Metabolic Basis of Hyperapobetalipoproteinemia

Turnover of Apolipoprotein B in Low Density Lipoprotein and Its Precursors and Subfractions Compared with Normal and Familial Hypercholesterolemia

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Abstract

The turnover of apolipoprotein B (apo B) in very low density, intermediate density, and low density lipoproteins (VLDL, IDL, and LDL) and in the light and heavy fractions of LDL was determined in seven patients with hyperapobetalipoproteinemia (hyperapo B), six normolipidemic subjects, and five patients with heterozygous familial hypercholesterolemia (FH). After receiving an injection of ^{125}I-VLDL, hyperapo B patients were found to have a higher rate of synthesis of VLDL-apo B than controls (40.1 vs. 21.5 mg/kg per d, $P < 0.05$) but a reduced fractional catabolic rate (FCR) (0.230 vs. 0.366/h, $P < 0.01$). After receiving an injection of ^{131}I-LDL, hyperapo B patients had higher rates of LDL-apo B synthesis than controls (23.1 vs. 13.0 mg/kg per d, $P < 0.001$), as did FH patients (22.7 mg/kg per d). The FCR of LDL was similar in hyperapo B patients and controls (0.386 vs. 0.366/d) but was markedly decreased in FH patients (0.192/d). Most subjects exhibited precursor-product relationships between VLDL and IDL, and all did between IDL and light LDL; an analogous relationship between light and heavy LDL was evident in most hyperapo B patients and controls but not in FH patients. Simultaneous injection of differentially labeled LDL fractions and deconvolution analysis showed increased light LDL synthesis with normal conversion into heavy LDL in hyperapo B, whereas in FH conversion of light LDL was reduced and there was independent synthesis of heavy LDL. These data show that the increased concentration of LDL-apo B in hyperapo B is solely due to increased LDL synthesis, which is secondary to increased VLDL synthesis; in contrast, in FH there is both an increase in synthesis of LDL (which is partly VLDL-independent) and reduced catabolism.

Introduction

Evidence that low density lipoprotein (LDL) is heterogenous in terms of particle size and composition has been published by several groups of workers, using a variety of methods of separation (1–6). These studies have shown that LDL can be separated into 3–5 subfractions in normal subjects (1, 3–5) and patients with type IV hyperlipoproteinemia (2) in whom, as pointed out by Fisher (7), heterogeneity seems especially marked. Incubation of LDL with postheparin plasma results in a shift in the distribution of LDL from lighter into heavier subfractions (1), which suggests the conversion of larger into smaller particles consequent on lipolysis (3, 5). Kinetic studies by Phair et al. (8) provide additional support for the existence of precursor-product relationships within the spectrum of LDL particles.

Recently we described a simple means of separating LDL into two major subfractions, fraction 1 or light LDL, and fraction 2 or heavy LDL, using discontinuous density gradient ultracentrifugation, and we documented differences in the densities and composition of these fractions between normal subjects and patients with increased plasma levels of LDL-apolipoprotein B (apo B) (6). The latter fell into two categories: patients with familial hypercholesterolemia (FH), in whom fraction 1 was cholesterol ester-enriched and less dense than normal, and patients with hyperapobetalipoproteinemia (hyperapo B), in whom fraction 2 was cholesterol ester-depleted and denser than normal. As defined previously (9, 10), the term hyperapo B denotes an increased concentration of LDL-apo B in plasma in the face of a normal concentration of LDL cholesterol, often accompanied by hypertriglyceridemia and associated with coronary heart disease (CHD). An increase in LDL-apo B, hypertriglyceridemia, and a predisposition to CHD are also features of familial combined hyperlipidemia (11), with which hyperapo B undoubtedly overlaps in some instances. The relationship between these two entities and the possibility that an abnormality of apo B metabolism is common to both has recently been discussed in detail elsewhere (12).

In the present study the turnover of apo B within the main subfractions of LDL and in its precursors, very low density lipoprotein (VLDL) and intermediate density lipoprotein (IDL), has been investigated in normal subjects, patients with hyperapo B, and patients with heterozygous FH; a major objective was to determine the mechanisms responsible for the increased levels of LDL-apo B and contrasting changes in LDL composition, which distinguish these two disorders.

Methods

Studies were performed in 17 male subjects: 7 patients with hyperapo B, 4 FH heterozygotes, and 6 normal controls; a female FH heterozygote was also studied (patient 18). Subjects 7, 11, and 14–18 were studied in London; the remainder were studied in Montreal. Their clinical and biochemical details are shown in Table I. The cholesterol and triglyceride

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1. *Abbreviations used in this paper:* ACR, absolute catabolic rate; apo B, apolipoprotein B; CHD, coronary heart disease; FCR, fractional catabolic rate; FH, familial hypercholesterolemia; hyperapo B, hyperapobetalipoproteinemia.

Table I. Clinical and Biochemical Data

Subject	Age	RBW	TC	TG	VLDL-C	LDL-C	HDL-C	LDL-B	WHO type
		%	*mg/dl plasma*	*mg/dl plasma*	*mg/dl plasma*	*mg/dl plasma*	*mg/dl plasma*	*mg/dl plasma*	
Hyperapo B									
1	33	107	280	198	40	200	40	147	N
2	57	127	309	533	96	162	51	143	IV
3	61	105	231	584	35	149	47	134	IV
4	53	89	183	113	23	111	49	124	N
5	56	116	261	236	47	162	52	146	IV
6	38	94	135	318	24	94	17	121	IV
7	61	95	250	226	—	186	19	150	IV
Mean±SD	51±11	105±13	236±59*	315±177*	44±27*	152±38	39±15	138±11‡	
Control									
8	40	95	204	113	23	139	41	85	N
9	46	105	191	84	9	131	51	94	N
10	50	105	216	136	27	147	42	95	N
11	47	96	182	72	3	129	49	98	N
12	63	104	152	54	6	100	46	77	N
13	40	97	160	127	5	105	30	73	N
Mean±SD	48±9	100±5	184±25	98±33	12±10	129±18	43±8	87±10	
FH									
14	27	102	550	142	—	489§	33	283	IIa
15	64	102	476	290	—	385§	33	242	IIb
16	52	92	491	139	—	422§	41	258	IIa
17	38	101	480	164	—	402§	45	270	IIa
18	62	128	526	186	—	398§	43	234	IIa
Mean±SD	49±16	105±14	505±32‡	184±62	—	419±41‡	39±6	257±20‡	

* $P < 0.05$; ‡ $P < 0.001$ versus controls; § calculated from formula of Friedewald et al. (14). *Abbreviations used in this table:* TC, total cholesterol; TG, triglyceride; VLDL-, LDL-, and HDL-C, VLDL-, LDL- and HDL-cholesterol; LDL-B, LDL-apo B in $d > 1.019$ fraction of plasma; RBW, relative body weight, calculated as $\frac{\text{kg}}{\text{cm} - 100} \times 100\%$.

content of samples was determined enzymatically, each value representing the mean of triplicate analyses of single samples or the mean of single estimates of two or more samples. LDL cholesterol was calculated by subtracting VLDL and high density lipoprotein (HDL) cholesterol from total cholesterol (13), except in the FH patients whose LDL cholesterol was calculated as described by Friedewald et al. (14) (see Table I). HDL cholesterol was determined after heparin-manganese precipitation of plasma (15), adapted for enzyme assay by the addition of 8 mmol/liter EDTA to the diluent line of the autoanalyzer. LDL-apo B was assayed by radial immunodiffusion, using a method designed to minimize any contribution from VLDL and IDL-apo B (16), as validated below. Upper limits of normality for serum triglyceride, LDL cholesterol, and LDL-apo B upon which lipoprotein phenotyping and the diagnosis of hyperapo B have been based were 200, 200, and 120 mg/dl, respectively, as defined previously (6, 10). All subjects consumed their usual diets except during studies of VLDL turnover when their fat intake was restricted to <5 g/d; meals were supplied by the diet kitchen during this 2-d period. Dietary intake was not assessed routinely but both groups of patients had received advice in the past aimed at achieving or maintaining ideal body weight and restricting the intake of saturated fat and cholesterol. None of the patients was on any lipid-lowering drug during the course of these studies with the exception of FH patient 18, who was on cholestyramine during one LDL turnover study. Hyperapo B patients 2 and 3 were on hydrochlorothiazide and patient 3 was also on propranalol but none of the other patients were on diuretics or β-blockers. All were ambulant and maintained their customary exercise habits. Relative body weight was within the normal range in all but two subjects, the exceptions being one hyperapo B and one FH patient, who were slightly obese. These studies were sanctioned by the Research Ethics Committees of the Royal Postgraduate Medical School and Hammersmith Hospital, London, and the Royal Victoria Hospital and McGill University, Montreal, and all subjects gave informed consent.

Measurement of LDL-apo B. This was performed as previously described (16); the samples were allowed to diffuse radially for 18 h into a 1.5% agarose gel containing a rabbit antibody to human LDL of d 1.025–1.050. To ascertain whether a significant contribution to the diameter of the rings was made by the apo B in VLDL and IDL, as has been claimed to occur with hypertriglyceridemic samples (17), blood samples were obtained from 50 fasting subjects undergoing health screening in London. All had a serum cholesterol of <260 mg/dl, 31 of which had a serum triglyceride of <177 mg/dl and 19 of which had a triglyceride of >220 mg/dl, the highest value being 526 mg/dl. A 4-ml aliquot of each subject's serum was adjusted to d 1.019 and ultracentrifuged, as described below.

The concentration of LDL-apo B was compared in each serum and its corresponding $d > 1.019$ fraction; the respective values were 106±19 vs. 100±19 mg/dl in normotriglyceridemic samples and 120±23 vs. 113±26 mg/dl in hypertriglyceridemic samples. In each group 94% of the apo B in serum was accounted for by apo B in the corresponding $d > 1.019$ fraction. Using an LDL apo B of 120 mg/dl or above as a

criterion, four of the normotriglyceridemic group had hyperapo B when serum was assayed, vs. three who had hyperapo B when the $d > 1.019$ fraction was assayed; in the hypertriglyceridemic group the corresponding numbers were 11 vs. 8. These data confirm that the contribution of VLDL and IDL-apo B to the measurement of LDL-apo B in plasma by radial immunodiffusion (RID) is slight in our hands and is no greater in hypertriglyceridemic than in normotriglyceridemic samples. It is possible, however, that LDL-apo B might be significantly overestimated by RID of plasma or serum at triglyceride levels of >1,000 mg/dl (17).

Family studies. Data was obtained on other family members of three of the seven hyperapo B patients. Patient 1, who had transiently exhibited a type IV phenotype in the past, had a mother and brother with elevated LDL cholesterols and type IIa phenotypes, a daughter age 4 yr with a plasma LDL-apo B of 137 mg/dl, and a son age 7 yr with a plasma LDL-apo B of 98 mg/dl, both with normal LDL cholesterols and lipoprotein phenotypes. LDL-apo B levels were 138–205 mg/dl in one son and three daughters, age 19–26 yr, of patient 3 and were 89–111 mg/dl in three other daughters, age 14–16 yr. This patient's wife had a value of 197 mg/dl. All these individuals had normal LDL cholesterols and lipoprotein phenotypes. Patient 5 had three adult first degree relatives with LDL-apo B levels of 122–129 mg/dl, a daughter age 14 yr with a value of 155 mg/dl, and a wife with a value of 172 mg/dl, all with normal LDL cholesterols and lipoprotein phenotypes. Thus, three families showed evidence of hyperapo B in first degree relatives, both adults and children; and in two families hyperapo B was present in a spouse.

Of the FH heterozygotes patient 16 had three hypercholesterolemic first degree relatives and the other four patients had tendon xanthomata, including patient 15, who had a type IIb phenotype.

Turnover of apo B in VLDL and IDL. The turnover of apo B in VLDL, IDL, and LDL was determined by simultaneously injecting ^{125}I-VLDL and ^{131}I-LDL, using standard techniques to isolate, label, and analyze lipoproteins, as described previously (18, 19). The amounts of ^{125}I and ^{131}I injected averaged 79 and 58 μCi, respectively. Lipid labeling was 20% for VLDL and <5% for LDL. All patients received oral potassium iodide, 180 mg/d throughout the 2 wk of the study period. Those undergoing VLDL turnovers were placed on an isocaloric, low fat diet for 48 h after receiving ^{125}I-VLDL. As a rule, ^{131}I-LDL was injected 10 min after ^{125}I-VLDL, after an overnight fast. In some instances light and heavy fractions of LDL were isolated, as described below, labeled with ^{125}I and ^{131}I, respectively, and then reinjected.

Blood samples were taken into EDTA at 2–4 hourly intervals during the first 24 h after the injection of labeled lipoproteins and at 12–24 hourly intervals for the next 9 d. Plasma was separated, stored at 4°C for 5–10 d, and then successively ultracentrifuged at d 1.006 and d 1.019 in a 50 Ti rotor (Beckman Instruments, Inc., Fullerton, CA; 40,000 rpm for 16 h at 4°C). The VLDL and IDL were recovered after tube slicing for analysis of apo B specific activities. This involved delipidation of samples with ether/ethanol, followed by column chromatography on Sephadex G-150 and assay of the leading edge of the apo B peak, which elutes with the void volume, for radioactivity and protein content, as originally described by Sigurdsson et al. (20). Sample count rates ranged from 100 to 550,000 cpm above background. The absolute catabolic rate (ACR) of apo B in VLDL was calculated as dose injected divided by area under the VLDL specific activity time curve, computed graphically, and the fractional catabolic rate (FCR) was calculated as ACR × body weight divided by pool size (21). Wherever possible the proportion of VLDL converted to LDL was determined by deconvolution analysis (20). The turnover of apo B in IDL derived from VLDL was calculated by the method of Zilversmit (22) where the FCR of a product is the increase in its specific activity over any given period of time, divided by the corresponding area between the precursor and product specific activity/time curves. This method can be used only where a precursor/product relationship exists and was applied only in those instances where the IDL specific activity curve intersected the VLDL specific activity curve at or not more than 2 h before the peak of IDL specific activity was reached. The sizes of VLDL- and IDL-apo B pools were calculated as plasma volume × concentration of tetramethylurea-insoluble protein (23) in the $d < 1.006$ and d 1.006–1.019 fractions, respectively; protein was measured by the method of Lowry et al. (24). Plasma volume was estimated as 4.5% of body weight rather than using the values obtained from the 10-min blood sample (20). In two FH patients 'broad spectrum' IDL (S_f 12–60) was isolated, labeled, and then injected, and its FCR was measured directly by monocompartmental analysis (25).

Turnover of apo B in LDL and its subfractions. The FCR of LDL was calculated by multicompartmental analysis of the plasma radioactivity/time curve, as described by Matthews (26), with the pool size of LDL being calculated as the mean LDL-apo B concentration of multiple samples of the $d > 1.019$ fraction × plasma volume, using radial immunodiffusion to determine LDL-apo B (16). ACR was calculated as FCR × pool size and since these were steady state studies synthesis was taken to equal ACR.

To obtain LDL subfractions, $d > 1.019$ samples were adjusted to d 1.071 with NaCl/KBr and centrifuged in a SW 50.1 rotor to isolate LDL of d 1.019–1.063; a portion of this sample was then dialyzed to d 1.050 and 1 ml was centrifuged on a discontinuous density gradient in a SW 50.1 rotor at 40,000 rpm for 40 h at 10°C (the temperature is critical since this influences the time taken for the gradient to reach equilibrium), as described in detail previously (6). At the conclusion the gradient was fractionated, using a tube piercer, into three 1-ml fractions (fractions 3–5) followed by visual separation of heavy LDL (fraction 2) usually recovered in ~1.25 ml (mean density, 1.0480), and light LDL (fraction 1), usually recovered in ~0.75 ml (mean density, 1.0405). The ^{125}I and ^{131}I in fractions 1, 2, and 3 were then counted in a dual channel gamma counter, appropriately calibrated to minimize spillover, and their apo B and cholesterol content were assayed, as described above. The 5–10% of counts and apo B recovered in fraction 3 (mean density, 1.063) were included with heavy LDL. Quenching of ^{125}I in these fractions was assessed by an internal standard and seldom exceeded 10%.

After injection of LDL fractions the FCR of light and heavy LDL were calculated by multicompartmental analysis (26) of the radioactivity/time curves of apo B in the light or heavy fraction of LDL, pool sizes were calculated by multiplying the total LDL pool by the mean percentage of apo B recovered in light or heavy LDL, and turnover (ACR) was calculated as FCR × pool of light or heavy LDL. The proportion of light LDL converted into heavy LDL was determined by deconvolution analysis (20) and total LDL turnover was derived as turnover of light LDL plus independent input of heavy LDL; the latter was calculated as turnover of heavy LDL minus turnover of light LDL × percent converted to heavy LDL. These calculations were based on the premise that light LDL can be converted into heavy LDL but that the reverse does not occur. The statistical significance of differences between means was calculated by the two-tailed t test (Hewlett-Packard 65 Statistical Program; Hewlett-Packard Co., Palo Alto, CA).

Results

The clinical and biochemical characteristics of the three groups of subjects are detailed in Table I. The hyperapo B patients did not differ greatly from the normal controls with respect to age and relative body weight but all had CHD, documented angiographically. They had significantly higher plasma levels of total cholesterol, triglyceride (five having a type IV phenotype), and VLDL cholesterol than the controls but there was no significant difference between the two groups with respect to LDL cholesterol and HDL cholesterol. However, the hyperapo B patients had significantly higher LDL-apo B levels than normal controls. All but one (No. 17) of the FH patients had CHD, for which three had previously undergone coronary artery bypass grafting. Their total and LDL cholesterol levels were greatly increased, as was the concentration of LDL-apo B in plasma, but their HDL cholesterol values were within the normal range. VLDL

cholesterol was not determined in FH patients but one of them had a raised triglyceride and type IIb phenotype.

Table II gives the distribution of apo B in the three fractions of LDL after density gradient ultracentrifugation and the cholesterol/apo B ratio of the two main fractions. The chief differences between the three groups are the reduced cholesterol/apo B ratio of heavy LDL (fraction 2) in hyperapo B patients and the increased cholesterol/apo B ratio of light LDL (fraction 1) in FH. In control subjects and hyperapo B patients, approximately two-thirds of LDL-apo B was found in fraction 2 and approximately one-fourth in fraction 1; the remaining 5–10% was recovered in fraction 3. Significantly more apo B was present in fraction 1, and less was present in fractions 2 and 3 in FH patients compared with the other two groups. Only trace amounts of apo B were detectable in the bottom 2 ml of the gradient.

Kinetics of apo B. Quantitative data on apo B turnover in VLDL, IDL, and unfractionated LDL are shown in Table III. Examples of turnover studies in individuals from each of the three groups of subjects are illustrated in Figs. 1–4.

VLDL-apo B turnover. Hyperapo B patients had significantly higher rates of VLDL synthesis than normal subjects, a lower FCR, and a larger VLDL-apo B pool; these abnormalities were especially marked in the hypertriglyceridemic patients. VLDL turnover was measured in only one of the FH patients (No. 14) but his values were comparable with those obtained in normal subjects.

IDL-apo B turnover. The rate of synthesis of IDL-apo B (S_f 12–20) from VLDL-apo B could be calculated in only three hyperapo B patients, three controls, and one FH patient, for reasons discussed below. In two other FH patients direct estimates of IDL turnover were obtained by injecting 'broad spectrum' IDL of S_f 12–60. Overall, the rates of synthesis, FCR, and pool size of IDL-apo B did not differ greatly between the three groups (Table III). However, unlike the controls, IDL synthesis rates were considerably lower than the corresponding LDL synthesis rates in both groups of patients, irrespective of whether indirect estimates of S_f 12–20 or direct estimates of S_f 12–60 apo B turnover were calculated.

LDL-apo B turnover. The rate of synthesis of LDL-apo B, analyzed in the conventional two-pool manner after injection of labeled LDL into all subjects, was higher in hyperapo B and FH patients than in controls; the FCR of hyperapo B patients was similar to controls, whereas that of FH patients was markedly reduced (Table III). The pool size of LDL apo B was even higher in FH than in hyperapo B patients, both being significantly greater than normal.

*Table II. Distribution of Apo B and Cholesterol/Apo B Ratios in LDL Subfractions**

	Apo B			Cholesterol/apo B	
Subject	LDL-1	LDL-2	LDL-3	LDL-1	LDL-2
	% of total	*% of total*	*% of total*		
Hyperapo-B (n = 6)‡	24.6±6.2	65.9±10.6	9.4±6.4	1.47±0.16	0.98±0.11§
Control (n = 6)	26.8±9.8	68.4±10.9	4.6±5.8	1.55±0.14	1.23±0.12
FH (n = 5)	43.2±6.3§	55.0±6.6‖	1.8±2.5	2.05±0.23¶	1.37±0.16

* 1, light; 2, heavy; 3, heavier; mean±SD.
‡ Samples from patient 7 were lost and mean values were used to calculate his pool sizes in Table IV.
§ $P < 0.01$; ‖ $P < 0.05$; ¶ $P < 0.001$ vs. controls.

Precursor-product relationships between VLDL, IDL, and LDL subfractions. Fig. 1 shows the changes in specific activity of apo B in the plasma of a control subject after injection of ^{125}I-VLDL. The classical precursor-product relationship between specific activity/time curves, where the precursor (VLDL) should intersect the product (IDL) at or just before the latter reaches its maximum, was not observed in this subject nor in another control and two hyperapo B patients, in all of whom the crossover occurred 3 h or more before IDL specific activity had reached its peak. However, in the remainder (see Fig. 2, left), where the delay was less than 2 h, an attempt was made to calculate the synthesis of IDL from VLDL, using the method of Zilversmit (22) but accepting that the values obtained (Table III) are only approximate estimates.

The proportion of VLDL-apo B converted into LDL-apo B, calculated by deconvolution analysis, averaged 58% (22–80%) in three control subjects compared with 49% (35–69%) in four hyperapo B patients. In one of the control subjects and two of the hyperapo B patients only 40–60% of the LDL synthesized could be accounted for on the basis of conversion of VLDL, but in the remainder all LDL was derived from VLDL.

Relationships between the specific activity/time curves of IDL and light LDL are shown in Figs. 1 and 2 (left) and between light and heavy LDL in Fig. 1 (right). All subjects studied exhibited precursor-product relationships between IDL and light LDL including the FH patients given ^{125}I-VLDL (Fig. 3) and ^{125}I-IDL (Fig. 4). However, in one control subject (No. 10) and one apo B patient (No. 4) there was no precursor-product relationship between light and heavy LDL, as exemplified in Fig. 2 (right); in both these individuals LDL synthesis was only partially accounted for by conversion of VLDL, as noted above. The lack of any such relationship between light and heavy LDL was even more evident in the three FH patients in whom this was studied, irrespective of whether the light LDL was originally derived from injected VLDL (Fig. 3) or from injected IDL of S_f 12–60 (Fig. 4).

Turnover of LDL subfractions. The turnover of apo B in the light and heavy fractions of LDL was qualitatively assessed in nine subjects after an injection of unfractionated, singly labeled LDL, which was given primarily to measure total LDL turnover (see Table III). As illustrated in Fig. 5, the early part of the specific activity/time curve for light LDL decays faster and is more curvilinear than that of heavy LDL in a control subject. These differences are accentuated in the hyperapo B patient but are much less evident in the FH patient. Quantitation of light and heavy LDL turnover was not possible during these studies because the proportion of light LDL converted to heavy LDL was not determined.

The turnover and interconversion of isolated LDL fractions was studied in six subjects who were injected with differentially labeled light and heavy LDL. As illustrated in Fig. 6 (left), there was a precursor-product relationship between light and heavy LDL when light LDL was injected into a hyperapo B subject but the reverse did not occur when heavy LDL was injected (Fig. 6, right). Similar findings were observed in a control subject in whom administration of intravenous heparin to stimulate lipolysis caused a transient dip in the specific activity/time curve of light LDL (Fig. 7, left), presumably reflecting conversion of

Table III. Kinetics of Apo B Turnover in VLDL, IDL, and LDL

	VLDL-apo B			IDL-apo B			LDL-apo B		
	ACR	FCR	Pool	ACR	FCR	Pool	ACR	FCR	Pool
	mg/kg per d		*mg*	*mg/kg per d*		*mg*	*mg/kg per d*	*per d*	*mg*
Hyperapo B									
1	46.0	0.261	615	—	—	123	20.8	0.343	5,538
2	50.2	0.168	822	19.3	0.244	217	32.1	0.521	4,063
3	32.7	0.127	810	—	—	172	20.8	0.355	4,002
4	28.4	0.355	199	7.6	0.258	81	20.3	0.362	3,487
5	43.3	0.237	543	4.8	0.095	148	22.1	0.350	4,430
Mean±SD	40.1±9.2*	0.230±0.088‡	598±254‡	10.6	0.199	148±51	23.1±5.1§	0.386±0.076	4,304±767‡
Control									
8	25.9	0.350	204	—	—	176	15.9	0.358	2,952
9	17.5	0.351	165	14.3	0.404	124	13.8	0.327	3,538
10	23.2	0.388	209	—	—	84	12.7	0.376	2,973
11	19.5	0.380	164	5.8	0.190	99	11.2	0.346	2,531
12	21.6	0.362	214	19.1	0.516	129	12.8	0.414	2,460
13	—	—	—	—	—	—	11.7	0.376	2,056
Mean±SD	21.5±3.3	0.366±0.017	191±25	13.1	0.37	122±35	13.0±1.7	0.366±0.030	2,752±515
FH									
14	27.6	0.383	316	6.5	0.203	97	33.0	0.259	9,233
15	—	—	—	11.44‖	0.088‖	381‖	22.1	0.192	8,095
16	—	—	—	5.92‖	0.167‖	91‖	19.7	0.168	7,203
17	—	—	—	—	—	—	21.0	0.173	9,161
18	—	—	—	—	—	—	17.8	0.169	6,308
Mean±SD							22.7±6.0§	0.192±0.039§	8,000±1,262§

* $P < 0.05$; ‡ $P < 0.01$; § $P < 0.001$ vs. controls; ‖ Sf 12–60.

unlabeled IDL into light LDL, without affecting heavy LDL turnover (Fig. 7, right). In FH, no precursor-product relationship was evident between light and heavy LDL, confirming earlier studies in which VLDL or IDL had been injected (Fig. 8, left). Indeed, the cross-over of specific activities 4 d after injection of heavy LDL (Fig. 8, right) suggests that some of the latter may be converted back to light LDL in this disorder, albeit at a slow rate. Alternatively, it may have been due to an influx of unlabeled heavy LDL.

Turnover rates of light and heavy LDL in the six subjects were determined by analysis of the respective radioactivity/time curves and the proportion of light LDL converted to heavy LDL was calculated by deconvolution analysis of the injected heavy LDL and the heavy LDL derived from injected light LDL. Total

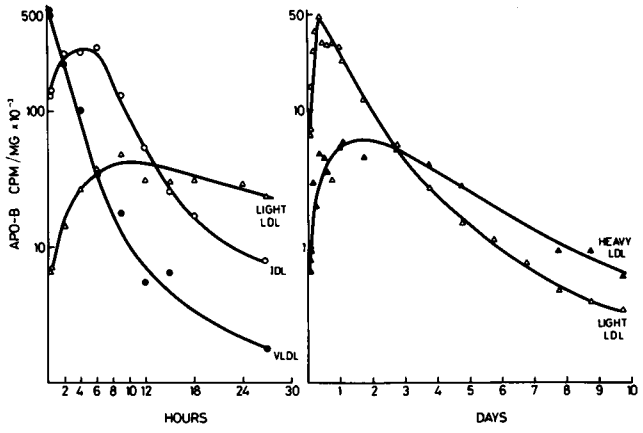

Figure 1. Specific activity of apo B in plasma lipoproteins of a control subject (No. 10) during the initial 27 h (*left*) and over the course of 10 d (*right*) after administration of ^{125}I-VLDL.

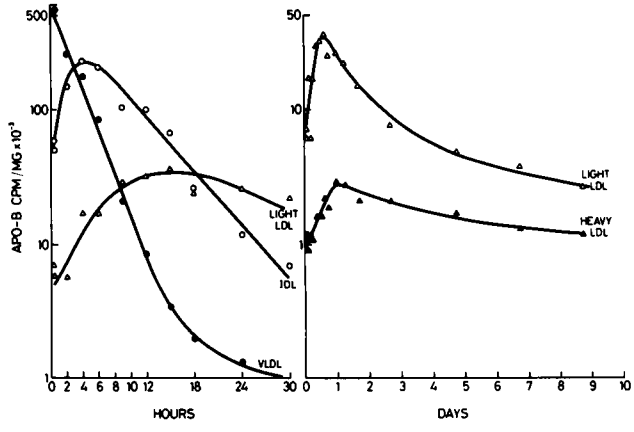

Figure 2. Specific activity of apo B in plasma lipoproteins of a hyperapo B patient (No. 4) during the initial 30 h (*left*) and over the course of 9 d (*right*) after administration of ^{125}I-VLDL. A similar pattern was observed in control subject No. 12.

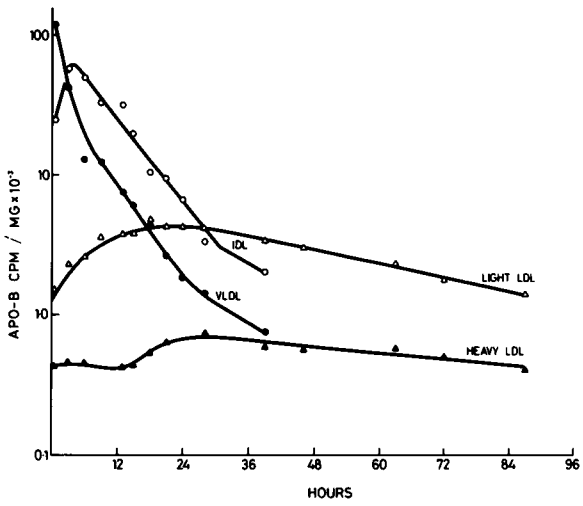

Figure 3. Specific activity of apo B in plasma lipoproteins of an FH patient (No. 14) during the 4 d after an injection of ^{125}I-VLDL.

LDL turnover was then derived, as shown under method A, Table IV. Four of these subjects also had total LDL turnover measured in the conventional manner on a different occasion (method B), three under similar conditions to those of the previous study, whereas the fourth was on cholestyramine during study A but not during study B. Values of FCR and ACR for total LDL were lower when calculated by method B, despite similar pool sizes during the two sets of studies.

Synthesis (ACR), FCR, and conversion rates of light LDL were highest in hyperapo B subjects whereas FCR and percent conversion were reduced in FH. FCR of heavy LDL was also reduced in FH but synthesis (ACR) of heavy LDL was increased as it also was in hyperapo B, although in FH the greater proportion was not derived from light LDL. In one FH patient administration of cholestyramine (Questran) for clinical reasons resulted in a decrease in the size of the light LDL pool.

Discussion

The hyperapo B subjects in this study were characterized by plasma levels of LDL-apo B in excess of 120 mg/dl in the face

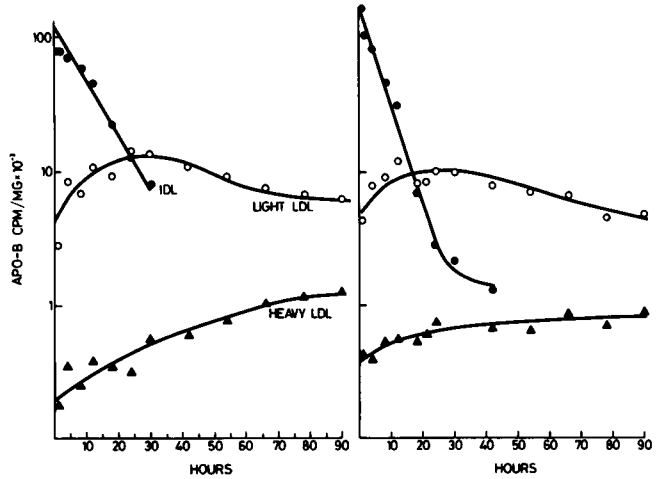

Figure 4. Specific activity of apo B in plasma lipoproteins of two FH patients (No. 15, *left* and No. 16, *right*) during the 4 d after injection of ^{125}I-IDL (S_f 12–60).

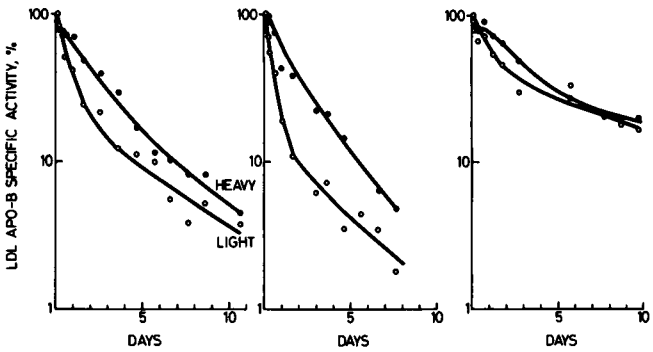

Figure 5. Specific activity of apo B in light (○) and heavy (●) LDL, expressed as percent of the specific activity of light LDL at 10 min after injection of ^{125}I- or ^{131}I-LDL, in a control subject (No. 9, *left*), a hyperapo B patient (No. 2, *center*), and an FH heterozygote (No. 17, *right*).

of LDL cholesterol levels of 200 mg/dl or less. This combination was due to an increased concentration of heavy LDL particles, denser than normal and with a reduced content of cholesterol ester (6). These features and a decrease in particle size tend to be especially marked in hypertriglyceridemic hyperapo B patients; five of our seven hyperapo B patients, all of whom had CHD, fell into that category. It was recently suggested that the RID assay we used over-estimates the concentration of LDL-apo B in hypertriglyceridemic plasma because of the penetration into the gel of small VLDL and IDL particles containing apo B (17). However, we could find no evidence of this since 94% of the apo B in 50 sera that we tested was attributable to LDL, both in normolipidemic and hypertriglyceridemic samples (see Methods). Another potential drawback to this method is the possibility that small LDL particles might diffuse faster into the gel than larger LDL particles, thus giving a spuriously high value in hypertriglyceridemic samples. However, comparison of the Lowry and RID methods of quantitating protein in light and heavy LDL, which differ markedly in size, failed to demonstrate any bias of the RID assay toward heavy LDL (6). Thus, overestimation of LDL-apo B by RID of plasma, at least in our hands, is relatively slight. Nevertheless, all the hyperapo B patients in this study had an LDL apo B of >120 mg/dl as measured both in plasma and in its $d > 1.019$ fraction.

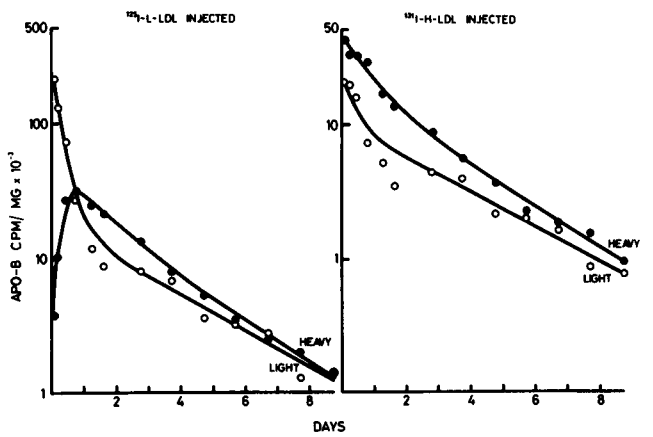

Figure 6. Specific activity of apo B in light and heavy LDL in a hyperapo B patient (No. 6) after administration of ^{125}I-light LDL (*left*) and ^{131}I-heavy LDL (*right*).

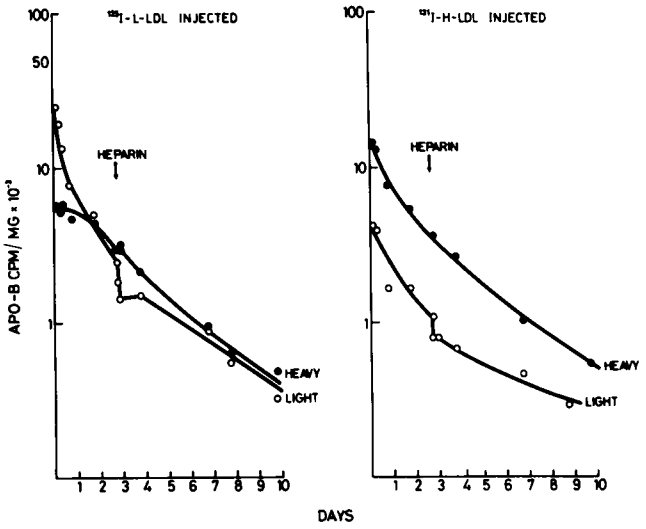

Figure 7. Specific activity of apo B in light and heavy LDL in plasma of a control subject (No. 11) after administration of ^{125}I-light LDL (*left*) and ^{131}I-heavy LDL (*right*). An intravenous injection of heparin 100 i.u./kg body weight was given at 2.75 d, as indicated by the arrow; blood samples were taken immediately before and 15 and 60 min later. Deconvolution analysis showed virtually complete conversion of light to heavy LDL before heparin was injected (77.0% at 2.75 d compared with 77.1% at 10 d) but both postheparin samples have been excluded from the turnover analysis.

Although each of the three hyperapo B families we studied contained first degree relatives with raised LDL-apo B levels, only one family exhibited the pattern of multiple lipoprotein phenotypes, which is characteristic of familial combined hyperlipidemia (11). The latter disorder includes patients with increased levels of LDL cholesterol and type IIa and IIb phenotypes, whereas such individuals were, by definition, excluded from the present study. Furthermore, LDL-apo B levels are often but not invariably raised (>120 mg/dl) in familial combined hyperlipidemia (11). VLDL-apo B synthesis is known to be increased in patients with the latter disorder (28–30), and a similar increase was evident in most of our hyperapo B patients, especially if they were hypertriglyceridemic. The area under the curve method of calculating VLDL-apo B turnover, which we used, gives a lower but more accurate estimate of synthesis than monocompartmental analysis (19), which fails to take into account the slowly turning-over tail of the specific activity/time

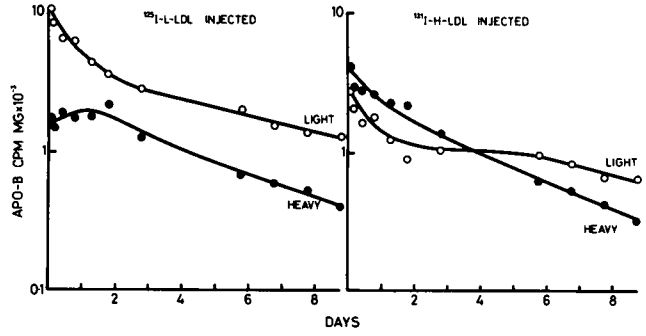

Figure 8. Specific activity of apo B in light and heavy LDL in an FH patient (No. 17) after administration of ^{125}I-light LDL (*left*) and ^{131}I-heavy LDL (*right*).

curve (30). The nearly two-fold increase in VLDL-apo B synthesis in hyperapo B patients was accompanied by a marked decrease in FCR and an expanded VLDL pool.

LDL-apo B synthesis was also nearly twice normal in the hyperapo B patients as was the size of the LDL pool. Similar results were obtained by Kesaniemi and Grundy (31), who found a strong correlation between LDL synthesis and the concentration of LDL-apo B in plasma. In both studies the FCR of LDL was normal in hyperapo B subjects. The same authors recently described another group of patients with CHD and overproduction of LDL, whose LDL-apo B levels remained normal because of an increase in FCR; these, they suggest, might represent normolipidemic variants of familial combined hyperlipidemia (32). Thus, it would seem that oversynthesis of apo B with normal or even increased LDL catabolism is common to both familial combined hyperlipidemia and hyperapo B. This contrasts with FH where increased synthesis of LDL is accompanied by a decrease in FCR, as observed in this and previous studies (33, 18).

Since hyperapo B exhibits similar phenotypic features to familial combined hyperlipidemia and both disorders seem to have the same metabolic defect, namely overproduction of apo B, this suggests that they may be identical. However, familial combined hyperlipidemia is considered to be a dominantly inherited disorder (34), whereas the pattern of distribution of hyperapo B within families is compatible with polygenic inheritance (35). Until such time as the genetic defect or defects responsible have been identified it seems reasonable to keep an open mind as to whether hyperapo B represents a subgroup of familial combined hyperlipidemia or whether it represents a phenotypically similar but genetically distinct disorder.

The increase in LDL synthesis in hyperapo B is accompanied in most instances by an increase in VLDL-apo B synthesis, as discussed above. Based on the estimate that almost 50% of VLDL was converted into LDL, this increase in VLDL synthesis accounted for most of the increase in LDL synthesis in hyperapo B. The absence of any increase in IDL synthesis in those hyperapo B patients in whom this could be calculated suggests that increased synthesis of LDL in this disorder seemingly involves direct conversion of VLDL to LDL, as has been postulated in hypertriglyceridemic subjects (36). In the latter study ~20–30% of labeled VLDL was converted directly to LDL without appearing in IDL, while a similar proportion was converted to LDL via IDL, the remainder being completely catabolized. These several fates of VLDL presumably reflect its heterogeneity in hypertriglyceridemic subjects (37). The possibility of a significant contribution to LDL-apo B levels being derived from a VLDL-independent synthetic pathway in hyperapo B cannot be excluded, however, especially in those subjects in whom a precursor-product relationship between light and heavy LDL was not demonstrable and in whom total LDL synthesis exceeded that derived from VLDL.

The specific activity/time curves of IDL provide useful qualitative information, but accurate quantitation of IDL turnover is dependent upon a valid estimate of pool size, which may be underestimated if S_f 12–20 concentrations of apo B are low or losses are high. This may be one explanation of why IDL synthesis rates were often lower than those of LDL. The discrepancy between IDL and LDL synthesis persisted even when 'broad spectrum' IDL (S_f 12–60) was directly injected into two FH patients. This observation, together with the lack of any precursor-product relationship between light and heavy LDL in all five

Table IV. Quantitation of Turnover of Apo B in Light (L) and Heavy (H) Fractions of LDL

Subject	Method of analysis	L-LDL				H-LDL				Total LDL		
		Pool	FCR	ACR	Conversion	Pool	FCR	ACR	Direct synthesis	Pool	FCR	ACR
		mg	*per d*	*mg/kg per d*	*%*	*mg*	*per d*	*g/kg per d*	*%*	*mg*	*per d*	*g/kg per d*
HyperapoB												
6	A	1,174	1.72	25.4	77.8	2,764	0.495	17.2	0	3,938	(0.513	25.4)
7	A	1,257	0.475	8.76	100	3,837	0.307	17.3	49.0	5,094	(0.231	17.3)
Control												
11	A	619	0.80	6.4	77.1	2,247	0.445	13.0	61.5	2,866	(0.386	14.4)
	B	—	—	—	—	—	—	—	—	2,531	0.346	11.2
13	A	563	0.628	5.34	73.8	1,522	0.55	12.65	69.2	2,085	(0.447	14.1)
	B	512	—	—	—	1,544	—	—	—	2,056	0.376	11.7
FH												
17	A	3,331	0.224	10.2	49.4	4,317	0.273	16.2	68.5	7,648	(0.203	21.3)
	B	3,023	—	—	—	6,138	—	—	—	9,161	0.173	21.0
18	A Q	2,047	0.239	8.0	39.4	3,034	0.240	11.9	73.9	5,081	(0.203	16.8)
	B	3,116	—	—	—	3,192	—	—	—	6,308	0.169	17.8

A, Light and heavy fractions were injected and turnover of each analysed by the method of Matthews (27); ACR of total LDL wsa derived as ACR of L-LDL plus direct synthesis of H-LDL, the latter being calculated as ACR of H-LDL minus ACR of light LDL × percent conversion. B, Unfractionated LDL injected and specific activity/time curves of whole LDL were analyzed by the method of Matthews (27); pool sizes of light and heavy LDL were measured after density gradient ultracentrifugation, as in Methods. Q, On Questran 16 g/d. Derived data are shown in parenthesis.

FH patients, supports the concept of a VLDL- and IDL-independent source of LDL in this disorder, which was originally based on our observation that LDL synthesis exceeded VLDL synthesis by 1.5 to 2-fold in three FH homozygotes undergoing simultaneous turnover studies (18). Subsequently, Janus et al. (38) performed similar studies in heterozygotes but, in addition, quantitated the proportion of VLDL converted to LDL. Their results suggested that 20–72% of LDL was synthesized independently of VLDL. More recently we showed reduced clearance of IDL in FH (19) and a similar finding was later reported in Watanabe Heritable Hyperlipidemic (WHHL) rabbits by Kita et al. (39). These authors showed that reduced clearance of IDL resulted in increased formation of LDL, which, they considered, was secondary to the LDL receptor deficiency that characterizes the WHHL rabbit as well as its human counterpart. There seems little doubt that a similar mechanism could contribute to increased LDL synthesis in FH patients but this does not exclude the possibility that 'direct' secretion of LDL also occurs (40).

Our studies not only explain the increase in LDL-apo B levels in hyperapo B but also have some bearing on the mechanism whereby light LDL is converted to heavy LDL. This process is accentuated in hyperapo B, especially in hypertriglyceridemic subjects in whom heavy LDL is denser, smaller, and more depleted of cholesterol ester than in control subjects (41) or normotriglyceridemic hyperapo B patients (6). Movement of cholesterol esters between lipoproteins in vitro has been shown to depend upon an exchange factor that is present in the $d > 1.25$ fraction of human plasma (42, 43). A reciprocal relationship exists between the decrement in LDL cholesterol and increment in VLDL plus HDL cholesterol that occurs in vivo during the transit of these lipoproteins through the liver (44). Reanalysis of those data, after excluding the contribution made by HDL cholesterol, gave almost as good a correlation between the decrease in LDL cholesterol and increase in VLDL cholesterol ($r = 0.57$, $P < 0.005$), the differences being confined to cholesterol ester.

Recently, Barter et al. (45) proposed that exchange of cholesterol ester between lipoproteins is a function of the pool size of the individual lipoprotein classes. This being so, an increase in the size of the VLDL pool relative to LDL, as occurs in hyperapo B, would accentuate movement of cholesterol ester from LDL to VLDL, whereas the reverse would occur in FH. The finding that light LDL is enriched in cholesterol ester and accumulates in the plasma of FH patients, who are deficient in LDL receptors, implies that the conversion of light to heavy LDL is normally dependent on efficient functioning of the receptor-mediated pathway. It is also possible that light LDL has a higher affinity for the LDL receptor than does heavy LDL, as suggested recently (46). The decrease in the cholesterol/apo B ratio of LDL and increase in FCR, especially of light LDL, induced by measures known to stimulate receptor-mediated LDL catabolism support these conclusions (47).

Lastly, the question arises as to which is the best method of analyzing LDL turnover. Berman (48) postulated the existence of at least two intravascular populations of LDL particles in equilibrium with an extravascular pool to explain apo B kinetics in humans and this concept is supported by our data. The method we have used to derive total LDL turnover (method A, Table IV) is to determine the turnover of each major fraction by the conventional two-pool model and to calculate the proportion of light LDL converted into heavy LDL by deconvolution analysis. In essence this is a twin two-pool model that allows for independent input into each intravascular pool as well as conversion of light into heavy LDL. Analysis of LDL turnover by

such a model would be expected to give higher values for FCR and thus ACR than the conventional two-pool model, which is in accord with our observations. Conversion of less dense into denser LDL particles has been well documented in hypertriglyceridemic subjects (49) but our data suggest the possibility that retroconversion of heavy to light LDL may occur in FH, which complicates the mathematical analysis. Computer modelling can resolve such problems and for this and other reasons may prove to be the most accurate means of quantitating apo B turnover in LDL as well as in VLDL, as discussed by Fisher (50) and, more recently, by Belz et al. (40).

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