less than 10% of normal receptor activity (13).

Therapy

FH_1, FH_2, FH_6, and FH_7 had been receiving biweekly plasmapheresis, and FH_3 and FH_4 were prescribed cholestyramine. These interventions were discontinued at least 6 weeks prior to initiation of the kinetic studies outlined below.

Protocol

The procedures that were used to examine the kinetics of apoB metabolism are detailed elsewhere (14, 15). Briefly, one unit of plasma was obtained from each fasting subject by plasmapheresis and used to prepare total VLDL ($d < 1.006$ kg·l^{-1}) by ultracentrifugation in a Beckman Ti60 rotor for 24 hr at 50,000 rpm (4°C). The supernatant VLDL was collected and its density was increased to 1.118 kg·l^{-1} by the addition of solid NaCl (170 mg/ml of VLDL solution). A 2-ml aliquot of this preparation was layered over a 0.5-ml cushion of d 1.182 kg·l^{-1} NaBr solution in a Beckman SW 40 rotor tube and a discontinuous salt gradient from d 1.0988–1.0582 kg·l^{-1} was constructed above it (16). The rotor was subjected to centrifugation at 39,000 rpm for 1 hr and 38 min at 23°C and decelerated without braking. $VLDL_1$ of S_f 60–400 was removed in the top 1.0 ml of solution which was replaced with 1.0 ml of d 1.0588 kg·l^{-1} solution before continuing with the separation. $VLDL_2$ (S_f 20–60) was then isolated from the top 0.5 ml of the gradient following centrifugation at 18,500 rpm for 15 hr and 41 min at 23°C. The S_f 60–400 and S_f 20–60 VLDL fractions were labeled with ^{131}I and ^{125}I, respectively, by the procedure of Bilheimer, Eisenberg, and Levy (17), and subsequently sterilized by membrane filtration (0.45 μm Amicon filters, Amicon Corp., Bedford, MA). Fifty μCi (approximately 0.5 mg VLDL protein) of each was then injected into the bloodstream of the respective donor. The tracers were routinely administered at 8:00 AM, and throughout the first day of the turnover the subjects were given a diet that contained less than 5 g of fat but their normal intake of carbohydrate and protein. This approach was designed to minimize chylomicron production and has been used in a number of previous studies (14, 15). Venous blood samples were collected at frequent intervals over the first 72 hr and thereafter on a daily basis. S_f 60–400 and S_f 20–60 VLDL were isolated directly from the plasma specimens at each time point, following the centrifugation schedule outlined above. Additionally, intermediate density lipoprotein (IDL, S_f 12–20) and LDL (S_f 0–12) were pre-

TABLE 3. Apolipoprotein B metabolism in normal and

Subject	Large VLDL ApoB (S_f 60-400)				Small VLDL ApoB (S_f 20-60)				
			Fractional Rate					Fractional Rate	
	Synthesis	Plasma Pool	Direct Catabolism	Transfer to $VLDL_2$	Synthesis Direct	Flux from $VLDL_1$	Plasma Pool	Direct Catabolism	Transfer to IDL
	mg/d	*mg*	*pools/d*		*mg/d*		*mg*	*pools/d*	
N_1	802	79	3.7	6.5	393	512	246	0.36	3.3
N_2	290	18	0.0	16.2	137	290	100	0.70	2.8
N_3	237	9	17.7	8.4	262	77	65	0.52	4.7
N_4	520	108	1.1	3.7	361	403	289	0.51	1.1
N_5	954	115	5.0	3.2	238	372	258	0.35	1.9
Mean ± SD	560 ± 280	66 ± 44	5.5 ± 6.4	7.6 ± 4.7	278 ± 91	331 ± 145	191 ± 91	0.49 ± 0.13	2.8 ± 1.2
FH_1	1045	108	3.7	6.0	390	648	660	0.57	1.11
FH_2	614	72	4.4	4.1	214	297	536	0.30	0.65
FH_3	398	110	1.2	2.4	1200	265	896	0.55	0.64
FH_4	181	28	3.7	2.8	983	78	475	0.83	1.23
FH_5	674	59	3.6	7.7	217	462	310	0.53	0.78
Mean ± SD	582 ± 289	75 ± 31	3.3 ± 1.1	4.6 ± 2.0	601 ± 411	350 ± 193	563 ± 192	0.56 ± 0.17	0.88 ± 0.24
FH_6	226	9	1.8	24.0	13	210	120	0.28	1.41
FH_7	165	8	3.6	18.0	74	137	75	0.28	1.41
Mean	196	9	2.7	21	44	174	98	0.36	1.64

pared by subjecting the SW 40 rotor contents to two further 23°C centrifugation steps of 39,000 rpm for 2 hr and 34 min and 30,000 rpm for 21 hr and 10 min, respectively. IDL was removed from the top of the gradient in a volume of 0.5 ml and LDL in a 1.0-ml aliquot.

Each lipoprotein fraction was treated with 1,1,3,3 tetramethylurea (18) and the resulting insoluble pellet was extracted with organic solvents (chloroform–methanol 1:1, vol/vol) to isolate apolipoprotein B prior to determination of its specific activity by previously published procedures (14). The mean apolipoprotein B present in each fraction derived from fasting blood specimens was used to calculate the plasma concentration of B protein in that density interval. To compensate for potential losses in the precipitation procedure, an independent estimate of this parameter was obtained as the difference between the total protein and tetramethylurea-soluble protein content of each fraction (18). Compositional analysis of the fractions (S_f 60-400, 20-60, 12-20, and 0-12) was performed by methods reported elsewhere (14, 15). This information permitted correction for losses incurred during centrifugation, viz: the cholesterol content of all four fractions was summed and the resulting value was compared with the total apoB-associated cholesterol (total cholesterol minus HDL cholesterol) measured by the standard Lipid Research Clinics methodology (19). Calculated losses of apoB during centrifugation were of the order of 10–15%.

Apolipoprotein B kinetic analysis

Kinetic analysis of the data was performed using the SAAM 29 program (20). Total apoB radioactivity decay curves and the mass of B protein associated with each lipoprotein fraction were used to derive rate constants and protein fluxes. The model (**Fig. 1**) is a development of an earlier published version (14):

1) Large VLDL apoB ($VLDL_1$, S_f 60-400) behaves as a single species which decays monoexponentially in both normal and FH subjects. This is either catabolized directly or transferred to the $VLDL_2$ (S_f 20-60) range.

2) Within the S_f 20-60 lipoproteins there is an arrangement akin to that described by Berman et al. (21). Some apoB enters a catabolic cascade and is converted to IDL (S_f 12-20) while other material is diverted into a slowly metabolized remnant species (compartment 6, Fig. 1).

3) There is input of newly synthesized apoB into the S_f 20-60 density range. This is required because not all of the S_f 20-60 apoB mass can be accounted for by transport from large VLDL, and when large (S_f 60-400) and small (S_f 20-60) VLDL are labeled separately the kinetics of appearance of these tracers in IDL and LDL apoB is different. Usually the radioactivity derived from labeled small VLDL appears more quickly in these denser fractions and accounts for a higher proportion of their mass. Provision is made for this phenomenon by incorporating in the model parallel pathways leaving small VLDL and appearing in IDL and LDL.

4) In the IDL range it was necessary to postulate the existence of a slowly metabolized species (compartment 9, Fig. 1).

in homozygous familial hypercholesterolemic subjects

IDL ApoB (S_f 12-20)					LDL ApoB (S_f 0-12)				
			Fractional Rate						
Synthesis Direct	Flux from $VLDL_2$	Plasma Pool	Direct Catabolism	Transfer to LDL	Synthesis Direct	Fluxfrom IDL + $VLDL_2$	Plasma Pool	Fractional Catabolic Rate	Total B Synthesis
mg/d		*mg*	*pools/d*		*mg/d*		*mg*	*pools/d*	*mg/d*
0.0	817	337	0.77	1.65	0.0	556	1750	0.32	1195
0.0	275	210	0.32	0.98	0.0	290	635	0.46	427
0.0	305	95	0.32	2.87	0.0	273	710	0.39	499
0.0	312	245	1.07	0.23	0.0	359	2110	0.17	881
0.0	499	496	0.15	0.85	9.0	423	2650	0.20	1282
0.0	441 ± 204	277 ± 134	0.52 ± 0.34	1.3 ± 0.9	18 ± 36	380 ± 102	1571 ± 788	0.31 ± 0.11	857 ± 349
0.0	668	1278	0.06	0.46	0.0	628	5698	0.11	1435
0.0	349	842	0.11	0.30	134	256	4644	0.084	962
0.0	577	1047	0.32	0.23	140	636	6748	0.11	1738
0.0	586	2222	0.06	0.21	315	543	5046	0.17	1479
94	241	1388	0.11	0.24	396	453	10186	0.083	1381
19 ± 38	484 ± 161	1355 ± 473	0.13 ± 0.096	0.29 ± 0.09	197 ± 141	503 ± 140	6464 ± 1992	0.11 ± 0.03	1399 ± 250
51	174	848	0.21	0.02	224	62	3587	0.080	514
163	140	1613	0.02	0.18	304	323	4964	0.128	706
107	157	1231	0.12	0.1	264	193	4276	0.10	610

5) LDL was distributed between two plasma compartments (compartments 10 and 11), only one of which was permitted to equilibrate with the extra vascular space. This was necessary to accommodate the observation that there were differential rates of appearance and removal of LDL apoB depending on whether the protein was derived from large or small VLDL.

The model provided an acceptable fit to the observed data in both the control and FH subjects, and the calculated masses derived from the kinetic analyses were within 20% of the measured values.

Ethical considerations

All subjects (or their parents) gave informed consent to the study which met the requirements of the Ethical Committee of each host institution. They were prescribed KI (60 mg thrice daily) for 3 days prior to and 1 month after isotope administration in order to minimize thyroidal sequestration of radioiodide.

RESULTS

Plasma lipids and lipoproteins

In familial hypercholesterolemia, plasma LDL cholesterol is characteristically elevated several-fold (1) while HDL concentrations are generally low (Table 1). Plasma triglyceride varies, and may be increased above normal. Portacaval shunt surgery is known to lower circulating VLDL (1, 5) and presumably was responsible for the low triglyceride concentrations recorded in FH_6 and FH_7.

Table 2 presents the mean compositional analyses of VLDL, IDL, and LDL subfractions in the control and FH groups. The composition of S_f 60-400 lipoproteins differed in the FH subjects only in their content of free cholesterol ($P < 0.02$). The smaller VLDL (S_f 20-60), on the other hand, was enriched in cholesteryl esters and depleted in triglyceride ($P < 0.005$ in each case) as was IDL. The depletion of free cholesterol in FH LDL ($P < 0.005$) probably resulted from the prolonged residence time of the particles in the plasma, increasing their exposure to lecithin:cholesterol acyltransferase.

Apolipoprotein B kinetic analysis

The metabolism of apolipoprotein B in the FH homozygotes not only differed substantially from normal in several major respects (**Table 3**) but also showed considerable variation within the group itself. Radioactive apoB initially present in large (S_f 60-400) $VLDL_1$ was metabolized rapidly in both FH and normal subjects (**Fig. 2** and **Fig. 3**, respectively), at rates that were not significantly different (fractional clearance in controls = 13.1 ± 7.5 pools · d^{-1} versus 7.9 ± 2.7 pools · d^{-1} in the FH group). The majority of this B protein transferred into the S_f 20-60 small $VLDL_2$ density interval, at which point the two groups showed divergent metabolic patterns. The clearance of small VLDL apoB in the FH

group was substantially delayed, resulting in a slow appearance of radioactivity in IDL and ultimately in LDL. This transfer, usually complete within 24 hr in control subjects, took 120–140 hr in the FH homozygotes, and examination of the metabolic behavior of directly labeled small VLDL apoB showed a similar pattern. The data obtained from the two tracers (^{131}I-labeled VLDL apoB (S_f 60–400) and ^{125}I-labeled $VLDL_2$ apoB (S_f 20–60)) were merged in the SAAM program to give a single set of kinetic parameters which permitted quantitation of the rates of transport of the B protein through these density classes (Table 3 and **Table 4**).

The parameters describing the metabolism of large S_f 60–400 VLDL apoB in the group of five medically treated FH subjects (FH_1–FH_5, Tables 3 and 4) were not significantly different from those of the controls. Both the rates of synthesis and catabolism and the plasma pool of apoB in the particles present in this density interval were normal. About 60% of this large VLDL transferred to the smaller, denser S_f 20–60 range. The remainder was removed directly from the plasma compartment. In the normal group, this transfer accounted for 54% of the B protein mass present in the S_f 20–60 interval. The rest of the $VLDL_2$ apoB came from direct synthesis, presumably by the liver. The magnitude of the de novo input of $VLDL_2$ apoB in the FH group was on average twice normal, but Table 3 shows that this was not a universal phenomenon but rather was the result of excessive B protein synthesis into S_f 20–60 VLDL in FH subjects 3 and 4. The 2.9-fold increase in the mass of $VLDL_2$ B protein in the FH subjects derived primarily from delayed clearance. The latter appeared to arise from two abnormalities. First, the rate of transfer of B protein out of $VLDL_2$ into IDL was only 31% of normal (Table 3). Secondly, more of the $VLDL_2$ apoB was diverted into a slowly metabolized remnant population (Fig. 1, compartment 6). In the control group, only 7% of the material in compartment 2 was diverted from the delipidation cascade into remnants, whereas 50% took this route in FH subjects 1 to 5. The mean computed mass of apoB in the remnant population was 232 mg in the FH subjects versus 24 mg in the normals (Table 4, M_6). This expansion was not due solely to increased remnant formation. Remnant removal was also defective in the FH group, $k_{0,6}$ being reduced by 50% in the latter.

FH subjects 1–4 showed no requirement for direct synthesis of apoB into IDL. They, as did the controls, derived this fraction entirely by transfer from VLDL. The flux of

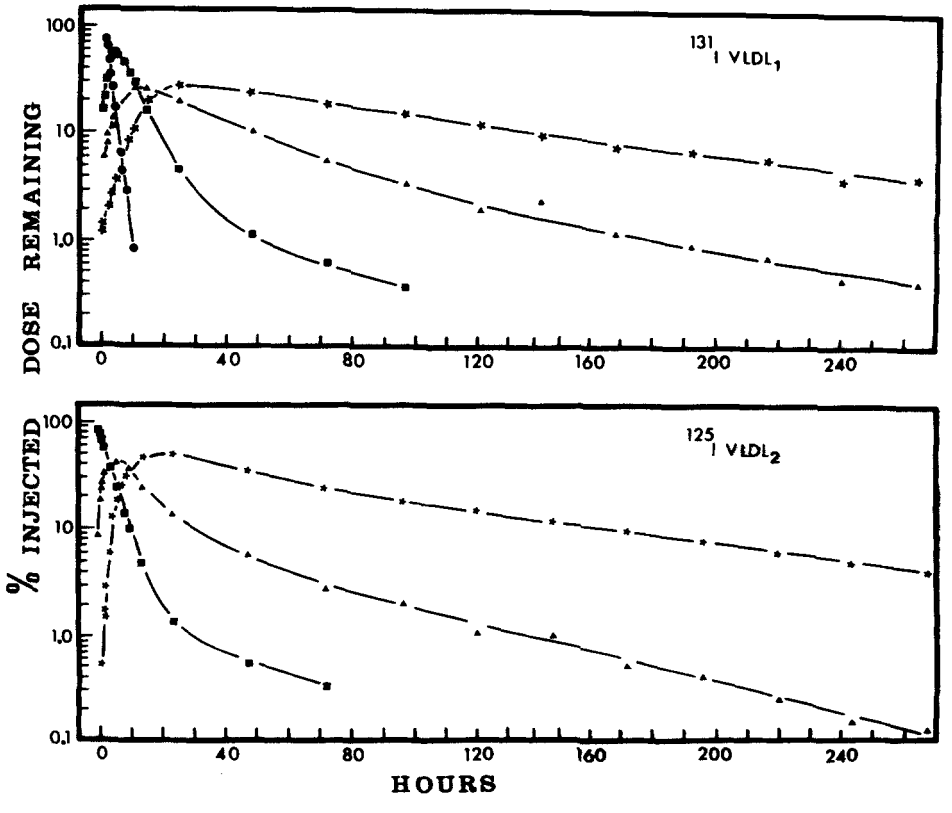

Fig. 2. Apolipoprotein B radioactivity decay curves of ^{131}I-labeled $VLDL_1$ (S_f 60–400) and ^{125}I-labeled $VLDL_2$ (S_f 20–60) in control subject N_1. Values are expressed as percent of initial dose. $VLDL_1$ (●), S_f 60–400; $VLDL_2$ (■), S_f 20–60; IDL (▲), S_f 12–20; LDL (*), S_f 0–12.

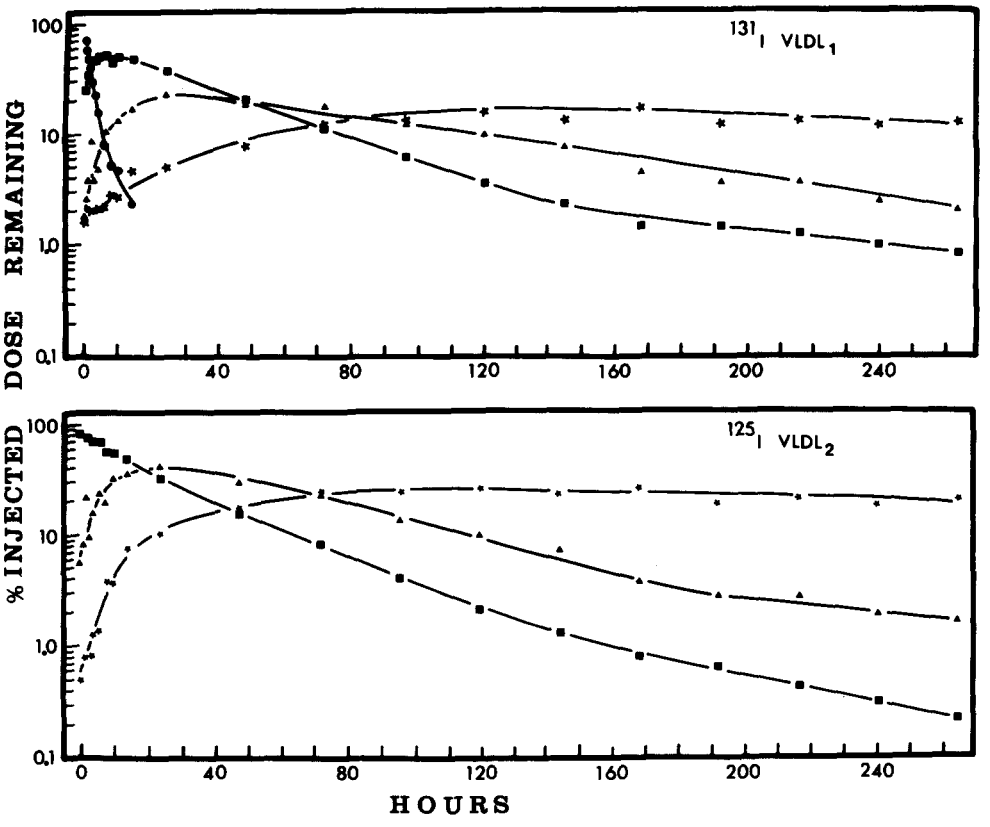

Fig. 3. Apolipoprotein B radioactivity decay curves of ^{131}I-labeled $VLDL_1$ (S_f 60-400) and ^{125}I-labeled $VLDL_2$ (S_f 20-60) in subject FH_2. Values are expressed as percent of initial dose. $VLDL_1$ (●), S_f 60-400; $VLDL_2$ (■), 20-60; IDL (▲), S_f 12-20; LDL (*), S_f 0-12.

B protein from small VLDL to IDL in all of the FH subjects was essentially normal. So, the marked (4.9-fold) increase in circulating IDL resulted from a major reduction in its rate of catabolism in FH. The fractional rates of direct removal and transfer of this lipoprotein to LDL were significantly retarded (by 72%, $P < 0.02$, and 78%, $P < 0.05$, respectively). However, despite the decrease in fractional transfer, the flux of IDL apoB into the LDL density interval was normal or even slightly increased, at a mean value of 503 $mg \cdot d^{-1}$. In most FH subjects, in addition to B protein transfer from IDL, it was necessary to specify direct LDL apoB input to account for the observed circulating mass of LDL. Total LDL production from both sources was increased to 700 ± 176 $mg \cdot d^{-1}$ in the FH group compared to 398 ± 116 $mg \cdot d^{-1}$ in the controls ($P < 0.02$). However, decreased B protein catabolism also contributed to the 4.1-fold expansion of the FH LDL apoB pool. The fractional catabolism of apoB from this lipoprotein was 0.11, versus 0.31 pools $\cdot d^{-1}$ in the controls ($P < 0.005$).

The data from the two portacaval shunt subjects FH_6 and FH_7 were handled separately since they were distinctly different from the others in the group (Table 3). They showed a substantial reduction in the rate of large VLDL apoB synthesis compared to both the controls and the other FH patients. Moreover, the fractional clearance rate from this pool was high and so its plasma concentration was very low. This was also true for small VLDL apoB whose rate of production either from large VLDL or by direct synthesis was reduced. Shunt surgery, however, did not seem to affect the distribution of B protein within small VLDL in that remnants again accumulated to about 45% of the total mass (cf M_6, Table 4, with the pool of small VLDL, Table 3). Interestingly, the rate of remnant removal ($K_{0,6}$, Table 4) was not apparently affected by surgery, whereas the fractional transfer of $VLDL_2$ apoB to IDL appeared to be higher (Table 3) even though the net amount transferred was low. The IDL apoB pools in FH_6 and FH_7 were as high as in the other patients in the group. Again, defective catabolism played a major role in generating this phenomenon, although in both of these subjects it was necessary to invoke direct IDL synthesis in order to account for the total mass of apoB in the fraction. The pattern was repeated in the LDL density interval. Expansion of the pool resulted primarily from defective catabolism although increased

TABLE 4. Computed compartment masses and rate constants for normal and FH subjects

Subject	M_1[a]	$k_{0,1}$[b]	$k_{2,1}$	M_2[c]	$k_{3,2}$	$k_{6,2}$	M_4	$k_{0,4}$	$k_{8,4}$	$k_{9,4}$	$k_{11,4}$	M_5	$k_{0,5}$	$k_{7,5}$	$k_{10,5}$
N_1	79	3.7	6.5	53	8.6	1.0	42	0.8	7.6	2.3	0.0	52	0.0	7.6	0.0
N_2	18	0.0	16.2	16	18.0	0.2	44	0.7	3.6	0.4	1.9	23	1.7	4.3	0.0
N_3	9	17.7	8.4	9	7.4	1.1	2	16.0	14.0	14.0	0.0	36	0.0	7.2	0.0
N_4	108	1.1	3.7	43	8.4	0.8	93	1.2	1.1	0.4	1.2	80	0.0	2.1	2.4
N_5	115	5.0	3.2	42	8.2	0.3	77	1.0	3.1	0.1	0.2	85	0.0	2.8	0.0
Mean ± SD	66 ± 44	5.5 ± 6.4	7.6 ± 4.7	33 ± 17	10.1 ± 4.0	0.7 ± 0.4	52 ± 32	3.9 ± 6.0	5.9 ± 4.6	3.4 ± 5.3	0.7 ± 0.8	55 ± 24	0.3 ± 0.7	4.8 ± 2.2	0.5 ± 1.0
FH_1	108	3.7	6.0	69	7.2	2.2	83	2.9	3.0	0.1	0.0	108	0.0	3.4	0.3
FH_2	72	4.4	4.1	45	2.4	4.2	81	0.1	1.2	0.0	0.0	59	0.0	3.6	0.0
FH_3	110	1.2	2.4	24	1.4	9.6	23	0.1	1.3	0.0	0.0	532	0.6	1.0	0.7
FH_4	28	3.7	2.8	12	0.48	5.8	63	0.0	0.0	0.1	0.0	261	1.2	2.2	0.3
FH_5	59	3.6	7.7	21	14.4	7.7	35	0.0	4.3	0.0	4.3	71	0.1	1.3	1.7
Mean ± SD	75 ± 31	3.3 ± 1.1	4.6 ± 2.0	34 ± 20	5.2 ± 5.2	5.9 ± 2.6	38 ± 24	0.6 ± 1.1	2.0 ± 1.5	0.04 ± 0.05	0.9 ± 1.7	206 ± 178	0.4 ± 0.5	2.3 ± 1.1	0.6 ± 0.6
FH_6	9	1.8	24.0	18	10.0	2.1	9	0.0	17.8	0.0	1.3	13	0.0	0.8	0.3
FH_7	8	3.6	18.0	13	9.6	0.7	9	1.9	10.8	0.0	1.9	13	0.5	3.5	1.6
Mean	9	2.7	21.0	16	9.8	1.4	9	1.0	14.3	0.0	1.6	13	0.3	2.2	1.0

Masses (M_i) refer to the numbering of sub-compartments given in Fig. 1. Rate constants K_{ij} indicate transfer from sub-compartment j to sub-compartment i.
[a]Masses in mg.
[b]Rate constants (d^{-1}).
[c]$M_3 = M_2$; $k_{4,3} = k_{3,2}$ was a constraint in the model.

TABLE 4. (continued)

Subject	M_6[a]	$k_{0,6}$[b]	$k_{9,6}$	M_7	$k_{0,7}$	$k_{10,7}$	M_8	$k_{0,8}$	$k_{11,8}$	M_9	$k_{0,9}$	M_{10}	$k_{0,10}$	M_{11}	$k_{0,11}$
N_1	66	0.8	0.0	115	0.5	2.9	66	1.53	3.38	160	0.6	680	0.49	1070	0.21
N_2	2	1.9	0.0	33	0.0	3.0	145	0.34	0.74	33	0.6	215	0.46	420	0.46
N_3	9	1.1	0.0	73	0.0	3.6	7	1.40	1.80	15	1.4	636	0.41	74	0.16
N_4	29	1.2	0.0	78	1.9	0.2	76	1.0	0.48	92	0.4	1380	0.15	728	0.20
N_5	12	1.0	0.0	219	0.0	1.1	270	0.23	0.65	7	1.0	1412	0.20	1171	0.20
Mean ± SD	24 ± 23	1.2 ± 0.4	0.0	104 ± 63	0.5 ± 0.7	2.2 ± 1.3	113 ± 90	0.90 ± 0.53	1.41 ± 1.09	61 ± 58	0.8 ± 0.4	865 ± 463	0.34 ± 0.14	693 ± 407	0.25 ± 0.11
FH_1	273	0.37	0.18	179	0.12	1.9	912	0.0	0.27	185	0.30	2994	0.13	2704	0.090
FH_2	302	0.50	0.12	513	0.05	0.37	184	0.17	0.36	144	0.25	3081	0.084	1563	0.084
FH_3	290	0.67	0.12	787	0.38	0.26	88	0.0	0.36	172	0.20	6166	0.12	532	0.060
FH_4	127	0.47	0.1	2175	0.05	0.21	0	0.0	0.0	47	0.37	5046	0.17	0	0.0
FH_5	166	0.96	0.0	770	0.0	0.24	618	0.24	0.0	0	0.0	8586	0.11	1600	0.072
Mean ± SD	232 ± 71	0.6 ± 0.2	0.1 ± 0.06	885 ± 681	0.1 ± 0.1	0.6 ± 0.7	360 ± 348	0.08 ± 0.10	0.20 ± 0.16	110 ± 73	0.22 ± 0.13	5174 ± 2086	0.12 ± 0.03	1279 ± 939	0.06 ± 0.03
FH_6	62	0.56	0.05	301	0.08	0.13	547	0.27	0.02	2	1.3	3230	0.10	357	0.057
FH_7	27	0.36	0.0	1140	0.02	0.18	473	0.03	0.17	0	0	4246	0.12	718	0.13
Mean	43	0.46	0.03	721	0.05	0.16	510	0.15	0.1	1	0.7	3738	0.11	538	0.09

direct synthesis was again contributory. The fractional catabolic rates of apoB in IDL and LDL did not appear to be affected by shunt surgery.

DISCUSSION

VLDL comprises a structurally heterogeneous spectrum of particles whose properties are the subject of continuing research. In normal individuals most VLDL is in the denser S_f 20–60 interval (18). Familial hypertriglyceridemic subjects, on the other hand, possess increased amounts of larger particles (14, 15, 18, 22) with greater triglyceride content, and a similar distribution can be induced in normal individuals by carbohydrate feeding (23). The size of the nascent VLDL may govern its subsequent metabolic behavior in the plasma. Large particles in the flotation range S_f 100–400 undergo rapid hydrolysis via lipoprotein lipase, acquiring in the process cholesteryl ester by transfer from HDL. Such particles, in fact, are the favored acceptors of sterol ester (24). The remnants that come from their lipolysis remain within the VLDL spectral distribution, although at its denser end. Here they resist further hydrolysis and are thought to be catabolized as a unit via receptor-mediated mechanisms. They therefore make little contribution to the production of LDL. The latter appears to come from smaller, denser VLDL which is secreted directly by the liver (14, 15). The extent to which remnants contribute to the total VLDL population can be assessed from the cholesteryl ester/triglyceride ratio in that fraction (25). Where they are abundant, as in Type III hyperlipoproteinemia, the ratio is increased. This compositional abnormality was found in the small VLDL and IDL of our FH patients, indicating that B protein metabolism was grossly perturbed in this group of subjects.

FH subjects 1–5 possessed large VLDL that was essentially normal both in terms of composition and metabolism. In particular, the rate of conversion of large to small VLDL, mediated by lipoprotein lipase, was not influenced by the receptor defect. The impact of the disease only became apparent at the level of small VLDL. A higher proportion of B protein in this fraction was channelled into slowly metabolized remnants, which presumably accounted for the elevated cholesteryl ester/triglyceride ratio found in FH S_f 20–60 $VLDL_2$. The mechanism underlying this phenomenon is not clear, although it may be postulated that the prolonged plasma residence time of small VLDL which followed from retardation of the whole delipidation process might expose the particle to increased cholesteryl ester transfer from HDL, thereby limiting its potential for further lipolysis. Defective catabolism of the remnants ($k_{0,6}$, Table 4) also played a part in their accumulation in these receptor-deficient patients. This observation suggests that the LDL receptor may be implicated in this process. The calculated 10-fold increase in the circulating VLDL remnant population in FH (M_6, Table 4) accounted for about one-half of the expanded S_f 20–60 VLDL pool. The remainder derived from an increment in direct synthesis of small VLDL, particularly evident in subjects FH_3 and FH_4 (M_5, Table 4).

An unexpected finding of this study, evident from the decay profiles (Fig. 3), was that the fractional rate of transfer of B protein from small $VLDL_2$ through IDL to LDL was very slow. These delipidation steps are thought to be mediated by lipases, and recent results from animal and human studies (26, 27) implicate hepatic lipase in the process. It is not immediately obvious why the VLDL–LDL conversion should be so slow in these LDL receptor-deficient subjects. Several possibilities exist. First, the delipidation pathway may be saturated, limiting the processing of apoB to about 500 $mg \cdot d^{-1}$, and reducing its fractional rate of transfer through the cascade. However, this would imply (Table 3) that the conversion process is virtually saturated in normal subjects also, and would not explain why patients FH_2, FH_6, and FH_7 exhibit such a low B protein flux from VLDL to LDL. A second alternative is that the greatly increased IDL and LDL pools act by product inhibition to suppress hepatic lipase activity. Or thirdly, it may be postulated that LDL receptors and lipase act cooperatively in the conversion process. It is known that the majority of LDL receptors are found in the liver (28) as is a lipase which expresses high affinity for smaller VLDL and IDL (29); and transhepatic measurements indicate that this organ is the most likely site of IDL–LDL conversion (30). Hepatic lipase is reported to be located on the surface of sinusoidal cells (31), which themselves are a particular variety of endothelial cells. The latter are known from tissue culture studies to express LDL receptors (32), although at confluence they do not necessarily participate in lipoprotein internalization and degradation, but may merely bind and release the particles (33). It is therefore possible that in the liver they assist lipolysis, bringing small VLDL and IDL into contact with hepatic lipase whose hydrolytic actions lead to the production of LDL. Subsequent loss of apolipoprotein E from the particle would reduce its affinity for the receptor and facilitate its release back into the circulation. Such a mechanism is, of course, highly speculative although it does explain the slow VLDL–LDL conversion that is seen in dysbetalipoproteinemia, in familial hypercholesterolemia, and in hepatic lipase deficiency (34).

As might be expected in FH homozygotes, direct catabolism of apoB-containing particles was retarded. LDL was cleared at one-third of the normal rate, presumably by receptor-independent mechanisms. The data presented in Table 3 and in an earlier publication by Soutar and her colleagues (9) show that IDL catabolism

is similarly retarded and indicate that receptors must play a role in the process. We have already shown (35) that this is likely to be the case in an experiment in which treatment of VLDL or IDL with 1,2 -cyclohexanedione, an agent that blocks potential interaction of the lipoprotein with receptors, slowed both the direct catabolism of IDL and its conversion to LDL. Thus the consequences of receptor deficiency are as profound for IDL as for LDL. Most of the differences observed between the normal and FH homozygotes are so marked that it is unlikely they can be explained by the necessary variation in age and body weight between the two groups. The influences of these factors, however, should be borne in mind when differences between the two groups are small.

A number of studies (3, 5) have indicated that homozygous FH is associated with overproduction of LDL. Table 3 shows that this also occurred in FH subjects 1-5 in whom total B protein synthesis was on average 63% higher than in controls ($P < 0.025$). The mechanism responsible for this effect is unknown, although recent in vitro experiments have linked the catabolism of LDL with the control of apoB synthesis. Cultured hepatoma cells, starved of the lipoprotein, show enhanced apoB mRNA production which is inhibited by addition of LDL to the culture medium (36). Extrapolation to the in vivo situation would suggest that failure of LDL to be assimilated by the liver via the receptor pathway may derepress apoB production in that organ.

We have already commented on the variability in apoB metabolism which was apparent in our seven homozygous FH subjects. Patient FH_1, who exhibited the highest plasma triglyceride levels, secreted excessive amounts of apoB into VLDL and showed no requirement for direct LDL synthesis. These results are reminiscent of the situation prevalent in Watanabe rabbits (7, 8). However, in our subjects with lower plasma triglyceride levels, an increasingly higher proportion of B protein was secreted directly into LDL. Clearly, the rabbit model is not applicable here.

If we assume that prior to shunt surgery FH subjects 6 and 7 resembled the other five in this study, then it may be surmised that the portacaval anastomosis primarily inhibits triglyceride synthesis, limiting secretion of VLDL into the plasma. In this situation, more apoB appears in the triglyceride-depleted IDL and LDL particles. The procedure itself seems to make no impact on the rates of catabolism of VLDL remnants, IDL or, as reported earlier, of LDL (9).

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REFERENCES

1. Goldstein, J. L., and M. S. Brown. 1983. Familial hypercholesterolemia. *In* The Metabolic Basis of Inherited Disease. J. B. Stanbury, J. B. Wyngaarden, D. S. Fredrickson, J. L. Goldstein, and M. S. Brown, editors. McGraw-Hill Book Co., New York. 672-712.
2. Simons, L. A., D. Reichl, N. B. Myant, and M. Mancini. 1975. The metabolism of the apoprotein of plasma low density lipoprotein in familial hyperbetalipoproteinaemia in the homozygous form. *Atherosclerosis.* **21:** 283-298.
3. Bilheimer, D. W., N. J. Stone, and S. M. Grundy. 1979. Metabolic studies in familial hypercholesterolemia. *J. Clin. Invest.* **64:** 524-533.
4. Sigurdsson, G., A. Nicol, and B. Lewis. 1975. Conversion of very low density lipoprotein to low density lipoprotein. A metabolic study of apolipoprotein B kinetics in human subjects. *J. Clin. Invest.* **56:** 1481-1490.
5. Soutar, A. K., N. B. Myant, and G. R. Thompson. 1977. Simultaneous measurement of apolipoprotein B turnover in very low and low density lipoproteins in familial hypercholesterolaemia. *Atherosclerosis.* **28:** 247-256.
6. Watanabe, Y. 1980. Serial inbreeding of rabbits with hereditary hyperlipemia. *Atherosclerosis.* **36:** 261-268.
7. Kita, T., M. S. Brown, D. W. Bilheimer, and J. L. Goldstein. 1982. Delayed clearance of very low density and intermediate density lipoproteins with enhanced conversion to low density lipoproteins in WHHL rabbits. *Proc. Natl. Acad. Sci. USA.* **79:** 5693-5697.
8. Hornick, C. A., T. Kita, R. L. Hamilton, J. P. Kane, and R. J. Havel. 1983. Secretion of lipoproteins from the liver of normal and Watanabe Heritable Hyperlipidemic rabbits. *Proc. Natl. Acad. Sci. USA.* **80:** 6096-6100.
9. Soutar, A. K., N. B. Myant, and G. R. Thompson. 1982. The metabolism of very low density and intermediate density lipoproteins in patients with familial hypercholesterolaemia. *Atherosclerosis.* **43:** 217-231.
10. Goldstein, J. L., and M. S. Brown. 1974. Binding and degradation of low density lipoproteins by cultured human fibroblasts: comparison of cells from a normal subject and from a patient with homozygous familial hypercholesterolemia. *J. Biol. Chem.* **249:** 5153-5162.
11. Farriaux, J. P., M. Ribet, M. Bertrand, M. Higgins, M. Mazzuca, and A. Luyckx. 1976. Traitement de l'hypercholesterolemie familiale de Type II par shunt porto-caval. *Arch. Fr. Pediatr.* **33:** 745-759.
12. Farriaux, J. P., and M. Ribet. 1978. Treatment of Type IIA hypercholesterolemia with portacaval shunt. *In* Treatment of Hyperlipoproteinemia. L. A. Carlson, editor. Raven Press, New York. 497-501.
13. Cuthbert, J. A., C. A. East, D. W. Bilheimer, and P. E. Lipsky. 1986. Detection of familial hypercholesterolemia by assaying functional LDL receptors on lymphocytes. *N. Engl. J. Med.* **314:** 879-883.
14. Packard, C. J., A. Munro, A. R. Lorimer, A. M. Gotto, and J. Shepherd. 1984. Metabolism of apolipoprotein B in large

triglyceride-rich very low density lipoproteins of normal and hypertriglyceridemic subjects. *J. Clin. Invest.* **74:** 2178–2192.
15. Shepherd, J., C. J. Packard, J. M. Stewart, R. F. Atmeh, R. S. Clark, D. Boag, H. G. Morgan, and T. D. V. Lawrie. 1984. Apolipoprotein A and B (S_f 100–400) metabolism during bezafibrate therapy in hypertriglyceridemic subjects. *J. Clin. Invest.* **74:** 2164–2177.
16. Lindgren, F. T., L. C. Jensen, and F. T. Hatch. 1972. The isolation and quantitative analysis of serum lipoproteins. *In* Blood Lipids and Lipoproteins: Quantitation, Composition, and Metabolism. G. J. Nelson, editor. John Wiley & Sons, New York. 181–274.
17. Bilheimer, D. W., S. Eisenberg, and R. I. Levy. 1972. Metabolism of very low density lipoproteins. I. Preliminary in vitro and in vivo observations. *Biochim. Biophys. Acta.* **260:** 212–221.
18. Kane, J. P., T. Sata, R. L. Hamilton, and R. J. Havel. 1975. Apoprotein composition of very low density lipoproteins of human serum. *J. Clin. Invest.* **56:** 1622–1634.
19. Manual of Laboratory Operations, Lipid Research Clinics Program. 1974. Volume 1: Lipid and Lipoprotein Analysis. National Heart and Lung Institute, National Institutes of Health, Bethesda, Maryland. DHEW Publication Number (NIH) 75-628.
20. Berman, M., and M. F. Weiss. 1974. SAAM Manual. USPHS Publication No. 1703. US Government Printing Office, Washington, DC.
21. Berman, M., M. Hall, R. I. Levy, S. Eisenberg, D. W. Bilheimer, R. D. Phair, and R. H. Goebel. 1978. Metabolism of apoB and apoC lipoproteins in man: kinetic studies in normal and hyperlipoproteinemic subjects. *J. Lipid Res.* **19:** 38–56.
22. Fisher, W. R., L. A. Zech, P. Bardalaye, G. Warmke, and M. Berman. 1980. Metabolism of apolipoprotein B in subjects with hypertriglyceridemia and polydisperse low density lipoprotein. *J. Lipid Res.* **21:** 760–774.
23. Kashyap, M. L., R. L. Barnhart, L. S. Srivastava, G. Perisutti, P. Vink, C. Allen, E. Hogg, D. Brady, C. J. Glueck, and R. L. Jackson. 1982. Effects of dietary carbohydrate and fat on plasma lipoproteins and apolipoproteins C-II and C-III in healthy men. *J. Lipid Res.* **23:** 877–886.
24. Eisenberg, S. 1985. Preferential enrichment of large-sized very low density lipoprotein populations with transferred cholesteryl esters. *J. Lipid Res.* **26:** 487–494.
25. Morganroth, J., R. I. Levy, and D. S. Fredrickson. 1975. The biochemical, clinical and genetic features of Type III hyperlipoproteinemia. *Ann. Intern. Med.* **82:** 158–174.
26. Goldberg, I. J., N-A. Le, J. R. Paterniti, H. N. Ginsberg, F. T. Lindgren, and W. V. Brown. 1982. Lipoprotein metabolism during acute inhibition of hepatic triglyceride lipase in the cynomolgus monkey. *J. Clin. Invest.* **70:** 1184–1192.
27. Breckenridge, W. C., J. A. Little, and P. Alaupovic. 1982. Lipoprotein abnormalities associated with a familial deficiency of hepatic lipase. *Atherosclerosis.* **45:** 161–179.
28. Dietschy, J. M. 1984. Regulation of cholesterol metabolism in man and in other species. *Klin. Wochenschr.* **62:** 338–345.
29. Nicoll, A., and B. Lewis. 1980. Evaluation of the roles of lipoprotein lipase and hepatic lipase in lipoprotein metabolism: in vivo and in vitro studies in man. *Eur. J. Clin. Invest.* **10:** 487–495.
30. Turner, P. R., J. Coltart, W. R. Hazzard, R. Bacchus, A. Nicoll, N. E. Miller, and B. Lewis. 1979. Production and conversion of lipoproteins: trans-splanchnic arteriovenous studies in man. *Eur. J. Clin. Invest.* **9:** 36–42.
31. Kuusi, T., E. A. Nikkila, I. Virtanen, and P. K. J. Kinnunen. 1979. Localisation of the heparin-releasable lipase in situ in the rat liver. *Biochem. J.* **181:** 245–246.
32. Vlodavsky, I., P. E. Fielding, C. J. Fielding, and D. Gospodarowicz. 1978. Role of contact inhibition in the regulation of receptor-mediated uptake of LDL in cultured vascular endothelial cells. *Proc. Natl. Acad. Sci. USA.* **75:** 356–360.
33. Fielding, P. E., I. Vlodavsky, D. Gospodarowicz, and C. J. Fielding. 1979. Effect of contact inhibition on the regulation of cholesterol metabolism in cultured vascular endothelial cells. *J. Biol. Chem.* **214:** 749–755.
34. Demant, T., L. A. Carlson, L. Holmquist, F. Karpe, P. Nilsson-Ehle, C. J. Packard, and J. Shepherd. 1988. Lipoprotein metabolism in hepatic lipase deficiency: studies on the turnover of apolipoprotein B and on the effect of hepatic lipase on high density lipoprotein. *J. Lipid Res.* **29:** 1603–1611.
35. Packard, C. J., D. E. Boag, R. Clegg, D. Bedford, and J. Shepherd. 1985. Effects of 1,2-cyclohexanedione modification on the metabolism of VLDL apolipoprotein B: potential role of receptors in IDL catabolism. *J. Lipid Res.* **26:** 1058–1067.
36. Monge, J. C., J. M. Hoeg, S. W. Law, R. E. Gregg, and H. B. Brewer. 1986. Human apolipoprotein B in mRNA regulation. Role of apoB-containing particles in the LDL receptor pathway. *Circulation.* **74:** II-107 (A).