Increased clearance of low density lipoprotein precursors in patients with heterozygous familial defective apolipoprotein B-100: a stable isotope approach

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Abstract In familial defective apolipoprotein B-100 (FDB) the presence of a mutant apolipoprotein (apo) B-100 ($FDB_{3500Q/W}$) in LDL markedly reduces their affinity for the LDL receptor, leading to elevated LDL cholesterol levels. However, the hypercholesterolemia in most FDB patients is relatively mild when compared with, e.g., familial hypercholesterolemia (FH). In order to study mechanisms that may partly alleviate the clinical consequences of FDB, we investigated the in vivo kinetics of apoB-100-containing lipoproteins in five FDB heterozygotes (total cholesterol: 7.84 ± 1.37 mmol/l; total apoB: 1.68 ± 0.37 g/l; mean ± SD) and six normolipidemic controls (4.61 ± 0.62 mmol/l; 0.98 ± 0.12 g/l) using a stable isotope approach. During and after a 10–12 h primed, constant infusion of either [$^{13}C_6$]phenylalanine or [$^{2}H_3$]leucine, tracer enrichment was determined in apoB-100 from ultracentrifugally isolated $VLDL_1$ (S_f 60–400), $VLDL_2$ (S_f 20–60), IDL (S_f 12–20), LDL_1 (S_f 7–12), and LDL_2 (S_f 0–7). The rates of apoB-100 production, catabolism, and transfer were estimated by model-based compartmental analysis. The overall fractional catabolic rate (FCR) of IDL apoB-100 in FDB was substantially increased (2.99 ± 0.68 pools/day vs. 1.70 ± 0.23 pools/day in controls, $P < 0.01$). The fractional rate of apoB-100 transfer from IDL to LDL in FDB was decreased (0.97 ± 0.13 pools/day vs. 1.24 ± 0.10 pools/day, $P < 0.05$). The FCR of LDL apoB-100 in FDB was decreased (0.18 ± 0.07 pools/day vs. 0.56 ± 0.05 pools/, $P < 0.01$). Finally, the input rate of LDL apoB-100 in FDB was markedly decreased (9.45 ± 2.96 mg/kg day^{-1} vs. 15.54 ± 1.70 mg/kg day^{-1}, $P < 0.05$). Our data suggest that the relatively small increase of LDL concentrations in FDB is due to an increased clearance of LDL precursor particles via the LDL-receptor and apoE-receptors as well as a decreased conversion of IDL to LDL - two mechanisms that distinguish FDB from FH.—**Pietzsch, J., B. Wiedemann, U. Julius, S. Nitzsche, S. Gehrisch, S. Bergmann, W. Leonhardt, W. Jaross, and M. Hanefeld.** Increased clearance of low density lipoprotein precursors in patients with heterozygous familial defective apolipoprotein B-100: a stable isotope approach. *J. Lipid Res.* 1996. **37:** 2074–2087.

Supplementary key words apoB-100 kinetics • stable isotopes • SAAM • mathematical compartmental modeling • $VLDL_1$ • $VLDL_2$ • IDL • LDL_1 • LDL_2

ApoB-100 is a major protein constituent of VLDL, IDL, and LDL (1). The interaction of apoB-100 with the LDL receptors enables the uptake of LDL cholesterol from blood by the liver and most other tissues. Mutations in the LDL receptor gene have been reported in familial hypercholesterolemia (FH), leading to highly elevated LDL cholesterol levels. In patients with moderately increased LDL cholesterol concentrations, the first indication that a mutation in the apoB gene can possibly lead to defective receptor-mediated removal of LDL was the in vivo assessment of markedly decreased fractional catabolic rate (FCR) of autologous LDL when compared with homologous LDL (2). A specific structural defect of the mature apoB-100 was then clearly identified in these patients (3–5). This metabolic disorder was designated familial defective apolipoprotein B-100 (FDB). FDB is an autosomal, dominantly inherited disorder caused by a rare single guanine → adenine transition at nucleotide 10580 of the human apoB cDNA, leading to an amino acid change (arginine → glutamine) at residue 3500 of the mature apoB-100 (FDB_{3500Q}) that disrupts

Abbreviations: VLDL, very low density lipoproteins; IDL, intermediate density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; apoB-100, apolipoprotein B-100; TC, total cholesterol; TG, triacylglycerols; FCR, fractional catabolic rate; PR, production rate; FSD, fractional standard deviation; SAAM, simulation analysis and modeling; CAD, coronary artery disease; FDB, familial defective apoB-100; FH, familial hypercholesterolemia.

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the binding of LDL to the LDL receptor (5, 6). More recently, Gaffney et al. (7) could also identify an arginine → tryptophan amino acid change at codon 3500 (FDB_{3500W}). The binding affinity of LDL with mutant apoB-100 is reduced by at least 95% (8). In FDB heterozygotes, this leads to an accumulation of the 'defective' LDL particles in the plasma. Under steady state conditions, about 75% of the LDL particles circulating in the blood of an FDB heterozygote are mutant and about 25% are normal (8). The LDL accumulation should have consequences with respect to the metabolic fate of the LDL precursors. Studies in patients homozygous for FDB have shown that there is residual affinity of 'defective' LDL, especially of less dense LDL (hydrated density 1.019–1.034 kg/l) to the LDL receptor, and normal or increased affinity of VLDL remnants to the LDL receptor, due to the presence of one or more apoE molecules in these particles (9, 10). However, a direct assessment of the in vivo metabolism of apoB-100 in FDB heterozygotes has not been carried out previously. The present work aimed at studying the behavior of the VLDL remnants and IDL in FDB heterozygotes. We assessed the in vivo kinetics of the apoB-100-containing lipoproteins using endogenous labeling of apoB-100 with L-[ring-$^{13}C_6$]phenylalanine and L-[5,5,5-$^{2}H_3$]leucine, respectively. The metabolic parameters for apoB-100 were subsequently estimated by model-based compartmental analysis using the SAAM 31 program.

METHODS

Subjects

Five heterozygous FDB patients attending our Lipid Clinic participated in this study. Their clinical and biochemical characteristics are given in Table 1 and Table 2. None of these patients had tendon xanthomas and they had no clinical cardiovascular diseases. Six normolipidemic volunteers (TC ≤ 5.25 mmol/l and plasma TG ≤ 1.7 mmol/l) served as controls. All subjects were free of renal, hepatic, hematologic, cardiovascular, and thyroid abnormalities, and all medications known to affect lipid levels were discontinued for at least 6 weeks before the study. All subjects were normoglycemic. None of the subjects was a smoker.

DNA analysis

For routine screening of FDB the method described by Motti et al. (11) was used. To verify the mutation we used the procedure published by Geisel et al. (12) and, in addition, we sequenced the region around residue 3500 (13). ApoE genotypes were ascertained as published by Hixson and Vernier (14).

Experimental protocol

We followed a study protocol as previously described with some modifications (15). In brief, all subjects received an isocaloric moderate fat diet (37% fats, 47% carbohydrate, 16% protein, and 200 mg of cholesterol/1000 kcal; four meals per day) for 3 days. They were then admitted to the metabolic ward for 2 days. Patients and controls fasted overnight and during the turnover procedure. All subjects gave informed written consent, and approval of the study was granted by the local Ethics Committee. During the tracer infusion, probands were studied in a recumbent position and were allowed to ingest water alone. The proteinogenic tracer amino acids L-[ring-$^{13}C_6$]phenylalanine (isotopic purity 99%) and L-[5,5,5-$^{2}H_3$]leucine (isotopic purity 98%; Cambridge Isotope Laboratories, Woburn, MA) were dissolved in 0.9% NaCl. The solutions were demonstrated to be sterile and free from pyrogens. An intravenous catheter was inserted into a superficial vein of each arm, one for tracer administration and the other for blood sampling. Patients #1, #3, and controls #1, #3, #4 received [$^{13}C_6$]phenylalanine as tracer amino acid, while patients #2, #4, #5, and controls #2, #5, #6 received [$^{2}H_3$]leucine as tracer amino acid. After a priming bolus of 550 μg • kg^{-1} [$^{13}C_6$]phenylalanine or 655 μg • kg^{-1} [$^{2}H_3$]leucine, a constant infusion of 12 μg • kg^{-1} • min^{-1} or 16 μg • kg^{-1} • min^{-1}, respectively, was continued for 10–12 h. Blood samples were obtained before the priming bolus, then at 10-min intervals for 2 h, and then after 2, 2.5, 3, 3.5, 4, 5, 6, 9, 10, 11, and 12 h. After the infusion was stopped, the patients remained fasting for 2 h and then resumed their usual diets. Blood samples were drawn for another 3 days (24, 48, and 72 h after the start of the tracer infusion). Blood was collected into vacutainer tubes containing EDTA at a final concentration of 0.1%. Plasma was recovered after centrifugation at 4°C. Sodium azide (0.01%) and aprotinin (200 KIU ml^{-1}) were added immediately. Samples were stored at 4°C. Plasma aliquots were used to determine plasma enrichments of free phenylalanine, tyrosine, leucine, and α-ketoisocaproic acid (KIC) and to measure TC, TG, and apolipoproteins B, E, and A-I in order to ascertain a metabolic steady state.

Isolation of apoB-100-containing lipoproteins by ultracentrifugation

ApoB-100-containing lipoproteins, i.e., large and small VLDL ($VLDL_1$, S_f (Svedberg units) 60–400 and $VLDL_2$, S_f 20–60), IDL (S_f 12–20), and buoyant and small-dense LDL (LDL_1, S_f 7–12 and LDL_2, S_f 0–7) were

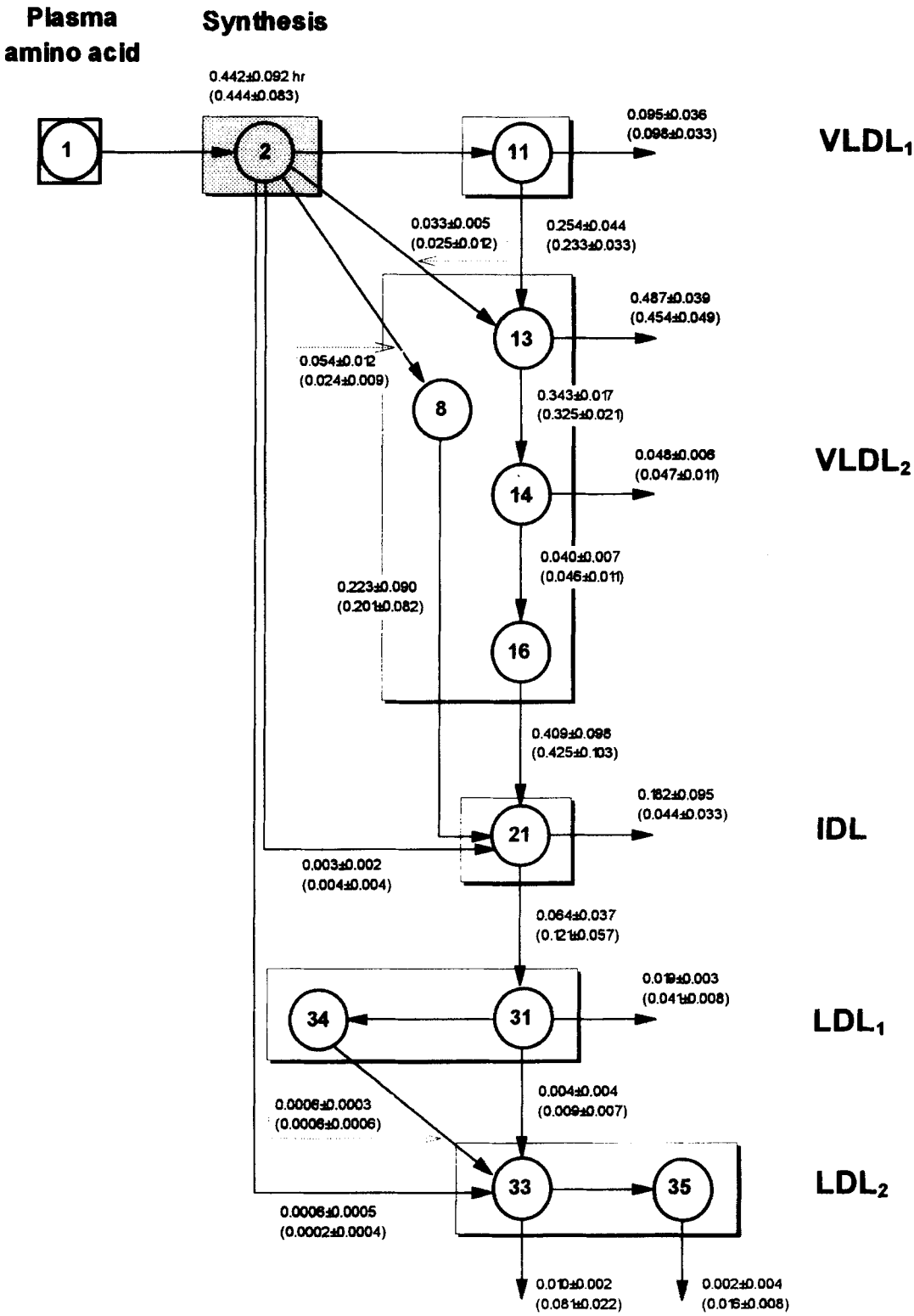

Fig. 1. Multicompartmental model for apoB-100 metabolism. Compartment 1: plasma phenylalanine or leucine (forcing function). Compartment 2: delay compartment (hepatic synthesis and secretion of apoB-100-containing lipoproteins). Compartment 11: $VLDL_1$. Compartments 8, 13, 14, 16: $VLDL_2$. Compartment 21: IDL. Compartments 31, 34: LDL_1. Compartments 33, 35: LDL_2. Numbers on arrows are the mean fractional rate constants ± SD in pools/h for FDB heterozygotes and normolipidemic controls (in parentheses). The numbers next to compartment 2 represent the time in hours necessary for the synthesis and secretion of apoB-100.

TABLE 1. Baseline characteristics of patients and controls

Subject Number	Age	Sex	Body Mass Index	ApoE Genotype	TG	TC	ApoB	ApoA-I	ApoE
	yr		*kg/m²*		*mmol/l*	*mmol/l*	*g/l*	*g/l*	*g/l*
FDB									
#1	51	M	26.5	3/3	0.81	7.08	1.52	1.40	0.054
#2	45	M	26.7	3/3	1.42	6.81	1.34	0.92	0.035
#3	27	M	23.8	2/3	0.86	7.03	1.40	1.52	0.066
#4	54	F	24.6	3/3	1.66	10.1	2.21	1.71	0.115
#5	63	F	24.5	3/3	1.55	8.16	1.92	1.25	0.045
Mean	48[a]	3/2	25.1	-	1.26	7.84[b]	1.68[b]	1.36	0.063
±SD	13		1.2		0.40	1.37	0.37	0.30	0.031
Normolipidemic controls									
#1	26	M	24.7	3/3	0.70	3.55	0.81	1.20	0.041
#2	26	M	24.5	3/3	0.97	4.23	0.88	1.73	0.052
#3	45	M	25.0	3/3	1.46	5.04	1.12	1.23	0.061
#4	30	F	21.0	3/3	0.81	4.78	1.05	1.46	0.078
#5	40	F	20.5	3/4	0.81	5.25	1.04	1.53	0.077
#6	20	F	21.0	3/4	0.97	4.82	1.00	1.43	0.061
Mean	31	3/3	22.8	-	0.95	4.61	0.98	1.43	0.062
±SD	9		2.2		0.27	0.62	0.12	0.20	0.014

The FDB patients #3 and #4 were first degree relatives. All lipid and protein values were determined at the time of the study where all medications known to affect lipid levels were discontinued for at least 6 weeks.

[a]$P < 0.05$; [b]$P < 0.01$ (Mann-Whitney test) for the difference between patients and normolipidemic controls.

isolated from plasma by a combination of both sequential and cumulative gradient ultracentrifugation techniques (16, 17).

All lipoprotein fractions were dialyzed against 10 mM Tris-HCl, pH 7.4, containing 1 mM Na_2-EDTA, 0.9% NaCl, 0.01% NaN_3, and 0.5 mM phenylmethylsulfonyl fluoride (PMSF) for 24 h and stored at 4°C.

Lipid and protein determinations

TC and TG in plasma and lipoprotein fractions were measured enzymatically as previously described (18, 19). Lipoprotein[a] was quantified by EIA using a commercially available kit from Immuno Diagnostics (Vienna, Austria). Notably, the Lp[a] concentrations of the samples we studied were not elevated and in some cases reached the detection limit of ≤ 30 mg/l. The concentrations of total protein in plasma and total and soluble protein in the lipoprotein fractions were determined by using the bicinchoninic acid protein assay from Pierce (Rockford, IL) with bovine serum albumin as protein standard. Serum apoB-100, A-I, and E were determined by immunoelectrophoresis using 'ready for use'-Hydragel-Apo® kits from Laboratoires Sebia (Issy-les-Moulineaux, France). Soluble protein in VLDL, IDL, and LDL was estimated after extraction with isopropanol (20). The content of apoB-100 was calculated as the difference between total and soluble protein.

Isolation and hydrolysis of apoB-100

ApoB-100 of all isolated lipoproteins was separated by preparative SDS-PAGE (5–15%) using a Tris-glycine buffer system (19). Immunoblotting served for definite identification of separated apolipoproteins (19). The stained apoB-100 bands were excised from polyacrylamide gels and hydrolyzed in 12 N HCL at 115°C for 24 h. The hydrochloric acid was subsequently evaporated and the samples were reconstituted in 0.5 ml of 50% acetic acid. The free amino acids were isolated from plasma or protein hydrolysates by cation exchange chromatography (21) using Dowex AG-50W-X8 (H^+, 100–200 mesh) resin obtained from Bio-Rad (Richmond, CA).

GC–MS analysis

The purified amino acids were converted to their N(O)-heptafluorobutyryl isobutyl ester derivatives (22) and analyzed by gas chromatography/methane negative

TABLE 2. Lipoprotein composition of patients and controls

Subject Number	$VLDL_1$-TC	$VLDL_1$-TG	$VLDL_2$-TC	$VLDL_2$-TG	IDL-TC	IDL-TG	LDL_1-TC	LDL_2-TC	HDL-TC
					mmol/l				
FDB									
#1	0.15	0.28	0.57	0.28	0.62	0.16	4.04	0.38	1.26
#2	0.25	0.62	0.54	0.35	0.61	0.08	3.11	1.18	0.98
#3	0.15	0.27	0.50	0.31	0.33	0.04	4.15	0.31	1.12
#4	0.21	0.51	0.58	0.30	1.05	0.16	6.51	0.57	1.16
#5	0.39	0.40	0.53	0.32	0.84	0.11	4.63	0.85	0.86
Mean	0.23	0.42	0.54	0.31	0.69	0.11	4.48[b]	0.66[a]	1.08
±SD	0.10	0.15	0.03	0.03	0.27	0.05	1.26	0.36	0.15
Normolipidemic controls									
#1	0.15	0.28	0.28	0.20	0.30	0.06	1.70	0.21	1.10
#2	0.11	0.25	0.25	0.20	0.37	0.07	1.94	0.24	1.30
#3	0.27	0.57	0.58	0.35	0.43	0.08	2.53	0.35	0.85
#4	0.17	0.23	0.58	0.22	0.62	0.09	2.00	0.28	1.18
#5	0.13	0.19	0.47	0.23	0.70	0.08	2.18	0.21	1.51
#6	0.18	0.34	0.41	0.22	0.30	0.10	2.51	0.35	1.00
Mean	0.17	0.31	0.43	0.24	0.45	0.08	2.14	0.27	1.16
±SD	0.06	0.14	0.14	0.06	0.17	0.01	0.33	0.07	0.23

[a]$P < 0.05$; [b]$P < 0.01$ (Mann-Whitney test) for the difference between patients and normolipidemic controls.

ion chemical ionization mass spectrometry (GC/CH_4–NICI–MS) on a model 5890 Series II gas chromatograph equipped with a 25 m × 0.20 mm × 0.33 μm HP-5 capillary column and interfaced with a model 5989A MS-Engine (Hewlett-Packard, Palo Alto, CA). For the determination of isotope ratios the selected isotope satellite peaks '[M]' (at *m/z* 397 for phenylalanine, *m/z* 609 for tyrosine, and *m/z* 363 for leucine), and '[M + 3]' (at *m/z* 366 for $[^2H_3]$leucine) or '[M + 6]' (at *m/z* 403 for $[^{13}C_6]$phenylalanine, and *m/z* 615 for $[^{13}C_6]$tyrosine) surrounding the base ion, [M-HF], were monitored (23). Plasma α-keto acids were converted to their pentafluorobenzyl ester derivatives and also analyzed by GC/CH_4–NICI–MS (24). The calculation of atom percent excess (APE) from the current isotope ratios and the conversion of enrichment values to tracer/tracee ratios was equivalent to the method of Cobelli et al. (25, 26). Because of the nonnegligible masses associated with stable isotope tracers, enrichment data were transformed to tracer/tracee ratios. Data in this format are analogous to the specific activity obtained in radiotracer experiments (26).

Compartmental analysis

The rates of production, catabolism, and transfer of apoB-100 were estimated by multicompartmental analysis using the SAAM (version 31) program package (27). The metabolic parameters are subsequently derived from the model parameters providing the best fit. Models depicting the metabolism of apoB have evolved substantially since the initial studies of Phair et al. (28). Based upon previously published and validated apoB models (29–31) a new model has been devised (**Fig. 1**) to explain the apoB-100 kinetics in normolipidemic controls and heterozygous FDB patients. The model contains a precursor compartment (compartment 1) of plasma free $[^{13}C_6]$phenylalanine or $[^2H_3]$leucine. Plasma tracer amino acid enrichment was fitted with the SAAM-QL-function representing a linear interpolation between data points (27). This function avoids the need of solution interrupts for fitting data from three phlebotomy timepoints after the infusion was stopped. We used QL as a forcing function to drive the appearance of tracer in the model. This approach decouples the kinetics of the plasma free amino acid from that of the tracer in the apoB-100 system. Mathematically, the effect of the forcing function is to substitute the value of QL for the amount of tracer in compartment 1 at the same time. Additionally, force fitting will account for tracer recycling, because the forcing function includes not only the infused tracer but also the recirculated tracer.

The current model is generally based on the following assumptions. *i)* The study subjects were in a metabolic steady state, and *ii)* There was a constant hepatic secretion rate of apoB-100 into the plasma pool. Further-

more, we assume that plasma amino acids are in a stable equilibrium with their specific hepatic aminoacyl tRNA pools. A delay (compartment 2) was used to account for the hepatic synthesis of apoB-100, lipoprotein assembly, and secretion of lipoproteins into the blood (32). The model proposes that the vast majority of the apoB-100 first enters the plasma with newly synthesized triglyceride-rich $VLDL_1$ and $VLDL_2$ via compartments 11 and 13, respectively. This pathway involves a series of delipidation steps (compartments 14 and 16) resulting in the formation of progressively less lipidated, small, triglyceride-poor $VLDL_2$ that are subsequently further delipidated to form IDL (compartment 21), and then LDL (compartments 31, 34, 33, and 35). Alternatively, VLDL particles can be removed directly from plasma. A second feature of the VLDL kinetics is an additional pathway where a smaller proportion of tracer entered and departed the $VLDL_2$ directly. These smaller VLDL particles are rapidly converted to LDL via IDL. This pathway requires a single compartment (compartment 8) to fit the sharp rise in the $VLDL_2$ tracer data satisfactorily. In all subjects studied, the major portion of apoB-100 is transferred to the IDL/LDL subsystem along these two pathways. An alternative is the direct synthesis of IDL or LDL apoB-100 (33, 34). In our protocol these pathways are poorly resolved due to the problems connected with the measurement of very early, extremely low enrichments, e.g., in the large LDL apoB-100 pools, and the relatively short duration of the experiment. In the current model these pathways are included because they provide an additional slight tracer input into the IDL and LDL pool, respectively, whose secretion cannot be completely explained by the output from VLDL compartments. IDL can be directly removed from plasma or can be converted into the LDL subsystem. In our studies, LDL were fractionated into two density subclasses of particles: buoyant LDL_1, (density range from 1.019 to 1.040 kg/l; compartments 31 and 34), and smaller, more dense LDL_2 (density range from 1.040 to 1.063 kg/l; compartments 33 and 35).

Here the enrichment curves clearly indicate that the labeling of the LDL_1 precedes that of LDL_2; hence, they are modeled as precursor and product. The LDL submodel proposed takes into account the more pronounced heterogeneity of the LDL subsystem in FDB. After fitting the model to the tracer mass data, apoB-100 fractional catabolic rates, input (production, appearance) rates, and transfer rates were determined with reasonable certainty based on the fractional standard deviations of the model parameter estimates (32, 35).

TABLE 3. Kinetic parameters of apoB-100 in $VLDL_1$, $VLDL_2$, IDL, and LDL of patients and controls estimated by multicompartmental modeling

Subject Number	Plasma volume	$VLDL_1$ Pool size	$VLDL_1$ FCR	$VLDL_1$ PR	$VLDL_2$ Pool size	$VLDL_2$ FCR	$VLDL_2$ PR	IDL Pool size	IDL FCR	IDL PR	LDL Pool size	LDL FCR	LDL PR
	l	mg	*d-1*	*mg/kg d-1*	*mg*	*d-1*	*mg/kg d-1*	*mg*	*d-1*	*mg/kg d-1*	*mg*	*d-1*	*mg/kg d-1*
FDB													
#1	3.78	311	5.48	20.29	328	7.27	28.39	665	2.22	17.57	3968	0.29	13.69
#2	3.60	273	4.80	16.38	367	8.16	37.43	601	3.84	28.85	4006	0.15	7.51
#3	3.24	421	7.41	43.33	291	6.89	27.85	379	2.71	14.27	2643	0.18	6.61
#4	3.01	373	5.79	32.23	307	5.10	23.37	705	2.61	27.46	4542	0.12	8.13
#5	2.92	391	8.86	53.92	292	6.30	28.30	342	3.56	18.73	4595	0.16	11.3
Mean	3.31	354	6.47	33.23	317[a]	6.74	29.07[b]	538	2.99[b]	21.38[b]	3951[b]	0.18[b]	9.45[a]
±SD	0.37	60	1.65	15.67	31	1.14	5.12	167	0.68	6.42	787	0.07	2.96
Normolipidemic controls													
#1	3.60	435	8.40	45.67	306	4.38	16.75	324	1.34	5.42	1908	0.53	12.64
#2	3.78	317	9.36	35.32	91	9.11	9.87	544	1.84	11.91	2154	0.62	15.90
#3	3.83	223	8.73	22.90	172	7.21	14.59	573	1.55	10.44	2776	0.51	16.65
#4	2.84	532	6.00	50.66	221	6.36	22.31	235	2.01	7.50	1702	0.63	17.02
#5	2.61	386	4.28	28.48	94	7.12	11.54	425	1.86	13.62	1604	0.52	14.38
#6	2.56	218	7.68	29.37	128	3.09	6.93	241	1.57	6.63	1692	0.56	16.62
Mean	3.20	352	7.41	35.40	169	6.21	13.67	390	1.70	9.25	1973	0.56	15.54
±SD	0.60	124	1.92	10.76	84	2.16	5.47	148	0.25	3.23	441	0.05	1.70

Pool sizes for apoB-100 in the lipoprotein fractions were calculated as the product of plasma volume (taken as 4.5% of body weight) and the plasma concentration of apoB-100 in each fraction. FCR (overall fractional catabolism) is the sum of fractional transfer to denser lipoproteins and direct catabolism out of system.

[a]$P < 0.05$; [b]$P < 0.01$ for the difference between patients and normal controls.

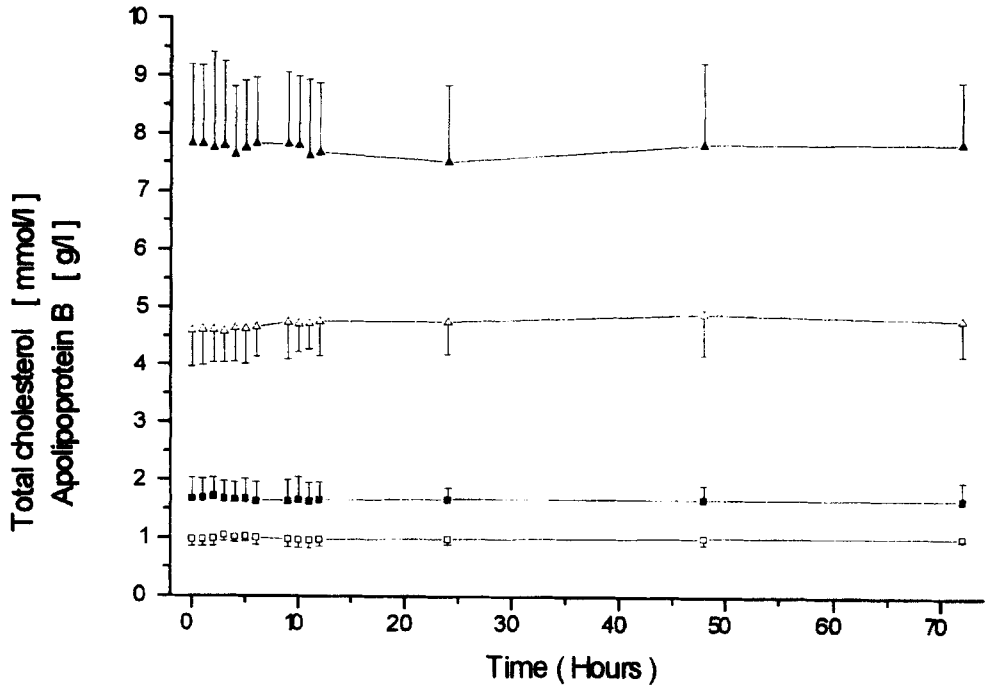

Fig. 2. Mean plasma cholesterol and apoB concentrations (±SD) in FDB heterozygotes (n = 5; cholesterol, solid triangles; apoB, solid squares) and normolipidemic controls (n = 6; cholesterol, open triangles; apoB, open squares) during the course of the turnover study.

Statistical analysis

Descriptive data were expressed as arithmetic means ± standard deviations (SD). Statistical analyses (Mann-Whitney tests) were calculated using SPSS/PC+. Fractional standard deviation (FSD) was calculated using the SAAM statistics.

RESULTS

Plasma lipids and apoB-100-containing lipoprotein concentrations

Baseline characteristics, plasma concentrations of lipids and lipoproteins for the five FDB patients and six normal subjects are presented in **Table 1** and **Table 2.** All lipid and apolipoprotein concentrations represent means of 16 separate samples taken during the period of fasting. FDB patients had significantly higher levels of plasma TC, plasma apoB-100, and all lipoprotein constituents of LDL_1 and LDL_2 whereas the concentrations of plasma TG, apoE, and apoA-I, and all lipoprotein constituents of $VLDL_1$, $VLDL_2$, IDL, and HDL, respectively, did not reach significant differences between the two groups. ApoB-100 pool sizes are presented in **Table 3.** In the FDB group the $VLDL_1$ apoB-100 pool size was not different from normal whereas the $VLDL_2$ apoB-100 pool size was significantly elevated (mean rise to 187%, $P < 0.05$). The IDL apoB-100 pool size in FDB was not different, but LDL apoB-100 mass was significantly elevated (mean rise to 200%, $P < 0.01$) in FDB. TC and apoB-100 concentrations remained constant in plasma (**Fig. 2**) and all lipoprotein fractions (data not shown in detail) during the course of the turnover study. The mean lipoprotein mass composition of $VLDL_1$, $VLDL_2$, IDL, LDL_1, LDL_2, i.e., the mass percentage of each lipid and protein constituent from total lipoprotein mass, did not differ significantly between the two groups (data not shown in detail).

ApoB-100 kinetics

Plasma steady state conditions of plasma free [$^{13}C_6$]phenylalanine and [$^2H_3$]leucine were achieved within 30 to 50 min in all study subjects. The enrichment levels of plasma free amino acid tracers remained in a steady state throughout the course of the primed, constant infusion. **Figure 3** shows the observed values and SAAM-derived fits (QL-function) to the plasma phenylalanine tracer/tracee ratio data for a patient with FDB (patient #3) and a normal control (control #3). Steady state conditions of endogenously formed [$^{13}C_6$]tyrosine and [$^2H_3$]KIC were reached within 30 to 60 min in all subjects. The latter indicates intracellular steady state enrichment conditions of phenylalanine and leucine as precursors for the protein synthesis. The mean enrichment plateaus (tracer/tracee ratio) of phenylalanine

(7.12 ± 0.67 in FDB vs. 7.34 ± 0.86 in controls), leucine (6.54 ± 0.71 vs. 6.37 ± 0.82), tyrosine (1.08 ± 0.21 vs. 1.12 ± 0.26), and KIC (4.98 ± 0.61 vs. 4.82 ± 0.77) did not differ significantly between the two groups. The shapes of these curves were independent of the amino acid injected.

Representative tracer appearance curves for apoB-100 of $VLDL_1$, $VLDL_2$, IDL, LDL_1, and LDL_2 during a 12-h period of primed constant infusion are illustrated in **Fig. 4A–4E.** The figures show the observed values and SAAM-derived fits to the $VLDL_1$-, $VLDL_2$-, IDL-, LDL_1-, and LDL_2-apoB-100 tracer/tracee ratio data using the current model for a patient with FDB (patient #3) and a normal control (control #3). The adjustable model parameters (fractional rate constants in pools/h (±FSD) and computed masses in mg) estimated by the models for these patients are presented in **Table 4.** The mean fractional rate constants (±SD) for both groups are shown in Fig. 1. The mean delay time until labeled apoB-100 was first detectable in the $VLDL_1$ and $VLDL_2$ fractions showed little variation in all studies (0.442 h ± 0.092 in FDB vs. 0.444 h ± 0.083 in controls) and was not dependent upon the amino acid tracer used. The fractional catabolic rates (overall FCR at steady state) derived by multicompartmental analysis for apoB-100 of the main lipoprotein classes are presented in Table 3. There were no differences in the total FCRs of VLDL apoB-100. The mean fractional rates of $VLDL_1$ and $VLDL_2$ apoB-100 direct catabolism, and of fractional rates of apoB-100 transfer from $VLDL_1$ to $VLDL_2$ and from $VLDL_2$ to IDL did not differ from normal (data not shown in detail). Most importantly, the mean fractional rate of IDL apoB-100 direct catabolism was drastically increased in FDB (2.02 ± 0.60 pools/day vs. 0.46 ± 0.18 pools/day in controls, $P < 0.01$) whereas the mean fractional rate of apoB-100 transfer from IDL to LDL was decreased in FDB (0.97 ± 0.13 pools/day vs. 1.24 ± 0.10 pools/day in controls, $P < 0.05$). The mean residence time (reciprocal of overall FCR at steady state) of IDL apoB-100 was 8.5 ± 1.9 hours in FDB vs. 14.4 ± 2.2 h in the control group. The overall FCR for LDL apoB-100 (LDL_1 plus LDL_2) was significantly decreased in FDB ($P < 0.01$). The mean residence time of total LDL apoB-100 was 6.1 ± 1.7 days in FDB vs. 1.8 ± 0.2 days in the control group. The mean residence times of LDL_1 and LDL_2 were 62 ± 24 h and 229 ± 68 h, respectively, in FDB vs. 29 ± 8 h ($P < 0.05$) and 52 ± 7 h ($P < 0.01$), respectively, in controls. In the FDB group the $VLDL_1$ apoB-100 production rate did not differ from normal. On the other hand, the production rate of $VLDL_2$ apoB-100 was significantly elevated ($P < 0.01$). The input rate of IDL apoB-100 was increased in all patients ($P < 0.01$), whereas the mean LDL input rates were decreased in FDB ($P < 0.01$). Thus, in the FDB group the increment above normal in $VLDL_2$ and IDL apoB-100 pools was the result of increased $VLDL_2$ production primarily from de novo synthesis, whereas the increment above normal in LDL apoB-100 pools was the result of de-

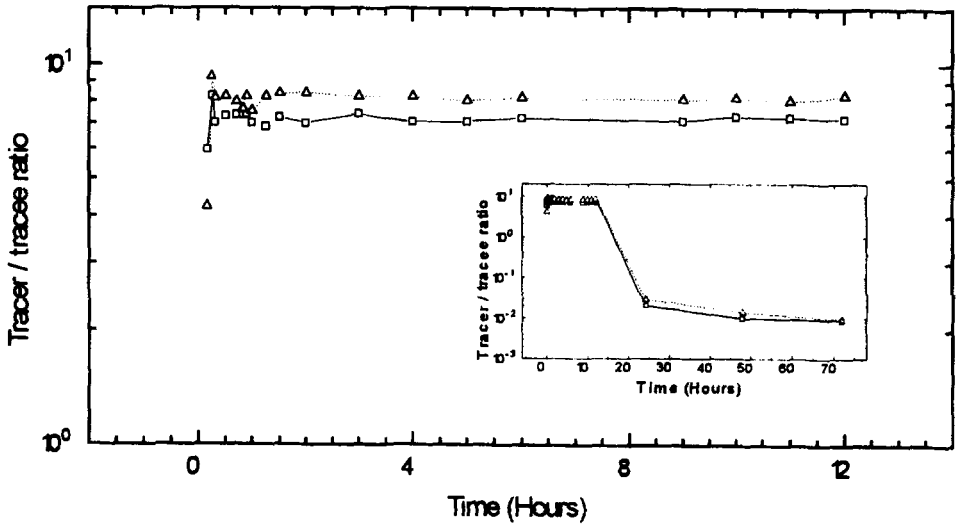

Fig. 3. Representative plasma enrichment curves for free [$^{13}C_6$]phenylalanine in FDB patient #3 (triangles, dotted lines) and control subject #3 (squares, solid lines) during the course of the primed, constant infusion (main graph) and during the whole study (insert graph). Symbols represent observed data; lines indicate linear interpolation between data points as calculated by using the SAAM-QL-function.

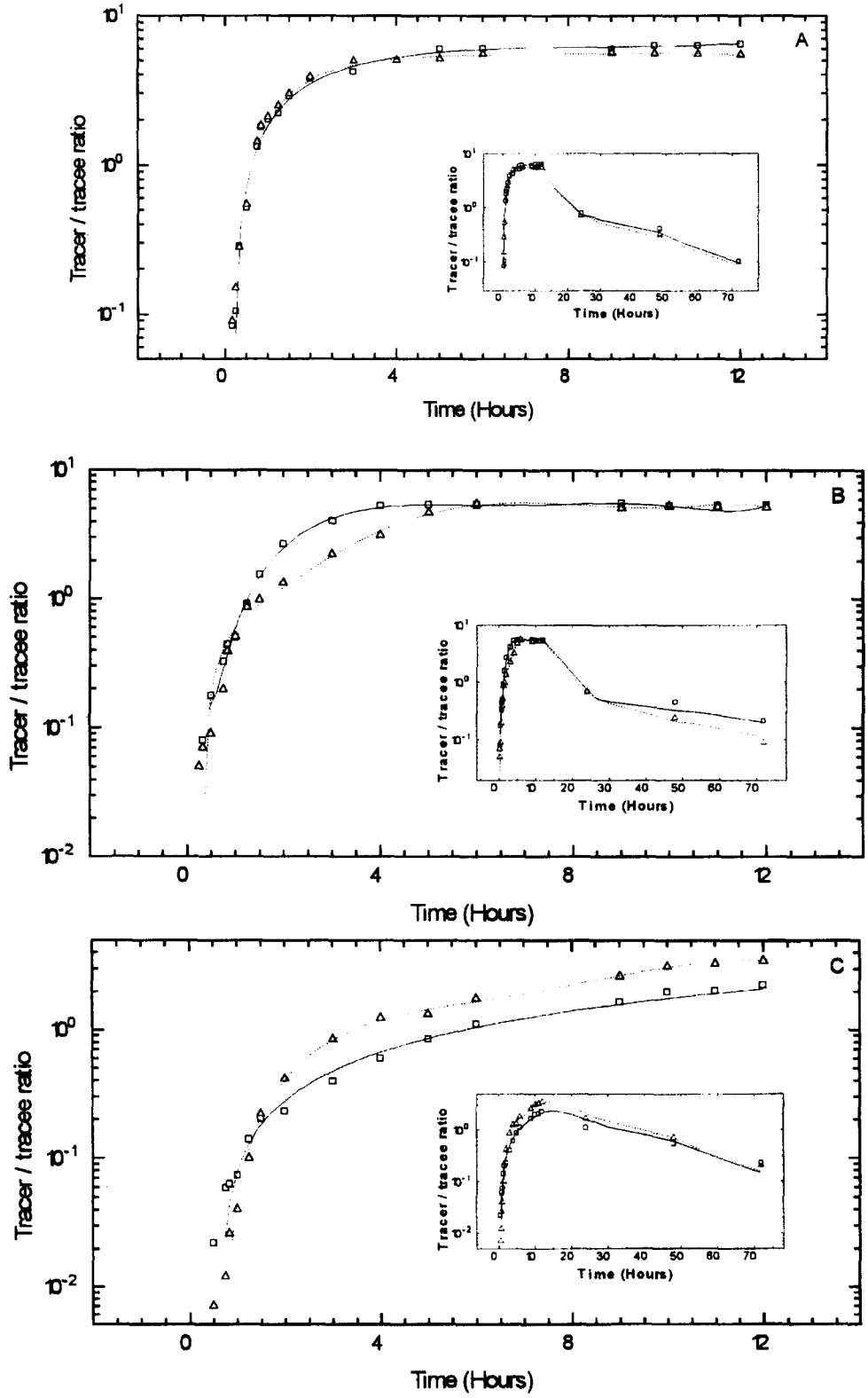

Fig. 4. Plots showing apoB-100 [$^{13}C_6$]phenylalanine enrichment curves for lipoproteins derived from $VLDL_1$ (A), $VLDL_2$ (B), IDL (C), LDL_1 (D), and LDL_2 (E) in FDB patient #3 (triangles, dotted lines) and control subject #3 (squares, solid lines) during the course of the primed, constant infusion (main graph) and during the whole study (insert graph). Symbols represent observed data; lines indicate computer-derived fits as calculated by compartmental modeling.

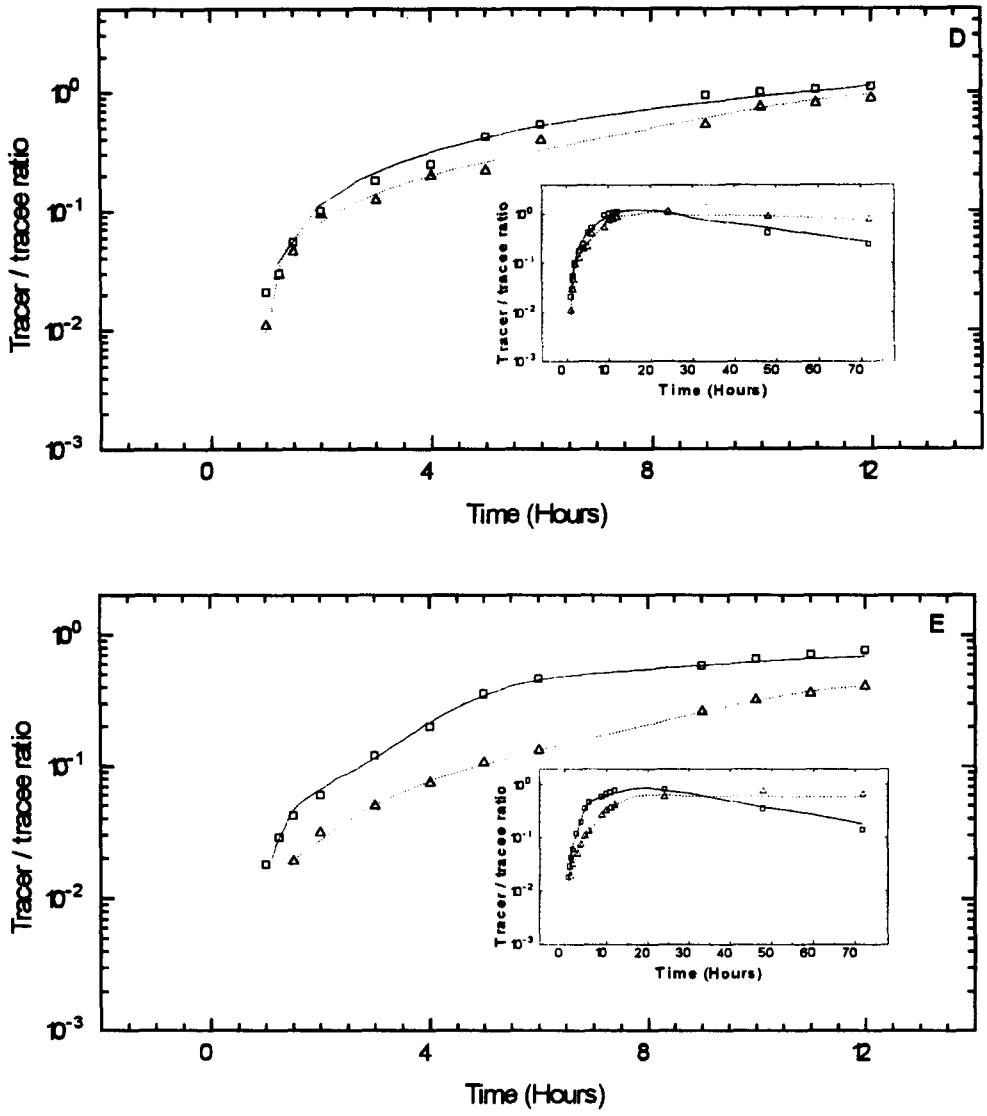

Fig. 4.

creased direct catabolism. Variation in LDL apoB-100, on the other hand, was associated with alterations in the fractional rate of IDL to LDL transfer.

DISCUSSION

This is the first study using a stable isotope approach to demonstrate in vivo that some FDB heterozygotes with mild forms of hypercholesterolemia show enhanced clearance of VLDL remnants and IDL. In order to study the in vivo apoB-100 turnover using labeled amino acids, a multicompartmental model has been devised to explain the apoB-100 kinetics in both heterozygous FDB patients and normolipidemic controls. The current model generated satisfactory fits to the isotopic enrichment data and masses that could be obtained from five distinct lipoprotein fractions differing by defined flotation intervals. Constant lipid and apolipoprotein levels during the course of the study in all participants reflect general steady state conditions. However, a more rigorous validation of this steady state assumption requires the determination of the intracellular precursor enrichment. Available evidence exists that the steady state labeling of endogeneously formed plasma free [$^{13}C_6$]tyrosine and [$^{2}H_3$]KIC, reflecting the intracellular steady state conditions of phenylalanine and leucine enrichment, respectively, can be used in this way (36, 37). Notably, both tyrosine and KIC rapidly achieved plasma steady state enrichment within 1 h under the conditions used. On the other hand, there are further assumptions to be taken into account when

TABLE 4. Model parameters (rate constants and computed masses) for patient #3 and control #3

	FDB Heterozygote (#3)		Control (#3)	
Parameter	Value	FSD	Value	FSD
L(0,11)	0.097	0.19	0.150	0.14
L(13,11)	0.211	0.29	0.213	0.11
L(13,2)	0.028	0.44	0.022	0.39
L(8,2)	0.040	0.31	0.004	0.34
L(0,13)	0.491	0.09	0.488	0.14
L(14,13)	0.331	0.21	0.310	0.11
L(0,14)	0.051	0.19	0.052	0.24
L(16,14)	0.034	0.12	0.041	0.13
L(21,16)	0.321	0.24	0.481	0.33
L(21,8)	0.178	0.22	0.200	0.31
L(21,2)	0.001	0.45	0.000	-
L(0,21)	0.082	0.08	0.021	0.10
L(31,21)	0.031	0.09	0.043	0.11
L(0,31)	0.016	0.08	0.031	0.16
L(33,31)	0.002	0.24	0.006	0.44
L(33,34)	0.001	0.12	0.001	0.21
L(33,2)	0.000	-	0.000	-
L(0,33)	0.009	0.14	0.052	0.19
L(0,35)	0.000	-	0.015	0.26
M(13)	80	-	30	-
M(14)	60	-	40	-
M(8)	50	-	50	-
M(16)	100	-	50	-
M(31)	1100	-	1300	-
M(34)	900	-	1000	-
M(33)	240	-	195	-
M(35)	400	-	55	-

Abbreviations: L (destination, source), fractional rate constants expressed in pools/h; M (compartment), computed masses expressed in mg; FSD, fractional standard deviation.

interpreting our kinetic data as discussed in the Methods section. LDL cholesterol levels in FDB heterozygotes are higher than expected for their age and sex from the overall population but lower than the average difference reported for patients who are heterozygous for FH (38, 39). März et al. (9) have clearly demonstrated that LDL from a patient homozygous for FDB had reduced binding and internalization, but no total abolition of LDL clearance. The receptor-mediated clearance of LDL from this patient revealed that the apoE-containing, buoyant LDL (density < 1.035 kg/l) could be removed efficiently, indicating normal affinity for LDL receptors, whereas more dense LDL that are apoE-poor or apoE-free were found to accumulate.

Gallagher and Myant (10) demonstrated in vitro the normal clearance of VLDL and VLDL remnants prepared from two other FDB homozygotes, and residual affinity of mutant LDL (13.4–14.7% of the affinity of normal LDL) to the LDL receptors. As expected, we could find a dramatic decrease of LDL FCR in FDB. In the present study the mean fractional rate for total LDL apoB-100 direct catabolism was decreased to 32% of the normal controls. This is consistent with studies showing in vitro 32–35.8% binding affinity of total LDL from FDB heterozygotes to the LDL-receptor when compared with normal LDL (40, 41). Notably, the LDL FCR presented in this study is the resulting FCR of the total, mixed LDL pool comprising both normal LDL and mutant LDL allotypes as characteristically present in FDB heterozygotes. Our main aim was to study the turnover of LDL precursors. In this context we did not take blood samples up to 2 weeks as is necessary when assessing LDL turnover in more detail. However, the estimated LDL subfraction parameters indicate that the catabolism of LDL_1 in FDB is relatively weakly decreased in comparison to the control group, whereas LDL_2

catabolism is extremely diminished. This is in agreement with the observations cited above (9). Of note, measurements of apoE in the LDL subfractions reached the detection limit in most cases. Nevertheless, both the LDL_1 and LDL_2 polyacrylamide gels contained detectable traces of apoE in all subjects. VLDL can be removed directly by receptor-dependent metabolic routes via the LDL receptor, recognizing not only apoB-100 of LDL but also binding apoE of VLDL, IDL, and competing defective LDL that contain still one or more apoE molecules. Other receptors also specifically recognize apoE, e.g., the LDL receptor-related protein (42, 43). Our data show that the fractional catabolism of both $VLDL_1$ and $VLDL_2$ apoB-100 does not differ between FDB and controls. On the other hand, the $VLDL_2$ apoB-100 production rate (PR at steady state) was increased in FDB. There is experimental evidence that VLDL overproduction in several hypercholesterolemic patients is channeled via triglyceride-poor, small $VLDL_2$ (35). This increase in $VLDL_2$ synthesis cannot be explained from the present data. The rate of hepatic apoB-100 production and secretion depends to some extent on lipid substrate availability (44, 45). Mechanisms that are responsible for enhanced availability of intracellular cholesterol used for a 'substrate-driven' VLDL secretion in FDB, comparable to the situation in FH, are still unclear (31, 46). Increased $VLDL_2$ apoB-100 production and normal fractional catabolism of VLDL apoB-100 lead consequently to an increased mass transfer of VLDL apoB-100 to the IDL pool in FDB. Despite this increased input the IDL pool size appears to be unaffected in FDB. This is explained by a dramatic increase of direct fractional catabolism of IDL in the FDB patients. This finding supports the hypothesis that impaired delivery of cholesterol to the liver, due to decreased uptake of the mutant LDL allotype, results in up-regulation of hepatic LDL receptors that may enhance the LDL precursor clearance via apoE as recognition ligand. In addition, there could be an increased clearance of apoE-carrying lipoproteins via the apoE receptors. In this study the influence of the apoE polymorphism on the lipoprotein metabolism in FDB (47) could be neglected (FDB: four $apoE_{3/3}$ and one $apoE_{2/3}$ vs. controls: four $apoE_{3/3}$, and two $apoE_{3/4}$). The significantly lower fractional transfer rates of IDL to LDL apoB-100 transfer in FDB due to the high direct catabolism is another significant modulator of the LDL apoB-100 mass. Possibly, the decrease of IDL conversion is due to a modulated hepatic lipase activity that governs this metabolic step (48). Despite the decreased LDL apoB-100 input rate in FDB, the LDL apoB-100 pool size was maintained at a supernormal level as a result of the significantly diminished LDL clearance. The LDL accumulated in FDB and contributing to the high levels of LDL cholesterol should be an apoE-poor, mutant entity of LDL. Our findings are consistent with the clinical evidence that statins lower the plasma LDL level in FDB patients partly by stimulating receptor-dependent catabolism of both normal and defect LDL precursors, in this way decreasing the input rate of LDL (39, 49). Additional investigation regarding apoB-100 turnover in FDB heterozygotes with moderate hypercholesterolemia is needed. The in vivo kinetic parameters related to clearly separated mutant and normal pools of LDL, using allele-specific antibodies (8) and separated apoE-rich and apoE-poor LDL as discussed by others (9), are of special interest. It would be of interest to distinguish the underlying mechanisms responsible for the existence of two distinct populations among FDB heterozygotes: one, exhibiting mild hypercholestero-lemia and consequently not affected by CAD and related disorders, and another that exhibits a more severe form of hypercholesterolemia and is affected by CAD and related disorders, similarly to heterozygous FH.

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