Metabolism of apolipoprotein B-100 in a kindred with familial hypobetalipoproteinemia without a truncated form of apoB

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Abstract Familial hypobetalipoproteinemia (FHBL) exists in three forms: *a*) FHBL genetically linked to truncated forms of apolipoprotein B (apoB); *b*) FHBL linked to the apoB gene but with no apoB truncations; and *c*) FHBL not linked to the apoB gene. Mean production rate (PR) of apoB-100 in FHBL subjects heterozygous for apoB truncations is ~30% of normal. In a 49-member D-kindred (FHBL phenotype defined as apoB <40 mg/dl), no apoB truncations were detectable either by immunoblotting of plasma or by sequencing of relevant stretches of the apoB gene. Herein we report on the kinetic parameters of apoB-100-containing lipoproteins in four affected members of the D-kindred, and compare their kinetic values to 14 normal subjects, and 8 previously reported FHBL subjects heterozygous for various truncated forms of apoB. After an 8-h primed intravenous infusion of [^{13}C]-leucine, enrichments of apoB-100 were assessed by gas chromatography–mass spectrometry and kinetic parameters were calculated by multicompartmental modeling. The affected members of the D-kindred had similar very low, intermediate, and low density lipoprotein (VLDL, IDL, and LDL) PRs as normal controls, but their fractional catabolic rates (FCR) for VLDL and LDL were ~2 and 3 times higher, respectively, than those of normals. By contrast in apoB truncation subjects, apoB-100 PRs were uniformly reduced, while apoB-100 FCRs were similar to normals. Thus, diverse physiologic mechanisms are responsible for the low apoB levels in these two different, genetically determined forms of FHBL.—**Latour, M. A., B. W. Patterson, J. Pulai, Z. Chen, and G. Schonfeld.** Metabolism of apolipoprotein B-100 in a kindred with familial hypobetalipoproteinemia without a truncated form of apoB. *J. Lipid Res.* 1997. **38:** 592–599.

Supplementary key words kinetics • lipids • lipoproteins • metabolism

Familial hypobetalipoproteinemia (FHBL) is an autosomal codominant disease characterized by low levels of total and LDL-cholesterol and apolipoprotein B (apoB) (1). Epidemiologic studies show that individuals with low cholesterol levels may have a lower than average risk for developing cardiovascular diseases, but a higher risk for a variety of cancers, pulmonary and gastrointestinal diseases than persons with higher cholesterol levels (2). Whether low cholesterol causes some of these diseases has been a contentious issue (2–4). In a recent review of epidemiologic data, low cholesterol levels are said to antedate illnesses such as cancer by several years, providing perhaps an early warning signal, but low cholesterol levels are not thought to be causally related to cancer (3, 4). The pathophysiology of most cases of hypocholesterolemia is unknown, including most cases of FHBL.

A small percentage of FHBL cases are associated with a truncated form of apoB and heterozygotes are usually asymptomatic (1). Three populations of apoB-containing lipoproteins circulate in fasting plasmas of FHBL subjects heterozygous for truncated forms of apoB. One population contains normal apoB-100, the second apoB-48, and the third, the mutant truncated form of apoB. Based on metabolic studies, using stable isotopically labeled amino acids, we have observed that heterozygotes produce apoB-100 at ~30% of the rates of matched normolipidemic controls (5). Heterozygotes also produce less of the truncated forms of apoB than of their own apoB-100 (6), suggesting that reduced production rates largely account for the low levels of circulating apoB in the plasma of FHBL hetorozygotes. Some of the longer truncated forms of apoB-containing lipoproteins (i.e., apoB-100/75, apoB-100/87 and

Abbreviations: FHBL, familial hypobetalipoproteinemia; apo, apolipoprotein; PR, production rate; GC/MS, gas chromatography–mass spectrometry; FCR, fractional catabolic rate; VLDL, IDL, LDL, and HDL, very low, intermediate, low, and high density lipoproteins; TC, total cholesterol; FC, free cholesterol; PL, phospholipid; TG, triglyceride; PRO, protein; CE, cholesteryl ester; FPLC, fast protein liquid chromatography; DGUC, density gradient ultracentrifugation.

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apoB-100/89) have increased fractional catabolic rates (FCR) (7–9).

We have recently identified a kindred, the D-kindred, in which the FHBL phenotype appears to be segregating as an autosomal dominant trait. Affected members of the D-kindred are asymptomatic. In contrast with other FHBL kindreds we have described (1, 5–8, 10), the affected members of the D-kindred do not display any detectable truncated forms of apoB in their plasmas. The molecular basis for the FHBL in this kindred is unknown. However, genetic linkage studies suggest that the low apoB values are linked to the apoB gene locus (J. Pulai, R. Newman, and G. Schoufeld, unpublished data). The objective of the present study was to determine the kinetic parameters of apoB-100-containing lipoproteins in affected members of the D-kindred in order to understand the physiological basis for their low levels of apoB. We compared the new data on the D-kindred to kinetic parameters previously reported from our laboratory on normolipidemic controls and FHBL subjects heterozygous for truncated forms of apoB.

MATERIALS AND METHODS

Study subjects

Four affected members and one unaffected member of the D-kindred participated in the present study. The clinical characteristics of the five members are shown in **Table 1**. Affected members were asymptomatic and taking no medication. The study protocol was approved by the Human Studies Committee at Washington University School of Medicine (WUMS). For comparison purposes, we used the kinetic values previously reported by this laboratory in normolipidemic controls and FHBL subjects with various apoB truncations (5–8, 10).

Characterization of plasma lipids and lipoproteins

Lipoprotein fractions very low density (VLDL, d < 1.006 g/ml), intermediate density (IDL, d 1.006–1.019 g/ml), low density (LDL, d 1.019–1.063 g/ml), and high density (HDL, d 1.063–1.21 g/ml) were isolated by sequential ultracentrifugation (11), and dialyzed against ammonium bicarbonate (5 mmol/L) for 24 h. Within each fraction, total cholesterol (TC), free cholesterol (FC), phospholipids (PL), triglycerides (TG), LDL-, and HDL-cholesterol were determined by the Lipid Research Center Core Laboratory at Washington University School of Medicine (12). Cholesteryl esters (CE) were determined as (TC-FC) × 1.67. The protein (PRO) concentration was determined by the method of Markwell (13) using bovine serum albumin as the standard. Plasma apoB concentrations were determined by immunonephelometry (Behring, Somerville, NJ).

Separation of plasma lipoproteins by FPLC

Fresh plasma from the four affected D-kindred members (1.5 ml) was applied to two 25-ml Superose 6 columns connected in series in a fast protein liquid chromatography (FPLC) system (Pharmacia Biotech, Uppsala Sweden) as previously described (14). The columns were equilibrated and eluted at ambient temperature with a buffer consisting of 1 mM EDTA and 0.15 M NaCl, pH 8.0. The column eluent fractions (0.5 ml/min) were analyzed for TC as described above.

Density gradient ultracentrifugation of plasma (DGUC)

DGUC was performed as described (15) with some modifications. Each of the plasma samples from the four affected D-kindred members was adjusted to a density of 1.040 g/ml with solid potassium bromide (KBr). The density gradient was prepared in 40 ml QuickSeal tubes (Beckman Instruments, Fullerton, CA) as follows. Six ml of solution A (0.195 M NaCl; 1 mM EDTA-NA_2 d 1.006 g/ml) was loaded into the tube using a syringe equipped with a 3.5-in long 18 gauge needle with the end bevel removed. This solution was carefully underlayered first with 10 ml of solution A adjusted to d 1.020 g/ml with KBr, followed by 14 ml of plasma (d 1.040

TABLE 1. Clinical characteristics of four affected and one unaffected member of the D-kindred

Subject	Gender	Age	BMI	CHOL	LDL	HDL	TG	ApoB	ApoAI	Lp[a]	Apo[a][a]	ApoE
		yr	kg/m^2				mg/dl					
Affected members												
III 10	F	38	21.6	77	24	46	47	32	161	1.1	16/11	E2/E3
III 18	M	31	27.4	81	31	38	87	36	134	1.1	14/11	E2/E3
III 20	M	22	28.7	72	28	37	35	34	139	n/a	n/a	E3/E3
IV 4	M	16	25.1	83	43	35	27	36	116	3.6	16/6	E2/E3
Unaffected member												
III 14	F	34	23.7	151	81	52	121	69	200	1.2	16/8	E2/E3

BMI, body mass index; CHOL, cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; TG, triglycerides; ApoB, apolipoprotein B; ApoA-I, apolipoprotein A-I; Lp[a], lipoprotein [a]; Apo[a], apolipoprotein [a]; ApoE, apolipoprotein genotype.

[a] Phenotype Isoform.

g/ml with KBr) and finally, by 10 ml of solution A adjusted to d 1.210 g/ml with KBr. A blank tube gradient containing 14 ml of solution A adjusted to d 1.040 g/ml instead of serum was used as the control density gradient. The tubes were centrifuged in a Beckman 50.2 Ti rotor for 24 h at 45,000 rpm at 12°C. The rotor was stopped without the use of brake and the gradient was pumped off from the top by pumping solution A, adjusted to 1.31 g/ml, into the bottom of the tube with a peristaltic pump. Fifty fractions of 0.5 ml were collected in a fraction collector (Gilson, Middleton, WI). Fifty fractions were also collected from the blank gradient tube. Consecutive pairs of blank gradient fractions were pooled and their densities were measured using a DMA 35 densitometer (PAAR, Graz, Austria). Each of the fractions were analyzed for TC, FC, calculated CE, PL, TG, and PRO.

Metabolic study

Detailed procedures on our metabolic protocols have been published (10). Briefly, each subject was requested not to change his/her diet 10 days prior to the study. Before the study began, each subject fasted for 10 h to clear their plasma of any chylomicrons or remnants. Then a primer bolus of [^{13}C]leucine (MSD Isotopes, Montreal, Canada, isotopic purity 99%) was given at 0.85 mg · kg^{-1} and immediately followed by an 8-h constant infusion of the tracer at 0.85 mg · kg · h^{-1}. The study was designed to last 5 days in length. During the study period, a total of 32 blood samples were drawn. Of these, 21 were used to assay apoB-leucine enrichment and the other 11 samples were used to describe the plasma leucine enrichment that served as the mathematical forcing function used in the kinetic analysis. Blood samples were taken at the following time periods (min): −30 (prior to the infusion), 0 (beginning of the infusion), then every 30 min for the next 8 h (480 min). Samples were then taken at 495, 510, 525, 540, 570, 600, 630, 660, 720, 780, 840, 960 min, and day 3 and day 5 after the onset of the experiment. Non-caloric drinks that contained no caffeine were permitted throughout the study period. Blood was collected into EDTA-containing tubes and plasma was separated by low-speed centrifugation.

Isolation of apoB and plasma amino acids

Plasma amino acids were isolated from 0.3 ml plasma by cation exchange chromatography (16). ApoB-100 was isolated by precipitation (17), and hydrolyzed in 6 N HCl for 24 h at 100°C. The resulting amino acids were derivatized to N-heptafluorobutyryl-n-propanol esters and enrichments were determined by positive or negative chemical ionization GC–MS by comparison to appropriate isotopic enrichment standards (18, 19) and expressed as tracer/tracee ratios (20).

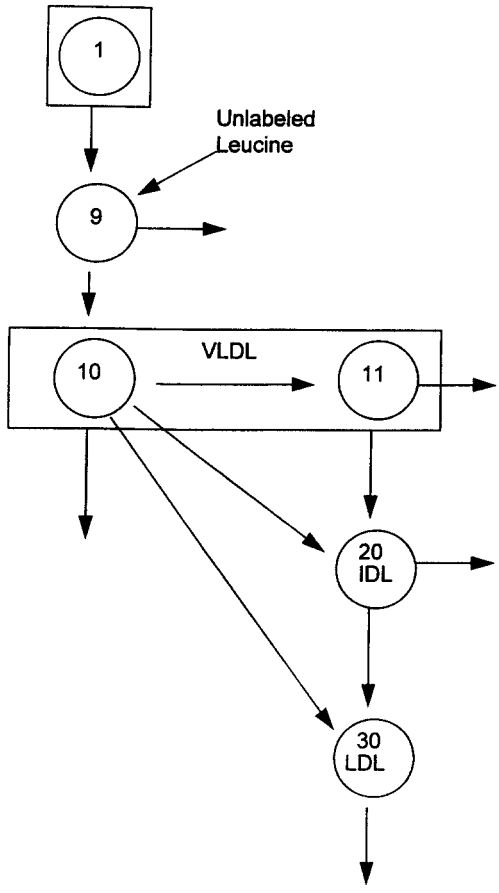

Fig. 1. Multicompartmental model for apoB-100. Compartment 1: plasma leucine tracer/tracee ratio; compartment 9 represents the input of unlabeled leucine for the assembly of apoB-100-containing lipoproteins. Compartments 10 and 11: very low density lipoprotein (VLDL)-apoB-100 fast and VLDL-apoB-100 slow, respectively. Compartment 20: intermediate density lipoprotein (IDL)-apoB-100; compartment 30: low density lipoprotein (LDL)-apoB-100 particles.

Kinetic analysis

A compartmental model was used to analyze the kinetics of apoB-100 using the SAAM II program (SAAM Institute, University of Washington, Seattle). This model (**Fig. 1**) has been used previously to describe apoB kinetics in VLDL, IDL, and LDL for both apoB-100 and various truncated forms of apoB (5–8, 10, 21). The model represents the minimal structural complexity that was both necessary and sufficient to account for the observed isotopic enrichment data and apoB-100 relative mass distribution among lipoprotein subfractions. Compartment numbering is arbitrary and follows the convention previously used. Although many models of greater complexity may be found in the literature which depict apoB-100 kinetics in greater detail, there is insufficient information in the data collected from a primed continuous infusion protocol to resolve any structural complexity beyond that which is shown.

The model includes a plasma compartment (com-

partment 1), described by a mathematical forcing function to describe plasma leucine enrichment during and after the termination of the 8-h primed continuous tracer infusion period. A rapidly turning-over intervening compartment, presumably intracellular, accounted for isotopic dilution of plasma tracer amino acid and a slight time delay (compartment 9). VLDL apoB-100 comprises two compartments to represent a minimal delipidation chain; rapidly turning-over VLDL apoB-100 (compartment 10) can leave the plasma or be converted to slowly turning over VLDL (compartment 11). IDL apoB-100 (compartment 20) arises from both fast and slow VLDL. LDL apoB-100 (compartment 30) is derived from the IDL fraction or directly from fast VLDL. The FCR of a given compartment is the sum of individual rate constants leaving that compartment; the FCR of VLDL apoB-100 is the weighted average (related to mass distribution) of the FCR of compartments 10 and 11. It is assumed that all apoB-100 enters plasma as fast VLDL; apoB-100 production rate was therefore determined as the size of compartment 10 [(total VLDL apoB-100 pool size) × compartment 10)] × the FCR of compartment 10. Plasma volume (dl) was assumed to be 0.45 × body weight (kg). The rate constants of the model were adjusted to minimize the sums of squares of predicted versus observed enrichment values for VLDL, IDL, and LDL.

Statistical analyses

Kinetic parameters for control, D-kindred, and truncated subjects were analyzed using analysis of variance. The Hartley F Max test was used to determine the differences in variation observed between data sets [i.e., present experiment vs. those previously reported (5, 6, 10)] for each variable. When significant differences were found, means were partitioned by Fisher's protected least significant difference. All data were analyzed using the General Linear Models Procedure of SAS® (22). Statements of significance were based on $P < 0.05$ unless otherwise noted.

RESULTS

For ease of presentation, the D-kindred will refer only to the four affected members. Analysis of parameters by the Hartley F Max test suggest there were no differences in the amount of variation observed between data sets and that pooling the numbers would be statistically valid. Additionally, as the one unaffected member in the D-kindred observed did not differ from controls with respect to body mass index (BMI), sex, age, or kinetic parameters (**Table 2**), she was included in the control group for comparison purposes. The mean BMIs for the normolipidemic controls, D-kindred members, and subjects with various truncations were statistically similar (Table 2). By definition, plasma TC, TG, apoB, and LDL concentrations were higher in controls when compared to either of the other low cholesterol groups (Table 2). No significant differences existed in mean plasma HDL concentrations among the three groups. The D-kindred and subjects with apoB truncations had similar mean total TG, apoB, and LDL levels, but mean TC levels were significantly lower in the D-kindred compared to subjects with apoB truncations (Table 2).

Although lipoprotein levels differed markedly between normals and members of the D-kindred, no differences were noted in the elution positions of their apoB-100-containing lipoprotein fractions on FPLC (**Fig. 2A** for D-kindred; Fig. 2B for normal). Flotation positions after DGUC for VLDL, IDL, and LDL also were similar between the D-kindred and normal controls (Fig. 2C for D-kindred; Fig. 2D for normal), which means the lipoproteins of the D-kindred had similar size and density distributions. We have previously reported that the apoB-100-containing lipoproteins of apoB truncation heterozygotes also have normal size and density distributions (8, 23). Thereby, indicating that isolated lipoproteins from the D-kindred by preparative, sequential ultracentrifugation techniques at the usual densities for compositional and metabolic studies would be valid. Within each of the fractions isolated (VLDL, IDL, LDL, and HDL) from the three groups there were no differences evident in their major constituents (**Table 3**).

During the kinetic study, apoB concentrations remained constant (coefficients of variation ~5%) suggesting that each subject remained in a metabolic steady state during the course of the study. The kinetic curves of the four affected members of the D-kindred are shown in **Fig. 3** and mean calculated kinetic parameters are shown in **Table 4**. The VLDL-, IDL-, and LDL-apoB-100 PRs were similar in the D-kindred and controls. The PR's in subjects with apoB truncations were significantly lower than Pr's of controls or the D-kindred. The mean FCRs in the D-kindred for VLDL- and LDL-apoB-100 were ~2 and 3 times higher, respectively, than the other two groups. The IDL apoB-100 FCRs were similar for the three groups. Mean LDL-apoB-100/VLDL-apoB-100 PR ratios were similar for the D-kindred and normals, whereas the mean PR ratio for the truncation heterozygote was lower.

DISCUSSION

The major finding of this study was that VLDL- and LDL-apoB-100-containing particles of affected mem-

TABLE 2. Mean plasma lipoprotein lipids and apoA-I and B levels of controls, affected D-kindred members, and heterozygous subjects for apoB truncations

Subject	n	BMI	Cholesterol	Triglycerides	Plasma ApoB	LDL	HDL
					mg/dl		
		kg/m^2					
Control[d]	14	23.8 ± .2	160.7 ± 4.8[a]	83.2 ± 6.7[a]	89.9 ± 3.5[a]	106.5 ± 5.2[a]	46.4 ± 2.7
D–kindred	4	25.7 ± .2	78.3 ± 9.1[c]	49.0 ± 12.8[b]	34.5 ± 5.4[b]	34.0 ± 7.9[b]	39.0 ± 4.1
Truncated[c]	8	25.2 ± .2	102.1 ± 6.4[b]	42.0 ± 9.1[b]	24.6 ± 3.8[b]	41.9 ± 5.6[b]	49.6 ± 2.9
P value		0.44	<0.0001	<0.0031	<0.0001	<0.0001	<0.0627

LDL, low density lipoprotein; HDL, high density lipoprotein; apoB, apolipoprotein B.
[a–c]Mean ± SE within a column with no common superscript are significantly different.
[d]Includes one unaffected member from the D-kindred plus subjects.
[c]Previously observed in this laboratory (5–8).

bers in the D-kindred had 2- to 3-fold increases, respectively, in their FCRs, while the PRs of VLDL- and LDL-apoB-100 particles were similar to those of controls. The in vivo metabolism of lipoproteins is distinct in terms of FCR's and site of clearance in different kindreds with FHBL. For instance, some truncations such as apoB-89, -87, and -75, which contain the LDL receptor recognition domain are cleared more rapidly as they interact with enhanced affinity with the receptor (7–9). ApoB-89, and -75, like apoB-100, are cleared mostly by the liver, whereas the shorter truncations such as apoBs-43.7, -38.9, and -31, which do not contain the LDL re-

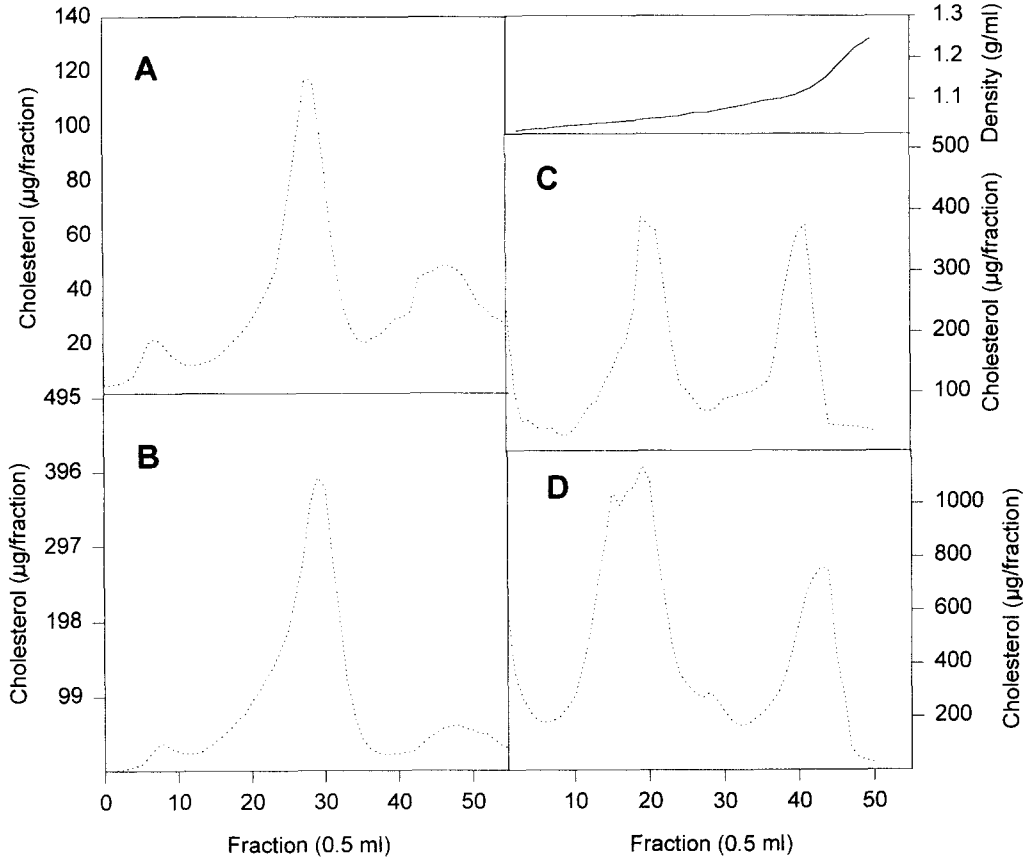

Fig. 2. Gel permeation chromatographic and density gradient profiles of plasma pools of controls and pools of affected members from the D-kindred (see Table 2). The dotted lines show cholesterol (µg/fraction). Panels A and B represent the gel permeation profile for controls and affect D-kindred members, respectively. Panels C and D represent the density gradient profile for controls and affected D-kindred members, respectively. On FPLC, peaks of VLDL, LDL, and HDL are in fractions 7, 29, and 47. On DGUC, respective fractions are 1, 19, and 40.

TABLE 3. Percent compositions of very low density (VLDL), intermediate density (IDL), low density (LDL), and high density lipoproteins (HDL) in controls, members of the D-kindred, and heterozygous subjects with apoB truncations

Subject	n	Protein	Triglycerides	Phospholipids	Free Cholesterol	Cholesterol Esters
				% of particle		
Control[a]	14					
VLDL		10.6	55.4	17.5	5.3	11.2
IDL		18.5	35.4	18.7	7.3	20.4
LDL		24.9	5.8	21.1	8.6	39.6
HDL		48.1	3.0	30.0	3.4	18.5
D-kindred	4					
VLDL		11.5	53.4	16.0	6.6	12.6
IDL		16.9	29.6	22.3	9.0	22.1
LDL		25.6	4.8	22.8	9.2	37.6
HDL		48.2	2.6	27.3	4.1	17.8
Truncations[b]	8					
VLDL		10.7	54.2	17.4	5.5	12.0
IDL		17.4	29.2	20.8	8.1	24.5
LDL		24.5	4.4	21.1	9.4	40.6
HDL		47.8	3.1	27.8	3.0	18.3

Among the different kindreds, no significant differences were noted between the measured parameters within a fraction type.

[a]Includes one unaffected member from the D-kindred plus subjects.

[b]Previously observed in this laboratory (5–8).

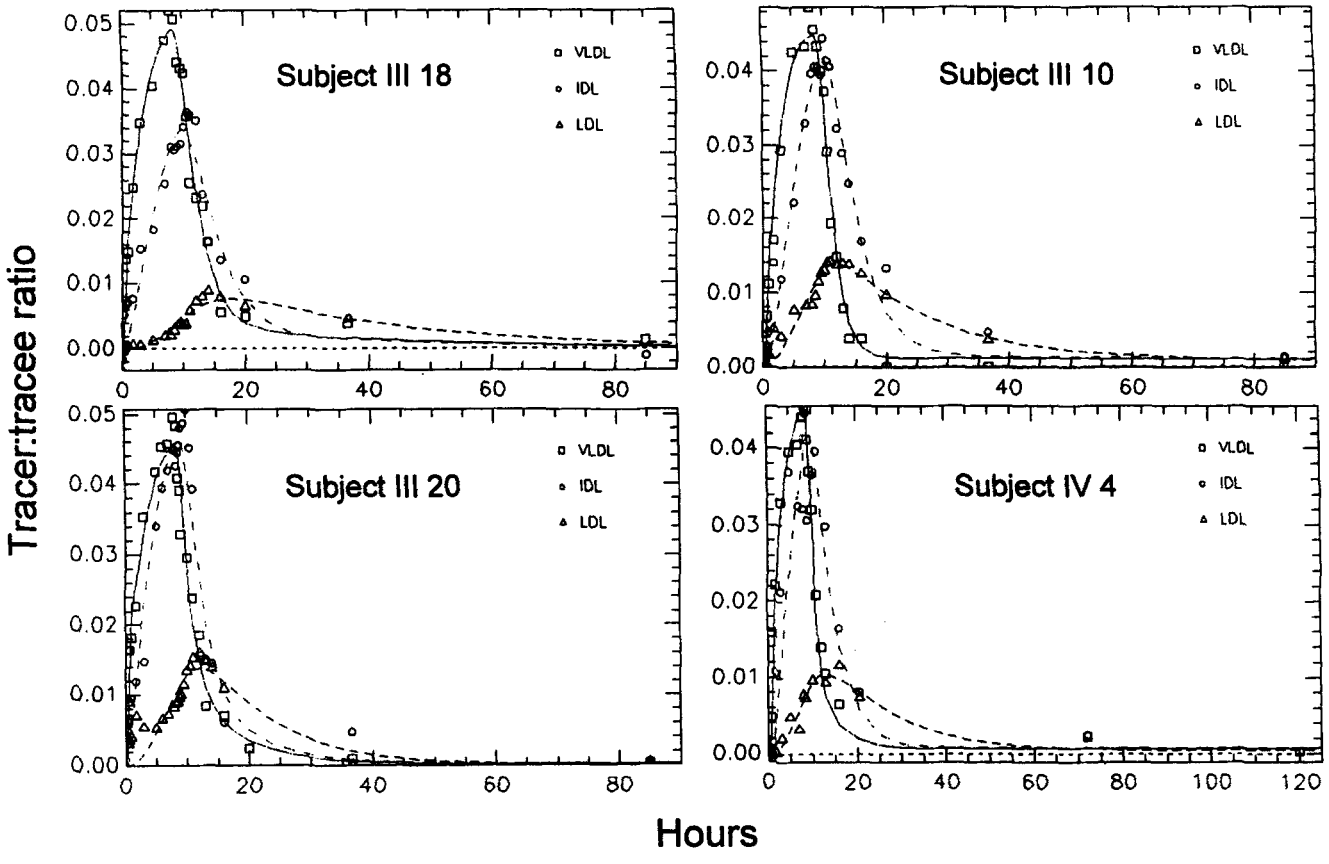

Fig. 3. Tracer/tracee ratios for the incorporation [^{13}C]leucine into VLDL-apoB-100, IDL-apoB-100, and LDL-apoB-100 in the four affected members of the D-kindred. Panels are labeled according to the subject identities in Table 1.

TABLE 4. Kinetic parameters of apoB-100-containing lipoproteins in controls, affected members of the D-kindred, and heterozygous subjects with various apoB truncations

Subject	n	Production Rates			Fractional Catabolic Rates		
		VLDL	IDL	LDL	VLDL	IDL	LDL
		mg/kg/day			*pools/day*		
Control[c]	14	18.3 ± 1.57[a]	10.1 ± 1.48[a]	13.4 ± 1.14[a]	8.8 ± 1.02[b]	8.9 ± 1.02	0.50 ± 0.10[b]
D-kindred	4	19.1 ± 2.94[a]	15.7 ± 2.78[a]	14.1 ± 2.13[a]	15.4 ± 1.90[a]	8.8 ± 1.91	1.78 ± 0.19[a]
Truncated[d]	8	7.7 ± 2.08[b]	3.2 ± 1.96[b]	4.5 ± 1.74[b]	9.6 ± 1.35[b]	5.5 ± 1.35	0.58 ± 0.16[b]
P values		0.0012	0.0031	0.0009	0.0180	0.1421	0.0001

[a–b]Means (± standard error) within a column with no common superscript are significantly different.
VLDL, very low density lipoprotein; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; apoB, apolipoprotein B.
[c]Includes one unaffected member from the D-kindred plus subjects.
[d]Previously observed in this laboratory (5–8).

ceptor domain (24), are cleared more rapidly by an unknown mechanism in the kidney (25). The enhanced LDL clearance exhibited in the D-kindred resembles the work reported in an FHBL kindred by Malmendier et al. (26). The organ loci of clearance have not been studied in either of these kindreds (i.e., in Ref. 26 or D-kindred). The FCR of ^{125}I labeled LDL-apoB in the Malmendier study showed a 215% in clearance when compared to normal subjects; data compatible with ours. Additionally, the mean FCR via non-LDL receptor pathways was increased by 60%. Unlike the D-kindred, the previous authors (26) did not observe any differences in the FCR of VLDL.

What is the physiological basis underlying the FHBL phenotype in the D-kindred? ApoB-100 enters the plasma exclusively in association with VLDL (direct secretion of IDL or LDL did not have to be invoked to fit the kinetic model in any of the three groups), and LDL-apoB-100 is derived from VLDL-apoB-100 (either directly or, to a lesser extent, indirectly from IDL-apoB-100), the implication is that the low levels of apoB-100 in plasma are, at least in part, due to low rates of production in some heterozygotes (5), but this was not the case in affected members of the D-kindred, where enhanced clearance seems to be the predominant mechanism. One possibility for the increased FCR's exhibited in the D-kindred may be due to a mutation in the apoB gene itself, such that apoB-100-containing lipoproteins interact with increased affinity with one or more types of lipoprotein receptors. This would be a gain-of-function mutation and opposite of that existing in the loss-of-function mutation present in familial defective apoB-100 (27). Another possibility is that lipoprotein receptors are up-regulated in the affected D-kindred members. Recently, Parhofer et al. (28) demonstrated that patients with familial hypercholesterolemia given apheresis as a treatment to lower cholesterol and apoB concentrations exhibited a 2-fold increase in their LDL-apoB FCR's, thereby suggesting that a reduction in pool size for LDL-apoBs may provoke higher receptor numbers. If this is the case, one wonders whether it is merely sufficient to invoke the presence of low plasma cholesterol levels as a cause, because low plasma cholesterol levels do not appear to raise FCRs for VLDL- or LDL-apoB-100 particles in apoB truncation heterozygotes (5). On the other hand, it is conceivable that a 24 mg/dl difference in total cholesterol (78 vs. 102 mg/dl) could affect the expression of some lipoprotein receptors. Thus, it is clear that the metabolic bases for the hypobetalipoproteinemia differ in these various genetic forms of FHBL.

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