Effects of weekly LDL-apheresis on metabolic parameters of apolipoprotein B in heterozygous familial hypercholesterolemia

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Abstract. Apheresis is a treatment option for patients with severe hypercholesterolemia and coronary artery disease. It is, however, unknown whether such therapy changes kinetic parameters of lipoprotein metabolism, such as apolipoprotein B (apoB) secretion rates, conversion rates, and fractional catabolic rates (FCR). We studied the long-term effect of regular apheresis therapy on metabolic parameters of apoB in five patients with heterozygous familial hypercholesterolemia (FH) using endogenous labeling with D_3-leucine, mass spectrometry, and multicompartmental modeling. Patients were studied prior to (study 1) and after 3–6 months of weekly apheresis therapy (study 2). LDL-apoB concentration was 183 ± 16 mg $\cdot$ d^{-1} prior to apheresis therapy (study 1), 135 ± 7 mg $\cdot$ dl^{-1} at the beginning of study 2, and 163 ± 10 mg $\cdot$ dl^{-1} at the end of study 2. VLDL-apoB and IDL-apoB were not different between the two studies and did not change during study 2. Separate modeling of the two studies revealed very similar parameters in each patient. In a second step simultaneous modeling of both studies was performed taking the changing pool size as a non-steady-state condition into account. ApoB tracer data of both kinetic studies and the change in pool size could be described with one set of kinetic parameters (VLDL-apoB FCR 4.32 ± 1.06 d^{-1}, LDL-apoB FCR 0.17 ± 0.05 d^{-1}, apoB secretion rate 11.9 ± 3.7 mg $\cdot$ kg^{-1} $\cdot$ d^{-1}). These parameters are well within the range of those previously published for FH heterozygotes in steady state. We conclude that regular apheresis therapy did not alter kinetic parameters of apoB metabolism in these patients with heterozygous FH in the long term and that the decreased rate of delivery of neutral lipids or apoB to the liver does not regulate plasma apoB metabolism.—**Parhofer, K. G., P. H. R. Barrett, T. Demant, W. O. Richter, and P. Schwandt.** Effects of weekly LDL-apheresis on metabolic parameters of apolipoprotein B in heterozygous familial hypercholesterolemia. *J. Lipid Res.* 1996. **37:** 2383–2393.

Extracorporeal elimination of LDL particles by apheresis is a widely accepted therapeutic measure for patients with familial hypercholesterolemia (FH) who do not respond sufficiently to dietary and/or drug therapy. All currently available apheresis techniques are based on the elimination of apolipoprotein B (apoB)-containing lipoproteins (1–4). Recently published data indicate that the decrease in cholesterol induced by apheresis has a beneficial influence on the course of coronary artery disease (5—9).

It is, however, unknown whether regular LDL apheresis alters kinetic parameters of apoB metabolism. Implying a feedback mechanism that includes plasma concentrations as a regulatory component, it is conceivable that apheresis changes the secretion rate and/or the catabolic rate of apoB-containing lipoproteins. Furthermore, the conversion rate of VLDL to LDL (delipidation cascade) may vary. Studying metabolic parameters before and during regular apheresis offers a unique opportunity to study the effect of a direct, exogenous perturbation of lipoprotein concentration on apoB metabolism. This is in contrast to studies examining the effect of dietary intervention or drug therapy. In the latter case, as for instance in a recently published study by Gaw et al. (10), changes in metabolic parameters are part of the intervention itself. The extracorporal removal of lipoproteins, such as with apheresis, is therefore the only in vivo method to examine whether kinetic parameters of apoB are regulated by plasma lipoproteins.

The decrease in plasma LDL concentration results in a decreased rate of delivery of neutral lipids and apoB

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to the liver. This, in turn, could lead to a decrease in the secretion of apoB-containing lipoproteins and to an up-regulation of LDL receptors. Numerous studies have shown that the availability of lipids has an important influence on the secretion rate of apoB in vitro (11–13). While earlier studies have focused on the role of fatty acids in regulating apoB secretion (14), the interest has shifted more towards the role of cholesterol in this process in recent years (15). Most of these data are based on in vitro studies, but VLDL-apoB secretion has been correlated to plasma mevalonic acid, an indicator of cholesterol biosynthesis, in normal subjects, obese patients, and patients with NIDDM (16–18). At the same time it has been shown that cholesterol biosynthesis can be stimulated by the reduction of plasma LDL with apheresis (19). However, more recent studies indicate that LDL-cholesterol must fall below a threshold level of 50–60 mg · dl^{-1} to induce such a stimulation (20). Taken together, these data indicate that apheresis therapy influences cholesterol biosynthesis, which in turn is correlated with apoB secretion in in vitro studies. Therefore, it would be conceivable that with apheresis therapy the secretion rate of apoB-containing lipoproteins decreases and/or the FCR increases.

Several previous studies were undertaken to establish whether LDL apheresis (21) or plasma exchange procedures (22–24) can alter parameters of apoB metabolism. Most of the previously published work assesses the effect of a single procedure, where in general no alteration in metabolic parameters was found. However, it is possible that only repetitive apheresis treatments result in a significantly altered apoB metabolism. More important, however, none of the previously published studies describes simultaneously VLDL-, IDL-, and LDL-apoB kinetics or takes into account the non-steady-state condition caused by apheresis.

In the experiments described in this report we study apoB metabolism in five patients with heterozygous FH before regular apheresis therapy and after 3–6 months of weekly treatment. ApoB metabolism was studied using endogenous labeling with D$_3$-leucine and multicompartmental modeling (25), taking the changing apoB pool size as a non-steady-state condition into account. The aim of the study was to determine whether regular apheresis alters the secretion rate of apoB and/or the catabolism of VLDL-, IDL-, or LDL-apoB and/or the conversion of VLDL-apoB to LDL-apoB.

METHODS

Study protocol

Four male and one female heterozygous FH patients participated in the study. The characteristics of these patients are shown in **Table 1.** All patients had coronary artery disease proven by angiography. They were resistant to dietary and drug therapy, and therefore were considered to be candidates for regular apheresis therapy. Patients with additional secondary causes of hyperlipidemia were excluded. Four to 6 weeks prior to each kinetic study the patients (except patient #1, AH, who continued simvastatin 40 mg qd throughout both studies) stopped taking hypolipidemic drugs (usually HMG-CoA reductase-inhibitor and/or resins), but continued their diet (AHA step 1 or 2) and other medications. The first kinetic study was performed after the patient had been accepted for regular apheresis therapy but before the first apheresis procedure was carried out. None of the patients had been treated with apheresis previously. The second study was performed after 3–6 months of weekly apheresis and approximately 5 to 7 days after the last procedure. None of the patients had a significant change in body weight between the two studies. Apheresis was performed using immunoadsorption (1) in three patients and heparin precipitation (2) in the other two patients. Such therapy eliminates LDL and to a lesser degree VLDL and IDL (1, 2). Immediately after apheresis LDL-cholesterol decreased by approximately 65% (Table 1), while serum triglycerides and HDL-cholesterol decreased by 45% and 19%, respectively. Triglycerides and HDL-cholesterol returned to baseline in less than 24 h (4). As estimated from the hematocrit plasma volume returned to baseline within 2–4 h.

Details of the procedure for the kinetic studies have been published previously (25, 26). After subjects had fasted for 10 h, a bolus of D$_3$-leucine (Cambridge Isotope Laboratories, Woburn, MA, isotopic purity 99%) was given (5.0 mg · kg^{-1}). After the leucine bolus, the subjects remained fasting for another 16 h, during which period 24 samples were drawn. The subjects were then allowed to continue their regular diet, and subsequent samples were drawn over the next 3–7 days after fasting for 10 to 12 h. In total, 30 plasma samples were drawn for assays of plasma amino acid enrichment, and 28 samples were drawn for VLDL-, IDL-, and LDL-apoB leucine enrichment. Aliquots for determination of VLDL-, IDL-, and LDL-apoB pool sizes were drawn on 5–8 occasions during the course of each kinetic study. The study was approved by the Ethics Committee of the Ludwig-Maximilians University and all patients gave written consent.

Analytical methods

VLDL (d < 1.006 g · ml^{-1}), IDL (1.006–1.019 g · ml^{-1}), and LDL (1.019–1.063 g · ml^{-1}) were isolated by sequential ultracentrifugation as previously described (25). ApoB concentrations were measured in VLDL, IDL, and LDL fractions by immunonephelometry using commercially available tests (Behring, Marburg, Ger-

TABLE 1. Characteristics of study patients

Patient	Age yr	BMI	Cholesterol[a]	Triglycerides[a]	LDL-Chol[a]	LDL-Chol[b]	LDL-Chol[c]	LDL-Chol[d]
		$kg \cdot m^{-2}$			$mg \cdot dl^{-1}$			
#1 AH	37	26.2	429 ± 15	127 ± 9	335 ± 23	62	206	243
#2 DD	45	35.0	281 ± 12	139 ± 13	233 ± 19	67	198	219
#3 GM	31	26.1	339 ± 14	112 ± 12	270 ± 26	62	204	235
#4 GS	37	26.2	353 ± 22	120 ± 12	304 ± 36	84	217	261
#5 WB	38	22.4	432 ± 15	120 ± 18	380 ± 28	92	245	316
Mean ± SD	37.6 ± 5.0	27.2 ± 4.7	367 ± 64	124 ± 10	304 ± 57	73 ± 14	214 ± 19	255 ± 37

[a]Concentrations obtained during dietary therapy before regular apheresis therapy was started; patient #1 (AH) also recieved HMG-CoA reductase inhibitor therapy.
[b]LDL-cholesterol concentration after apheresis (5–7 days prior to start of study 2).
[c]LDL-cholesterol concentration at beginning of study 2.
[d]LDL-cholesterol concentration at end of study 2.

many). Cholesterol and triglycerides were measured by commercially available tests (Boehringer, Mannheim, Germany). VLDL-, IDL-, and LDL-apoB pool sizes were determined by multiplying the measured apoB concentration by the estimated plasma volume (0.04 × body weight). ApoB-100 was isolated at each time point from each lipoprotein fraction by precipitation with butanol–isopropylether as previously described (27). The precipitated apoB was dried under nitrogen and then hydrolyzed in 12 N HCl for 16 h at 110°C. Plasma amino acid enrichment was determined from 0.2 ml plasma after isolation by cation exchange chromatography (AG50W-X8, Bio-Rad, Richmond, CA). Amino acids obtained from the plasma samples or from the hydrolyzed apoB precipitates were derived with N-tert butyl-dimethylsilyl-N-methylfuoracetamid (Fluka, Buchs, Switzerland). Leucine isotope ratios were determined by gas chromatography–mass spectrometry (Trio 1000 Fisons Instruments, Manchester, UK) (28). Enrichment was calculated using the method of Cobelli, Toffolo, and Foster (29) and converted to tracer/tracee ratios. Data in this format are analogous to specific activity in radiotracer experiments.

Kinetic analysis

VLDL-, IDL-, and LDL-apoB tracer data were analyzed by a multicompartmental model (**Fig. 1**). The model consists of a precursor compartment (compartment 1) and an intracellular delay compartment accounting for the synthesis of apoB and the assembly of lipoproteins (compartment 2). Compartments 10 through 14 are used to account for the kinetics of the VLDL-apoB fraction. Compartment 10 represents rapidly turning over lipoproteins. Particles in this compartment can be transported into the delipidation chain (compartments 11 through 13) or shunted directly into the IDL fraction (compartment 20) or the LDL fraction (compartment 30). Compartments within the delipidation chain can either be transferred into a slowly turn-

ing over VLDL compartment (compartment 14) or to the next compartment of the chain. As in previous models using a delipidation chain (30–34) it was assumed that the same fraction of each compartment is transferred to the next compartment of the chain and to the slowly turning over compartment. These constraints are necessary to ascertain the system identifiability of the model (35). IDL can either be formed from the last compartment of the delipidation chain (compartment 13) or directly from the shunt compartment. IDL data were modeled similarly to VLDL, i.e., with a delipidation chain (compartments 20–22) and a slowly turning over compartment (compartment 23). This feature was necessary to describe the relatively broad peak of the IDL data. For the same reasons as given above with the VLDL fraction, the fraction of apoB removed from the chain was the same for each compartment. LDL apoB was described by one compartment (compartment 30) and originated either from the IDL delipidation or was shunted directly from the fast turning over VLDL.

It is assumed that plasma leucine (compartment 1) is the source of the leucine that is incorporated into apoB. A triexponential function (25) was used to fit the plasma leucine tracer/tracee ratios and was used as a forcing function (36) in the model. This model is the simplest model that is consistent with our tracer data and the current understanding of lipoprotein metabolism. Compared to previously published models using the methodology (25, 26, 30), structure was added to the IDL section to account for the broader peak of the IDL tracer curve. Because we are comparing the results of two kinetic studies in the same individual, our results are to some degree model independent. In this study the SAAM II (SAAM Institute Inc., Seattle, WA) program was used to fit the model to the observed tracer data. Metabolic parameters for apoB-100 were subsequently derived from the best fit. The fractional catabolic rate (FCR) of VLDL-apoB is the weighted average (related to mass distribution) of the FCR of the individ-
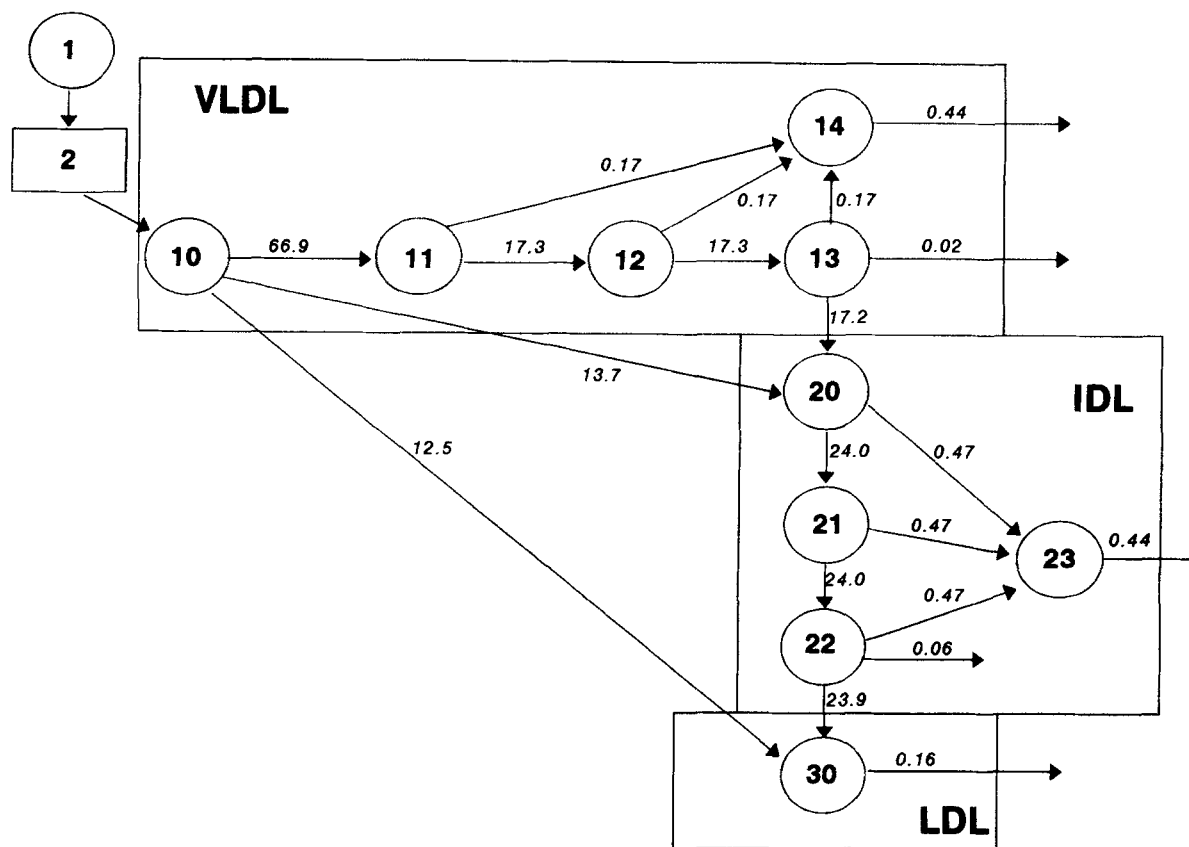
Fig. 1. Multicompartmental model of apoB metabolism. Compartment 1: plasma leucine tracer/tracee ratio (forcing function); compartment 2: delay compartment (synthesis of apoB and secretion); compartments 10 through 14: VLDL-apoB; compartments 20 through 23: IDL-apoB; compartment 30: LDL-apoB (for details see Methods). In addition, rate constants (d^{-1}) obtained by stimultaneous modeling of both tracer studies (Table 3) for a representative subject (WB) are shown.

ual VLDL pools. The FCR of each VLDL pool is the sum of individual rate constants, thus including conversion to IDL and LDL as well as removal from plasma.

Patients were considered to be in a steady state condition with regard to apoB metabolism and apoB pool sizes during the first study as evidenced by the stable apoB concentrations in VLDL, IDL, and LDL. For this first study, data were analyzed as previously described (25). For the second study, non-steady-state conditions had to be assumed. VLDL- and IDL-apoB concentrations were not different between the two studies and did not change during the second study. However, as the last apheresis was only 5–7 days prior to the second study, LDL-apoB pool size was increased in the course of that kinetic study. The submodel used to describe LDL-apoB concentrations (which parallels total-apoB, cholesterol, LDL-cholesterol) is shown in **Fig. 2.** After initiation of apheresis, LDL-apoB is decreased further with each apheresis until a new equilibrium is reached where the amount of apoB removed during one procedure corresponds to the increase in pool size between treatments. In this phase the second tracer study was

performed. The dotted line in Fig. 2 represents the submodel for LDL-apoB pool size. It was assumed that one "superapheresis" reduced LDL-apoB concentration from steady state (study 1) to a typical post-apheresis value, and that thereafter LDL-apoB increases and eventually reaches the initial steady state. The model is based on the measured values (steady state concentration, post-apheresis concentration, and concentrations during study 2) and on the hypothesis that if no further apheresis is performed the initial steady-state will be reached eventually.

To model the non-steady-state condition using the SAAM II program, two experiments were performed on the model described above. One, the tracer experiment, described the movement of tracer through the model. The other experiment on the model, also setup as a tracer experiment, described the movement of apoB through the model. The link between the two experiments, tracer and apoB, comes from the data that are expressed as the tracer/tracee ratio. Therefore, the sample points in the model, corresponding to VLDL, IDL, and LDL data, were functions of the mass in the
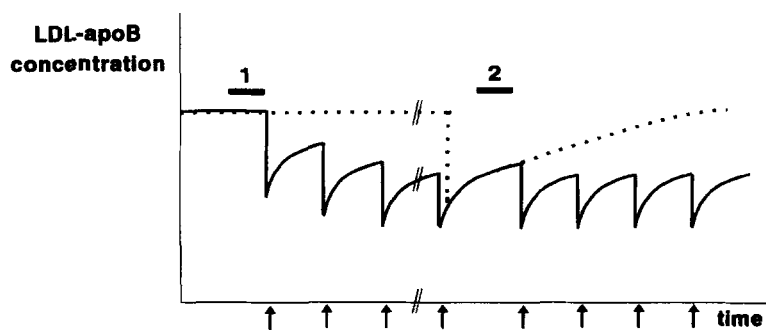
Fig. 2. Submodel for LDL-apoB pool size. The solid line corresponds to LDL-apoB concentration during regular apheresis therapy; the dotted line corresponds to the hypothetical LDL-apoB concentration used in the modeling process. The two bars, labeled 1 and 2, correspond to the periods during which the two studies were performed, arrows at the x-axis indicate apheresis (for details see Methods).

tracer compartments divided by the means of apoB in the respective compartments. In the steady-state, equations describing the relationship between compartment mass and the fractional rate constants can be constructed. This set of equations was used to assign apoB mass to each compartment while the steady-state condition held. After apheresis the mass of apoB on the LDL, fraction was instantly lowered to its post-apheresis level using a change condition function in SAAM II. No non-linear fractional rate constants were required to fit the data acquired after apheresis or the subsequent rise in LDL apoB concentrations.

Statistical analysis

All data are expressed as mean ± standard deviation, except where stated differently. Parameters between study 1 and study 2 were compared with paired *t*-tests.

RESULTS

ApoB concentrations during both kinetic studies are shown in **Table 2.** Except for LDL-apoB in study 2, these values represent means ± SD of 5–8 samples obtained during each study. During study 1 apoB concentrations remained stable indicating steady-state conditions. During the second study VLDL- and IDL-apoB concentrations also remained stable and were not different to those in study 1. As the last apheresis was performed 5–7 days prior to the second study, LDL-apoB had not yet reached steady state and was increasing during the study period (Fig. 2). The first and the last measured values are each indicated in the table. The other 3–6 values are not shown, but were used during modeling.

Plasma leucine tracer/tracee ratios were very similar in all subjects and did not differ from previously published data concerning hyperlipidemic and normolipidemic subjects (25, 30). Furthermore, in four of the five patients, plasma leucine tracer/tracee ratio curves were identical during both kinetic studies. In the fifth patient, the only female, the tracer disappeared faster from plasma during study 2. The fact that she was in a different phase of her menstrual cycle may explain this difference. The plasma leucine tracer/tracee ratio curves were fitted with triexponential functions which were used to define the forcing functions in the modeling process.

Initially in each patient the two studies were analyzed separately (**Table 3, Fig. 3**). As can be seen, this analysis

TABLE 2 ApoB concentration in VLDL, IDL, and LDL

Patient	Study 1 (before regular apheresis)			Study 2 (after 3–6 months of weekly apheresis)			
	VLDL-ApoB[a]	IDL-ApoB[a]	LDL-ApoB[a]	VLDL-ApoB[a]	IDL-ApoB[a]	LDL-ApoB[b]	LDL-ApoB[c]
	$mg \cdot dl^{-1}$			$mg \cdot dl^{-1}$			
#1 AH	8.5 ± 3.1	9.9 ± 3.5	197 ± 14	8.0 ± 3.3	7.9 ± 4.1	127	165
#2 DD	7.9 ± 2.1	2.5 ± 0.2	161 ± 18	9.8 ± 1.6	2.1 ± 0.5	137	151
#3 GM	6.2 ± 0.3	3.5 ± 0.6	193 ± 5	8.4 ± 2.6	3.2 ± 0.6	146	168
#4 GS	5.8 ± 1.5	3.7 ± 0.6	171 ± 27	4.7 ± 1.7	2.5 ± 0.9	131	156
#5 WB	6.1 ± 1.3	7.9 ± 1.9	193 ± 14	5.2 ± 1.1	8.0 ± 2.3	135	176
Mean ± SD[d]	6.9 ± 1.2	5.5 ± 3.2	183 ± 16	7.2 ± 2.2	4.7 ± 3.0	135 ± 7	163 ± 10

[a]Indicates mean ± SD of 5–8 values obtained during the study.
[b]Indicates LDL-apoB concentration at the beginning of study 2 (5–7 days after last apheresis).
[c]Indicates LDL-apoB concentration at the end of study 2; 4–7 values were obtained during the course of study 2, these values are not shown here, but were used for the modeling.
[d]Mean of the means.

TABLE 3. Metabolic parameters obtained by separate and combined modeling of both tracer studies

Patient	SR			VLDL-FCR			LDL-FCR			Conversion		
	Study 1	Study 2	Combined	Study 1	Study 2	Combined	Study 1	Study 2	Combined	Study 1	Study 2	Combined
	$mg \cdot kg^{-1} \cdot d^{-1}$			d^{-1}			d^{-1}			%		
#1 AH	21.0	16.1	17.6	6.0	4.8	5.16	0.26	0.26	0.25	98	83	97
#2 DD	9.3	10.6	9.1	3.0	3.3	2.88	0.14	0.19	0.17	94	96	95
#3 GM	13.9	10.6	10.4	5.5	4.3	4.20	0.14	0.12	0.13	95	59	94
#4 GS	9.1	9.6	8.9	3.9	4.0	3.84	0.14	0.16	0.14	96	86	97
#5 WB	11.9	13.1	13.3	4.8	5.2	5.52	0.12	0.20	0.16	87	93	93
Mean ± SD	13.0 ± 4.9	12.0 ± 2.6	11.9 ± 3.7	4.6 ± 1.2	4.3 ± 0.7	4.32 ± 1.06	0.16 ± 0.06	0.19 ± 0.05	0.17 ± 0.05	94 ± 4	83 ± 15	95 ± 2

Metabolic parameters obtained by separate modeling (studies 1 and 2) and combined modeling of tracer data obtained before and during regular apheresis therapy. No significant differences (paired test) were observed between study 1 and study 2. SR, secretion rate; FCR, fractional catabolic rate; conversion relates to percentage of apoB that is converted from VLDL to LDL.
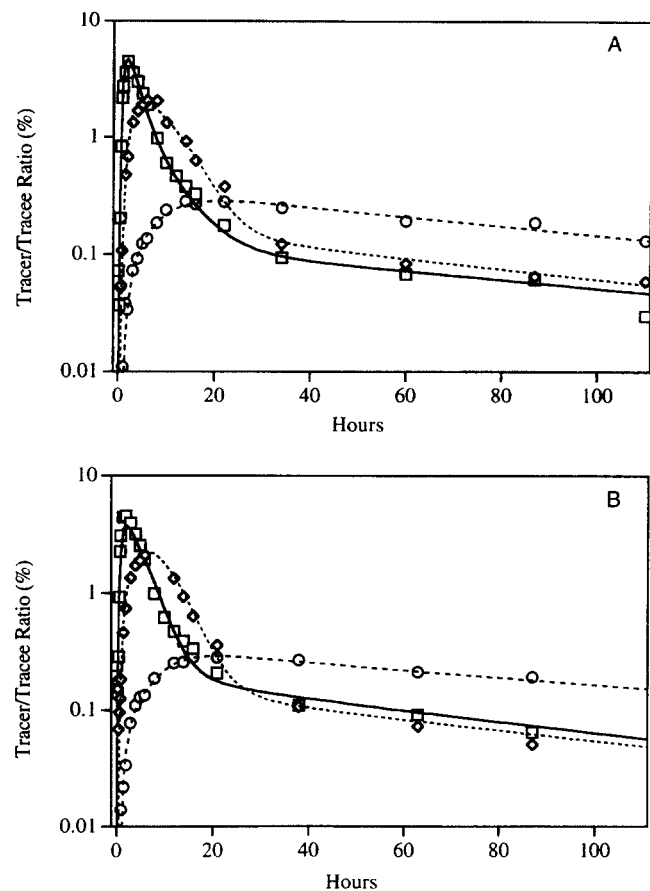

Fig. 3. VLDL- (squares), IDL- (diamonds), and LDL-apoB (circles) leucine tracer/tracee ratios in a representative subject. Observed values (symbols) and model predicted values (lines) are shown. In panel A, study 1 (prior to regular apheresis) and in panel B, study 2 (during regular apheresis therapy) is presented. The model predicted values (lines) were obtained by independent modeling of studies 1 and 2.

revealed that parameters of study 1 (before regular apheresis) and study 2 (after 3–6 months of weekly apheresis) were similar in all patients. This indicates that apheresis did not induce changes in metabolic parameters of apoB. Individual rate constants of all patients are shown in **Table 4.** Despite considerable variability among patients there was no consistent change for any specific parameter. Because of this, tracer data of both studies were modeled simultaneously for each patient. This exercise revealed that the same model parameters describe the tracer data of study 1 and study 2 (Table 3). The secretion rate of apoB, which corresponds to VLDL-apoB secretion, was 11.9 ± 3.7 mg · kg^{-1} · d^{-1}. The FCRs of VLDL-, IDL-, and LDL-apoB were 4.32 ± 1.06, 2.32 ± 1.53, and 0.17 ± 0.05 d^{-1}, respectively. It should be noticed that subject #1 (AH), who was studied on concomitant HMG-CoA reductase inhibitor therapy, had a considerably higher LDL-apoB

TABLE 4. Rate constants of all compartments obtained before and during regular apheresis

Rate	#1 AH		#2 DD		#3 GM		#4 GS		#5 WB		Mean ± SD		P[b]
	Study 1	Study 2	Study 1	Study 2	Study 1	Study 2	Study 1	Study 2	Study 1	Study 2	Study 1	Study 2	
Delay[a]	0.58	0.48	0.41	0.59	0.52	0.65	0.53	0.83	0.50	0.50	0.51 ± 0.06	0.61 ± 0.14	0.21
k(0, 13)	0.09	5.17	0.12	0.12	0.14	31.6	0.37	10.5	0.01	0.02	0.15 ± 0.13	9.5 ± 13.1	0.19
k(0, 14)	0.28	0.36	0.24	0.24	0.18	0.44	0.36	0.12	0.56	0.52	0.32 ± 0.15	0.34 ± 0.16	0.89
k(0, 22)	0	0	0	0	0.16	0.31	0.04	0.12	0.10	0.06	0.06 ± 0.07	0.10 ± 0.13	0.33
k(0, 23)	0.28	0.36	0.24	0.24	0.18	0.44	0.36	0.12	0.55	0.52	0.32 ± 0.14	0.34 ± 0.16	0.87
k(0, 30)	0.26	0.26	0.14	0.19	0.14	0.12	0.14	0.16	0.12	0.20	0.16 ± 0.06	0.19 ± 0.05	0.20
k(2, 1)	0.04	0.05	0.02	0.02	0.02	0.02	0.01	0.01	0.01	0.01	0.02 ± 0.01	0.02 ± 0.02	0.37
k(10, 2)	81.6	88.6	68.2	60.2	63.3	147.4	92.6	115	64.1	106	74.0 ± 12.7	103 ± 32.3	0.14
k(11, 10)	2.26	5.98	2286	2383	28.5	6.0	2.7	3.1	66.5	96.0	477 ± 1011	499 ± 1054	0.35
k(12, 11)	9.1	14.6	12.0	12.4	17.0	38.0	13.7	12.1	13.4	18.7	13.0 ± 2.9	19.1 ± 10.8	0.19
k(14, 11)	0.14	0.30	0.17	0.10	0.26	0.81	0.40	0.50	0.14	0.19	0.22 ± 0.11	0.38 ± 0.28	0.21
k(20, 10)	5.74	4.62	133	123	16.8	2.69	3.57	3.12	15.5	9.08	34.9 ± 55.1	28.5 ± 52.9	0.07
k(20, 13)	9.12	6.4	11.9	12.3	16.8	6.58	23.3	3.14	13.4	18.7	14.9 ± 5.4	10.0 ± 5.9	0.35
k(21, 20)	13.1	13.2	49.0	50.4	18.5	14.8	18.8	16.6	41.5	21.5	28.2 ± 15.9	23.3 ± 15.5	0.28
k(23, 20)	0.11	0.11	0.28	0.20	0.15	0.26	0.10	0.12	1.82	0.39	0.49 ± 0.75	0.22 ± 0.12	0.40
k(30, 10)	4.70	4.39	394	223	8.64	4.28	1.70	12.2	10.4	12.6	83.8 ± 173.4	51.3 ± 96.1	0.40
k(30, 22)	13.1	13.2	49.0	50.4	18.3	14.4	18.7	16.5	41.5	21.5	28.1 ± 16.0	23.2 ± 15.5	0.28

[a] Refers to the delay in compartment 2 (hours), all other values are rate constants (d⁻¹) between individual compartments. The numbers refer to the model shown in Fig. 1. The first number defines the destination compartment, the second number the source compartment, i.e., k(11, 10) indicates the fraction of apoB in compartment 10 transferred to compartment 11 per day. To ascertain the system identifiability, the model was constrained in that k(14, 12) and k(14, 13) correspond to k(14, 11), k(13, 12) corresponds to k(12, 11), k(23, 21) and k(23, 22) correspond to k(23, 20), and k(22, 21) corresponds to k(21, 20).
[b] Values were compared by paired t-test.

FCR than the other subjects not taking lipid lowering medication (0.25 d⁻¹ vs. 0.15 ± 0.02 d⁻¹). The conversion rate of VLDL-apoB to LDL-apoB was 95 ± 2%, i.e., almost all apoB secreted was converted to LDL. While secretion rate and FCR of VLDL- as well as LDL-apoB did not vary extensively among individuals, the FCR of IDL-apoB varied between 0.71 and 4.2 d⁻¹. This variation reflects the fact that, despite inclusion of a delipidation chain and a slowly turning over compartment, the IDL fraction was modeled to account for the necessary delay and loss resulting from the conversion of VLDL to LDL. In Fig. 1 kinetic parameters of all compartments in the model are shown for a representative subject (WB). The values within the compartment represent pool sizes (mg), the values along the pathways rate constants (d⁻¹).

DISCUSSION

Our results show that in this group of patients with heterozygous FH 3–6 months of weekly apheresis therapy did not change kinetic parameters of apoB metabolism. Metabolic parameters established before and during regular apheresis were not significantly different. This lack in difference is underlined by the fact that the same parameters that characterize apoB metabolism in steady state, before initiation of regular apheresis, describe apoB metabolism in non-steady state, i.e., during

weekly apheresis. This indicates that in these patients apoB metabolism is not regulated through LDL plasma concentration. Furthermore, it indicates that in the long term the beneficial effects of LDL-apheresis are not counterregulated through changes in metabolic parameters.

The kinetic parameters established in this study are well within the range of previously published parameters of apoB metabolism in FH patients (33, 37–39). A review in 1982 (33) revealed a VLDL-apoB FCR of approximately 5 d⁻¹, a VLDL-apoB secretion rate of approximately 14 mg · kg⁻¹ · d⁻¹, and a LDL-apoB FCR of 0.15 d⁻¹. Further kinetic studies in FH patients, some of which were performed with endogenous tracers, revealed similar parameters (37–39). Compared to normolipidemic subjects in whom apoB metabolism was studied with the same methodology and a similar model (25), heterozygous FH patients are characterized by a decreased VLDL-apoB FCR (4.3 ± 1.1 vs. 8.4 ± 1.9 d⁻¹ in normal subjects), a slightly decreased VLDL-apoB secretion rate (11.9 ± 3.7 vs. 14.8 ± 5.7 mg · kg⁻¹ · d⁻¹ in normal subjects), a significantly decreased LDL-apoB FCR (0.17 ± 0.05 vs. 0.62 ± 0.43 d⁻¹ in normal subjects), and an increased conversion rate (95 ± 2 vs. 75 ± 12% in normal subjects). These differences between normal subjects and FH heterozygotes are in good agreement with a recently published paper by Fisher, Zech, and Stacpoole (39). This study characterized apoB metabolism in FH by a decrease in apoB secretion, an increase to VLDL to LDL conversion, and a decrease in LDL-

apoB FCR. Of note is the high conversion rate of VLDL-apoB to LDL-apoB; this could be related to the model used to analyze the data (Fig. 1). In a model allowing direct secretion of apoB into the LDL fraction, the conversion rate (VLDL to LDL) will be lower than in a model not including this pathway. However, the ratio of VLDL and IDL-apoB removed from plasma to total LDL-apoB production is similar in models with and without direct apoB production (32). However, it is still unclear whether significant direct secretion of apoB into the LDL fraction exists in humans. In a recent study (32) it was shown that the same tracer data can be modeled equally well with and without such a direct input.

Although the model used to describe the tracer data (Fig. 1) appears relatively complicated, it can be reduced to a few important features: a) a rapidly turning over pool of VLDL, which shunts apoB directly into the IDL and LDL fractions, and thereby accounts for the rapid appearance of tracer in these lipoprotein fractions; b) a VLDL and IDL delipidation chain; c) slowly turning over compartments for VLDL and IDL; and d) a single compartment for LDL. Compared with previous models structure was added to the IDL fraction to account for the broad peak of the IDL tracer curve (Fig. 3). This fraction was modeled similarly to the VLDL fraction, i.e., with a slowly turning over compartment and a delipidation chain. The rate constants of the compartments within the VLDL and IDL delipidation chain were constrained to be identical for corresponding pathways. This is necessary to ascertain the identifiability of the model, as such constraints allow an increase in the number of compartments within the delipidation chain without increasing the number of unknown parameters. It is, however, unlikely that the parameters associated with the individual compartments of the IDL fraction are by themselves meaningful. As in other studies using endogenous tracers to describe apoB metabolism, the LDL fraction is oversimplified, as the use of a single endogenous tracer does not permit complex modeling of the LDL fraction. Numerous studies using exogenous tracer have demonstrated that LDL-apoB metabolism is considerably more complex than could be described with a single compartment (40). Despite this simplistic approach, LDL-apoB parameters obtained in this study agree very well with previously published data in FH heterozygotes (33, 37–39).

As in all kinetic studies, the calculation of metabolic parameters relies as much on the modeling of the tracer data as on the estimation of the pool size, i.e., the apoB concentration and plasma volume. Independent of the method, the determination of apoB concentrations is associated with considerable error. This notion is also supported by the finding that the recovery rate of apoB from the individual lipoprotein fractions is usually in the range of 85–90%. However, these uncertainties are of lesser importance in studies such as this one, where apoB metabolism is determined twice under different conditions in the same individual, as there is no reason to believe that apoB concentration measurements are affected systematically by apheresis. Therefore, statements concerning comparisons before and during apheresis are valid. However, calculated absolute secretion rates could be in error to the extent that estimated pool sizes are under or overestimated.

While we could not find any differences in rate constants established before and during regular apheresis therapy, it cannot be excluded that differences in some parameters may only become significant when considerably larger groups of patients are studied. However, the fact that both tracer studies could be described with one set of metabolic parameters in all patients also indicates that there is little if any difference between apoB metabolism before and during regular apheresis.

We also included one patient (AH) on concomitant HMG-CoA reductase inhibitor therapy. This patient had a considerably higher FCR of LDL-apoB than the other four patients (0.25 d^{-1} vs. 0.15 ± 0.02 d^{-1}), underlining that these drugs primarily increase the FCR of LDL-apoB (41). Despite this concomitant drug therapy, apheresis did not change the metabolic parameters of apoB. This is in accordance with the effect of such combined therapy on cholesterol biosynthesis, when an effect was only observed when LDL-cholesterol fell below a threshold level of 50–60 mg $\cdot$ dl^{-1} (19, 20). It is unknown whether kinetic parameters of apoB metabolism would change if LDL-cholesterol is decreased below this level with combined drug and apheresis therapy.

The decrease in plasma LDL concentration results in a decreased rate of delivery of neutral lipids and apoB to the liver. As numerous studies have shown that the availability of lipids regulates the secretion rate of apoB (11–13), this decreased rate of delivery could theoretically induce the following changes: a) an increased activity of the LDL receptor, b) an increased hepatic cholesterol synthesis, and c) a decreased secretion rate. Some of these points have been addressed in previous studies. Thus, it has been shown that cholesterol biosynthesis can be stimulated by apheresis-induced reduction of plasma LDL (19). However, more recent studies indicate that LDL-cholesterol must fall below a threshold level of 50–60 mg $\cdot$ dl^{-1} to induce such a stimulation (20). Furthermore, VLDL-apoB secretion has been correlated to plasma mevalonic acid, an indicator of cholesterol biosynthesis, in normal controls, obese patients, and patients with NIDDM (16–18).

The question whether the exogenous concentration of lipoproteins can regulate apoB secretion has also

been evaluated with in vitro experiments showing ambiguous results (11). While Sato et al. (42) described a decrease of apoB secretion when VLDL and LDL were added to the medium of HepG2 cells, other investigators did not (43, 44). In vivo, this phenomenon can only be addressed using a system in which apoB is removed exogenously from the plasma, as changes induced by drugs or diet (endogenous changes) necessarily result in altered metabolic parameters. However, compared to in vitro experiments, only small differences of apoB concentration can be studied in vivo. This study shows that apoB metabolism is regulated independently of plasma LDL concentration, at least over the range between 135 ± 7 mg $\cdot$ dl^{-1} and 183 ± 16 mg $\cdot$ dl^{-1}, and that the recurrent reduction to very low apoB levels does not induce changes of apoB metabolism in the long term. Despite the fact that this phenomenon was evaluated in patients with heterozygote FH, the results are probably also valid for the normolipidemic population. Because patients with heterozygous FH can regulate their receptor activity when stimulated (41, 45) it can be expected that they would also react to alterations of plasma lipoprotein concentrations, if those are part of the regulatory circuit of apoB metabolism.

As our studies were performed 5–7 days after the last apheresis, we cannot exclude that apoB metabolism is altered immediately after such a procedure. Indeed, recent data indicate that cholesterol synthesis, and hence possibly also apoB metabolism, is affected when LDL-cholesterol is decreased below a threshold of 50–60 mg $\cdot$ dl^{-1} (20). The same mechanism that increases cholesterol biosynthesis may also decrease apoB secretion or up-regulate receptor activity. Further studies will have to determine whether apheresis changes apoB metabolism, if with each apheresis an LDL-cholesterol below this threshold is reached. Furthermore, apoB metabolism should be described immediately after apheresis, particularly when LDL-cholesterol is decreased below 50–60 mg $\cdot$ dl^{-1}. It is also conceivable, though not very likely, that changes in VLDL concentration observed after apheresis influence apoB secretion. However, the analysis of tracer data obtained immediately after apheresis is considerably more complex, as apheresis does not only remove LDL but to a lesser degree VLDL and IDL also. Therefore, VLDL-, IDL-, and LDL-apoB concentrations will have to be described by a non-steady-state system. VLDL and presumably also IDL concentrations return to baseline within 24 h after apheresis, which in this study allowed us to consider the patients to be in steady state concerning their VLDL- and IDL-apoB concentrations.

A previously published study observed effects of a single apheresis on apoB metabolism using a similar approach (21). Immediately after apheresis, secretion rate was unchanged but the FCR was increased. In interpreting the data, the non-steady-state condition was not accounted for. In order to interpret the data correctly, it is mandatory to take the changing apoB pool size into account. In steady state conditions fractional catabolic rate, secretion rate, and pool size are directly linked to each other. Therefore, either FCR or secretion rate are inevitably affected when apoB pool size is altered (for example through apheresis) and steady state conditions are erroneously assumed. However, this is not necessarily true, as the externally induced decrease of the pool size does not necessarily alter either of the metabolic parameters. In that case the increase of apoB concentration after apheresis might simply represent the refilling of the pool with unchanged metabolic parameters.

In 1977 it was shown (23) that LDL FCR does not change with plasmapheresis in FH patients using a different approach: 7 days after reinjection of radiolabeled LDL a plasmapheresis was performed. This induced a shift but no change of the slope of the LDL decay curve. In another study Eriksson et al. (24) assumed that apoB metabolism remained unaffected by plasmapheresis and calculated metabolic parameters from the rebound of the plasma concentration after such a procedure. As these parameters are very close to those determined under steady-state conditions using a tracer technique, this study gave indirect evidence that apoB metabolism was not affected by plasmapheresis or apheresis. However, these studies evaluated the effect of a single apheresis or plasmapheresis procedure and it would be conceivable that only regularly performed apheresis results in changes of apoB metabolism. Our results show that even after 3–6 months of weekly therapy, apoB metabolism remains unchanged. Furthermore, previously published studies focused on LDL-apoB metabolism, while in this study the long-term effect of apheresis therapy on VLDL-, IDL-, and LDL-apoB metabolism was evaluated.

Our results indicate that 1 week after apheresis the rebound of plasma apoB concentration reflects the refilling of the apoB pool without change of metabolic parameters, i.e., FCR and secretion rate. As the same FCR results in a decreased absolute removal rate, if pool size is decreased, and secretion rate is unchanged, pool size will increase until the absolute removal rate is equal to the secretion rate, indicating steady-state.

In summary our studies show that 3–6 months of regular apheresis did not alter secretion or catabolism of apoB-containing lipoproteins in this group of patients with heterozygous FH. We conclude that the beneficial effect of apheresis is not counterregulated by changes in apoB metabolism.

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