Acute effects of low density lipoprotein apheresis on metabolic parameters of apolipoprotein B

Klaus G. Parhofer,[1,*] P. Hugh R. Barrett,[§] Thomas Demant,[†] and Peter Schwandt[†]

Department of Internal Medicine II* and Department of Clinical Chemistry,[†] Klinikum Grosshadern, Ludwig-Maximilians University, Marchioninistr. 15, 81377 Munich, Germany; and Department of Medicine,[§] University of Western Australia, Perth, Western Australia, Australia 6000

Abstract **Apheresis is a treatment option for patients with severe hypercholesterolemia and coronary artery disease. It is 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 acute effect of apheresis on metabolic parameters of apoB in five patients with drug-resistant hyperlipoproteinemia, using endogenous labeling with D_3-leucine, mass spectrometry, and multicompartmental modeling. Patients were studied prior to and immediately after apheresis therapy. The two tracer studies were modeled simultaneously, taking into account the non-steady-state concentrations of apoB. The low density lipoprotein (LDL)-apoB concentration was 120 ± 32 mg dl^{-1} prior to and 52 ± 18 mg dl^{-1} immediately after apheresis therapy. The metabolic studies indicate that no change in apoB secretion (13.9 ± 4.9 mg kg^{-1} day^{-1}) is required to fit the tracer and apoB mass data obtained before and after apheresis and that in four of the five patients the LDL-apoB FCR (0.21 ± 0.02 day^{-1}) was not altered after apheresis. In one subject the LDL-apoB FCR temporarily increased from 0.22 day^{-1} to 0.35 day^{-1} after apheresis. The conversion rate of very low density lipoprotein (VLDL)-apoB to LDL-apoB is temporarily decreased from 76 to 51% after apheresis and thus less LDL-apoB is produced after apheresis. We conclude that an acute reduction of LDL-apoB concentration does not affect apoB secretion or LDL-apoB FCR, but that apoB conversion to LDL is temporarily decreased. Thus, in most patients the decreased rate of delivery of neutral lipids or apoB to the liver does not result in an upregulation of LDL receptors or in decreased apoB secretion.**—Parhofer, K. G., P. H. R. Barrett, T. Demant, and P. Schwandt. **Acute effects of low density lipoprotein apheresis on metabolic parameters of apolipoprotein B.** ***J. Lipid Res.*** **2000.** 41: **1596–1603.**

Supplementary key words apheresis • lipoprotein metabolism • apoB-100 • non-steady-state kinetics • kinetics • compartmental models

In patients with drug-resistant hyperlipoproteinemia and coronary artery disease extracorporeal elimination of low density lipoprotein (LDL) by apheresis is a therapeutic option to lower elevated cholesterol levels. In such patients, apheresis is usually performed on a weekly or biweekly basis to achieve an average LDL-cholesterol concentration below 100 mg dl^{-1}. Different techniques are available for extracorporeal elimination of LDL particles, all of which are based on the elimination of apolipoprotein B (apoB)-containing lipoproteins (1). Clinical studies indicate that the decrease in cholesterol induced by apheresis has a beneficial influence on the course of coronary artery disease (1).

Apheresis also offers the opportunity to study the effects of exogenously induced modifications of plasma lipoprotein concentrations on apoB metabolism. We have previously shown that regular LDL apheresis does not alter apoB metabolism in the long term (2). From this study, however, it cannot be deduced whether or not LDL apheresis acutely and reversibly alters apoB metabolic parameters.

There is in vitro evidence that LDL receptor activity increases after apheresis (3). If the catabolic pathways of LDL operate at saturation at baseline (before apheresis) then a decrease in LDL pool size (status after apheresis) might be expected to increase the LDL fractional catabolic rate (FCR). However, even if the catabolic pathways are not saturated at baseline, an abrupt decrease in LDL plasma concentration will reduce the supply of lipid substrate to the liver. This in turn could result in an upregulation of LDL receptor activity and thus an increased FCR.

In the experiments described in this article we study apoB metabolism in five patients with drug-resistant hyperlipoproteinemia before and immediately after apheresis. ApoB metabolism was studied using endogenous labeling with D_3-leucine and multicompartmental modeling (4), taking into account the changing apoB pool size. The aim of the study was to determine whether acute changes in LDL-apoB concentration would affect apoB metabolic parameters.

Abbreviations: apo, apolipoprotein; FCR, fractional catabolic rate; FSR, fractional synthetic rate; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein.

[1] To whom correspondence should be addressed.

MATERIALS AND METHODS

Study protocol

Five male patients with drug-resistant hyperlipoproteinemia participated in the study. On the basis of clinical findings, family history, lipid concentrations, and exclusion of apoB-3500 (familial defective apoB), patients 2, 4, and 5 were classified as having heterozygous familial hypercholesterolemia, while patients 1 and 3 were diagnosed with severe combined hyperlipoproteinemia. The characteristics of these patients, including apoE phenotype, are shown in **Table 1**. All patients had coronary artery disease proven by angiography. In all patients LDL concentrations were resistant to dietary and drug therapy [no achievement of NCEP (National Cholesterol Education Program) goal (LDL-cholesterol $<$ 100 mg dl^{-1}) despite maximal combination therapy], and had therefore been included in our apheresis program (weekly apheresis). Patients with additional secondary causes of hyperlipidemia were excluded from this study. Four to 6 weeks prior to each kinetic study the patients stopped taking lipid-lowering drugs (simvastatin or atorvastatin), but continued their diet [AHA (American Heart Association) step 1 or 2] and other medications. In each patient apoB metabolism was studied twice. The first kinetic study was performed 7 days after the last apheresis, characterizing the status prior to apheresis. The apheresis scheduled for that day was canceled. The second kinetic study was started 2 h after the end of a regular, fully completed apheresis. The 2-h gap was chosen to allow plasma volume to adjust. The two studies were separated by at least 6 weeks. In patient 5 the order of the studies was reversed. None of the patients had a significant change in body weight between the two studies. Apheresis was performed by immunoadsorption (5) in three patients and heparin precipitation (6) in the other two patients. Both systems preferentially eliminate LDL and only to a small degree very low density lipoprotein (VLDL) and intermediate density lipoprotein (IDL). Immediately after apheresis LDL-cholesterol is decreased by approximately 60%, while serum triglycerides and HDL-cholesterol are decreased by 40 and 15% respectively (Table 1). Triglycerides and HDL-cholesterol return to baseline in less than 24 h (7).

Details of the procedure for the kinetic studies have been published previously (2, 4). After subjects had fasted for 10 h, a bolus of D_3-leucine (isotopic purity, 99%; Cambridge Isotope Laboratories, Woburn, MA) was given (5.0 mg kg^{-1}). After the leucine bolus, the subjects remained fasting for another 16 h, during which period 20 samples were drawn. The subjects were then allowed to continue their regular diet, and subsequent fasting samples were drawn over the next 3–7 days. Samples were drawn for assays of plasma amino acid and for VLDL-, IDL-, and LDL-apoB leucine enrichment. Aliquots for determination of VLDL-, IDL-, and LDL-apoB pool sizes were drawn on three to five occasions during the course of each kinetic study. The Ethics Committee of the Ludwig-Maximilians University (Munich, Germany) approved the study and all patients gave written informed consent.

Analytical methods

VLDL (d $<$ 1.006 g ml^{-1}), IDL (d 1.006–1.019 g ml^{-1}), and LDL (d 1.019–1.063 g ml^{-1}) were isolated by sequential ultracentrifugation as previously described (4). ApoB concentrations were measured in VLDL, IDL, and LDL fractions by immunonephelometry, using commercially available tests (Behring, Marburg, Germany). Cholesterol and triglycerides were measured using 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 $\times$ body weight). ApoB-100 was isolated at each time point from each lipoprotein fraction by precipitation with butanol-isopropyl ether as previously described (8). 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 of plasma after isolation by cation-exchange chromatography (AG50W-X8; Bio-Rad, Hercules, CA). Amino acids obtained from the plasma samples or from the hydrolyzed apoB precipitates were derivatized with *N-tert*-butyl-dimethylsilyl-*N*-methylfluoracetamide (Fluka, Buchs, Switzerland). Leucine isotope ratios were determined by gas chromatography-mass spectrometry (Trio 1000; Fisons Instruments, Manchester, UK) (9). Enrichment was calculated by the method of Cobelli, Toffolo, and Foster (10) and converted to tracer/tracee ratios.

Kinetic analysis

VLDL-, IDL-, and LDL-apoB tracer data were analyzed by a multicompartmental model (**Fig. 1**). The model is identical to the model used to describe the long-term effects of apheresis on apoB metabolism (2). The model consists of a precursor compartment (compartment 1, forcing function) 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

TABLE 1. Characteristics of study patients

Patient	Age	ApoE Phenotype	Cholesterol[a]	Triglycerides[a]	LDL-Chol.[a]	HDL-Chol.[a]	LDL-Chol.[b]	LDL-Chol.[c]
	y				*mg dl^{-1}*			
1	61	3/3	311	119	246	41	196	59
2	43	3/4	469	221	387	38	245	112
3	58	3/4	298	414	193	23	126	56
4	47	3/3	233	84	173	42	138	60
5	51	3/3	328	86	272	39	220	105
Mean ± SD	52 ± 8	NA	328 ± 87	185 ± 140	254 ± 84	37 ± 8	185 ± 52	79 ± 29

[a] Concentrations were obtained before regular apheresis therapy was started; in subjects 2 and 5 concentrations were obtained without concomitant lipid-lowering drug therapy, while patients 1 and 3 were receiving lovastatin (40 mg day^{-1}) and patient 4 was receiving simvastatin (40 mg day^{-1}) therapy; however, all patients were without concomitant lipid-lowering drug therapy during both turnover studies.

[b] LDL-cholesterol concentration during preapheresis study (7 days after last apheresis).

[c] LDL-cholesterol concentration at beginning of postapheresis study; at that time patients were without concomitant lipid-lowering drug therapy for 4–6 weeks.

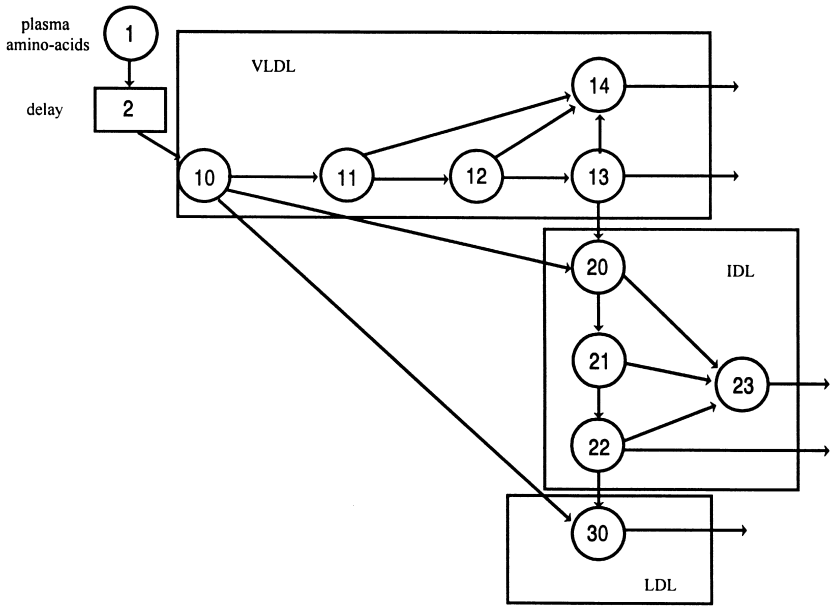

Fig. 1. Multicompartmental model of apoB metabolism. Compartment 1, plasma leucine tracer/tracee ratio (forcing function); compartment 2, delay compartment (synthesis of apoB and secretion); compartments 10 through 14, VLDL-apoB; compartments 20 through 23, IDL-apoB; compartment 30, LDL-apoB (for details see Materials and Methods).

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 turning over VLDL compartment (compartment 14) or to the next compartment of the chain. As in previous models using a delipidation chain (11–14) 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 (15). 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, that is, 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 four-compartment model was used to fit the plasma leucine tracer/tracee ratios. These parameters were then used as a forcing function in the apoB model.

The SAAM II program (SAAM Institute, Seattle, WA) was used to fit the model to the observed tracer data. Metabolic parameters for apoB-100 are 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 individual 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.

In those studies performed immediately after apheresis the apoB pool size (plasma concentration) increased throughout the study. To account for this non-steady-state condition, the apoB tracer data were modeled simultaneously with the apoB mass data. Therefore, the sample points in the model, corresponding to VLDL, IDL, and LDL tracer/tracee data, were func-

TABLE 2. ApoB concentration in VLDL and LDL fractions

	VLDL-ApoB		LDL-ApoB			
Patient	Preapheresis	Postapheresis	Preapheresis	Postapheresis[a] (2 h)	Postapheresis[b] (45–65 h)	Postapheresis[b] (108–145 h)
	mg dl^{-1}		*mg dl*$^{-1}$			
1	4.5	4.5	136	52	83	116
2	8.7	5.9	143	68	110	138
3	18.1	3.8	110	36	48	82
4	5.1	5.1	68	32	58	74
5	5.4	5.4	145	72	128	143
Mean ± SD	8.4 ± 5.7	4.9 ± 0.8	120 ± 32	52 ± 18	85 ± 34	111 ± 32

[a] LDL-apoB concentration 2 h after completion of apheresis (start of kinetic study).
[b] LDL-apoB concentration determined 45–65 h (108–145 h, respectively) after completion of apheresis.

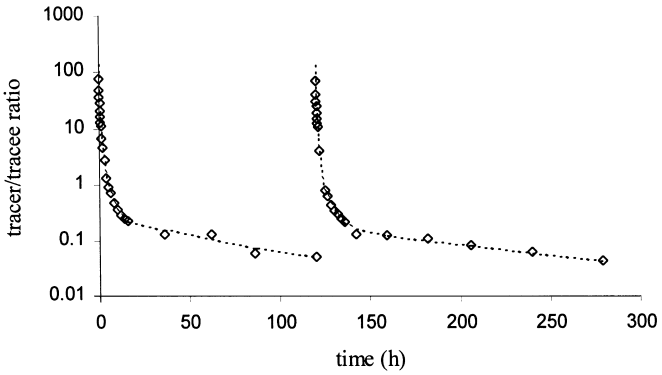

Fig. 2. Plasma leucine tracer/tracee ratio in a representative subject (patient 3). Observed values (symbols) and model-predicted values (lines) are shown. Although the two tracer studies were separated by 6 weeks it was assumed for the modeling that the apheresis was performed at 120 h, and thus the data between 0 and 119 h represent preapheresis data, while the data shown between 120 and 280 h represent postapheresis data.

tions of the tracer mass in each compartment divided by the mass of apoB (leucine equivalent) in the corresponding compartment.

Nonlinear fractional rate constants were not required to fit the tracer/tracee data obtained before apheresis (7 days after last apheresis). Because apheresis reduces LDL concentration, the FCR of LDL-apoB may also change. To account for this possibility a nonlinear fractional rate constant was included in the compartment model to fit the data obtained immediately after apheresis. The parameters determined with the study before apheresis were used as baseline values for the study performed after apheresis. It was assumed that the LDL-apoB FCR may increase temporarily and return to baseline (preapheresis study). The following function describes possible increases in LDL-apoB FCR:

$$k(0,30) = A_1 + A_2\exp^{(-at)} \qquad \textit{Eq. 1)}$$

where $k(0, 30)$ equals the LDL-apoB FCR, A_1 equals the LDL-apoB FCR at baseline, A_2 equals the incremental increase in the LDL-apoB FCR, $A_1 + A_2$ equals the maximal LDL-apoB FCR after apheresis, and a is an exponent describing how rapidly the LDL-apoB FCR returns to baseline. Both the maximal FCR and the rate at which the FCR returns to baseline are adjustable parameters estimated during model fitting.

RESULTS

ApoB concentrations obtained during both kinetic studies are shown in **Table 2**. Although LDL-apoB concentrations were increasing during the turnover study performed immediately after apheresis in all patients, there was some variation with respect to the rebound kinetics (Table 2).

Plasma leucine tracer/tracee ratios were similar in all subjects and did not differ from previously published data in hyperlipidemic and normolipidemic subjects (4, 16). Furthermore, in all five patients plasma leucine tracer/tracee ratio curves were not different between the two kinetic studies (data for subject 3 are shown in **Fig. 2**).

For each patient the preapheresis tracer/tracee data were analyzed first. Linear fractional rate constants provided good model fits to the tracer and apoB concentration data. The postapheresis study was analyzed with the parameters obtained during the preapheresis study as baseline values. Furthermore, the LDL-apoB FCR could increase transiently above that value (returning to baseline with time), and thus be nonlinear. However, only in one of the five patients was an increase in LDL-apoB FCR necessary to simultaneously describe the tracer and apoB mass data (**Table 3**). In this patient (patient 1), the LDL-apoB FCR temporarily increased from 0.22 day^{-1} to 0.35 day^{-1} but returned to baseline within 4 days. In the four other patients a constant LDL-apoB FCR described both sets of tracer and apoB mass data (Table 3, **Fig. 3**). In all subjects, the LDL-apoB leucine tracer/tracee ratio increased to a higher value in the postapheresis study, reflecting the smaller pool of LDL-apoB postapheresis.

While the FCR and fractional synthetic rate (FSR) are identical in the steady state these parameters may differ in non-steady-state situations such as described here. The FCR as expressed above refers to the fraction of apoB being removed irreversibly from the LDL fraction, while the FSR refers to the ratio of LDL-apoB production (flux

TABLE 3. Metabolic parameters

Patient	Secretion	FCR			Conversion (VLDL to LDL)[c]	
		VLDL-ApoB	LDL-ApoB Preapheresis	LDL-ApoB Postapheresis	Preapheresis	Postapheresis
	mg kg^{-1} day^{-1}		*day^{-1}*		*%*	
1	12.5	6.96	0.22	0.35	97	61
2	22.2[a]	6.38[b]	0.23	0.23	61	97
3	13.9	1.93[b]	0.21	0.21	67	16
4	10.7	5.28	0.22	0.22	56	36
5	10.0	4.65	0.17	0.17	97	46
Mean ± SD	13.9 ± 4.9	5.04 ± 1.96	0.21 ± 0.02	0.24 ± 0.07	76 ± 20	51 ± 30

Metabolic parameters were obtained by modeling of tracer and mass data; FCR, fractional catabolic rate; conversion relates to percentage of apoB that is converted from VLDL to LDL.

[a] ApoB production was different during both studies (22.2 vs. 15.8 mg kg^{-1} day^{-1}).

[b] VLDL-apoB FCR was different during both studies (6.38 vs. 6.69 day^{-1} and 1.93 vs. 9.12 day^{-1}) because VLDL-apoB concentrations changed (Table 2; for details see text).

[c] Fraction of secreted apoB that eventually reaches the LDL fraction.

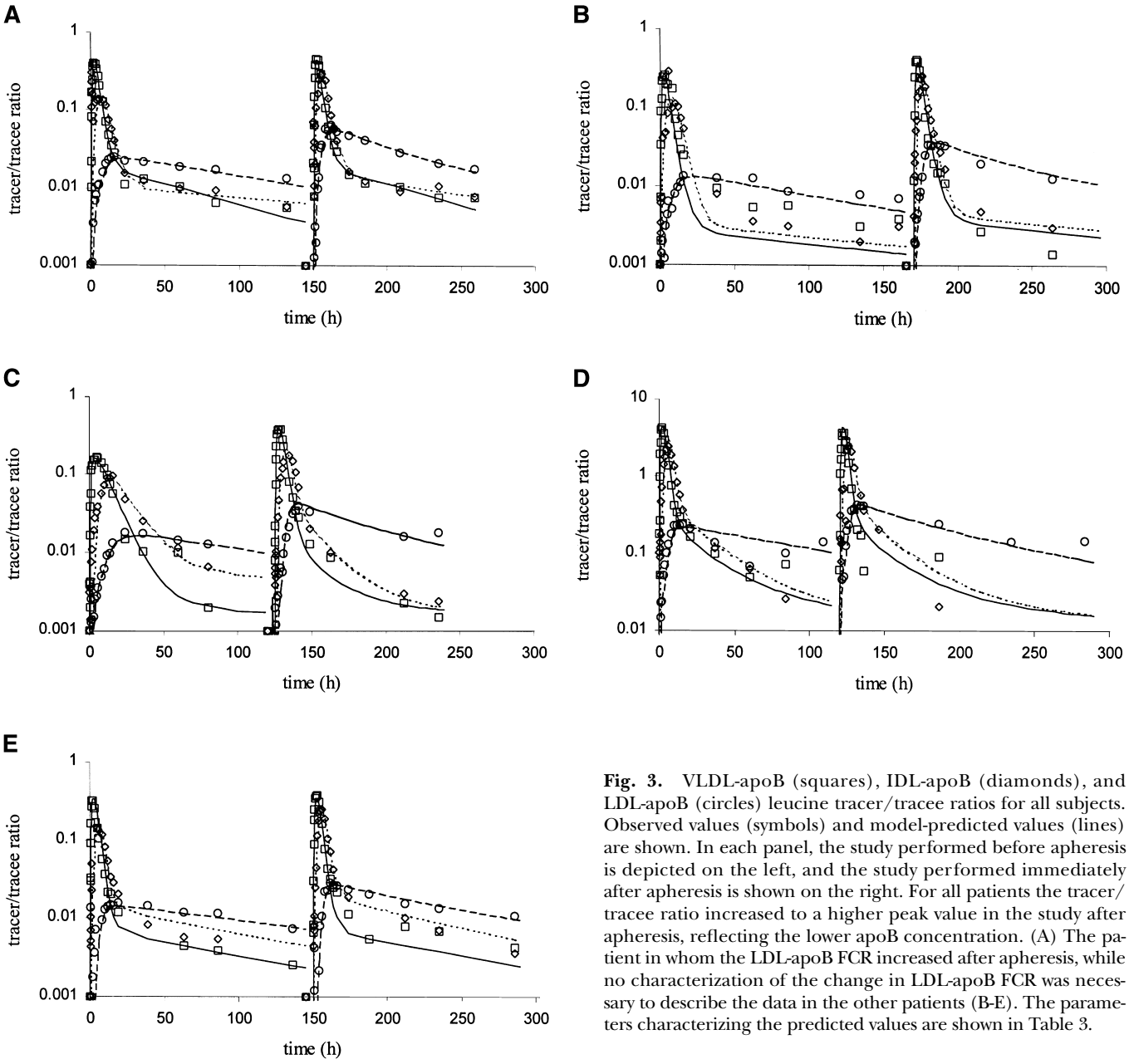

Fig. 3. VLDL-apoB (squares), IDL-apoB (diamonds), and LDL-apoB (circles) leucine tracer/tracee ratios for all subjects. Observed values (symbols) and model-predicted values (lines) are shown. In each panel, the study performed before apheresis is depicted on the left, and the study performed immediately after apheresis is shown on the right. For all patients the tracer/tracee ratio increased to a higher peak value in the study after apheresis, reflecting the lower apoB concentration. (A) The patient in whom the LDL-apoB FCR increased after apheresis, while no characterization of the change in LDL-apoB FCR was necessary to describe the data in the other patients (B-E). The parameters characterizing the predicted values are shown in Table 3.

of apoB into the LDL fraction) and the LDL pool size. Although the FCR remains constant in four patients, FSR increases after apheresis (0.21 ± 0.02 vs. 0.31 ± 0.17 day^{-1}). The FSR returns to baseline as LDL-apoB concentration reaches steady state.

Because of the non-steady-state conditions after apheresis the net amount of apoB leaving the LDL fraction is decreased postapheresis (**Table 4**). Thereafter, this flux gradually increases and returns to baseline. The LDL-apoB concentration rebounds because the flux of apoB from the LDL fraction (FCR × pool size) is less than the flux of apoB into the LDL fraction from VLDL and IDL (apoB secretion × conversion rate).

The fraction of VLDL-apoB secreted that eventually reaches the LDL fraction decreased after apheresis in four of five patients (76 ± 20% vs. 51 ± 30%). At the same time the amount of VLDL-apoB irreversibly removed from plasma and thus not converted to LDL-apoB increased. In one patient (patient 2) whose VLDL-apoB concentration decreased after apheresis, VLDL-apoB production also fell. In this patient the conversion rate of VLDL- to LDL-

TABLE 4. ApoB flux from the LDL fraction (mg kg^{-1} day^{-1})

		Postapheresis		
Patient	Preapheresis	After 2 h	After 24 h	After 48 h
1	12.1	7.4	7.5	8.7
2	13.4	6.3	9.5	11.1
3	9.4	3.1	4.5	5.5
4	6.0	2.8	3.9	4.8
5	9.7	4.8	6.2	7.2
Mean ± SD	10.1 ± 2.8	4.9 ± 2.0	6.3 ± 2.3	7.5 ± 2.5

apoB increased and the amount of apoB removed from plasma as VLDL decreased. In another patient (patient 3), who also displayed a fall in VLDL-apoB concentration, VLDL apoB production did not change (Table 3).

DISCUSSION

In this study we describe the acute effects of LDL apheresis on apoB metabolism. In four of five patients the acute reduction in LDL-apoB mass by almost 60% did not affect apoB secretion or catabolism. In one subject the LDL-apoB FCR temporarily increased by 50%. Furthermore, the fraction of secreted apoB that finally reaches the LDL fraction is temporarily reduced after apheresis, from 76 to 51%, and thus LDL production is temporarily decreased.

LDL apheresis results in an acute reduction in LDL pool size that decreases the flux of lipid substrate to the liver. Theoretically, this could induce an upregulation of LDL receptors and thus an increased LDL-apoB FCR. Alternatively, a decreased flux of lipid to the liver could also result in a decreased secretion of apoB-containing lipoproteins. Finally, intrahepatic regulatory mechanisms could compensate for the decreased net flux of lipid. If such compensation were complete, no changes in intravascular apoB metabolism would be observed. Our results indicate that in most patients the acute reduction in plasma LDL-apoB neither upregulates LDL receptors nor downregulates apoB secretion, which is consistent with previous studies describing an upregulation of intrahepatic lipid synthesis after apheresis (17, 18). However, this also indicates that acute regulation of the LDL receptor is not coupled to that of cholesterol synthesis in these patients with low basal LDL-apoB FCR. In patients with normal LDL receptor function this may be different and it cannot be excluded that an acute change in LDL concentration will result in an increased removal of the remaining LDL.

The finding that less VLDL-apoB is converted to LDL-apoB after apheresis probably relates to the fact that VLDL and LDL particles are competing for the same removal mechanisms (19). Because after apheresis there are relatively more VLDL than LDL particles (compared with baseline), VLDL are preferentially removed from plasma rather than converted to LDL, and thus less apoB reaches the LDL fraction.

The study also emphasizes that it is necessary to strictly differentiate between FCR and FSR in non-steady-state situations, such as the one examined here. While the FCR (the fraction of apoB being irreversibly removed from the LDL fraction) remains constant in four of the five patients, the FSR (ratio of apoB flux into the LDL fraction to apoB mass in LDL fraction) necessarily increases after apheresis to warrant the rebound of LDL-apoB concentration. The FSR is increased despite the decreased conversion rate of VLDL to LDL. The change in conversion rate (VLDL to LDL) also affects LDL-apoB production. While total apoB secretion remains constant after apheresis, LDL production (in absolute terms) is reduced and returns to baseline to the extent that the VLDL-to-LDL conversion rate returns to baseline.

The question of whether lipoprotein concentration regulates apoB secretion has also been evaluated with in vitro experiments (20), although the results are not unequivocal. In vivo, this hypothesis can be addressed only by using a system in which apoB is removed exogenously from the plasma, because changes induced by drugs or diet (endogenous changes) necessarily result in altered metabolic parameters. Compared with in vitro experiments, however, only the effect of relatively small changes in apoB concentration can be studied in vivo.

A previously published study looked at the effects of a single apheresis on apoB metabolism, using a similar approach (21). Immediately after apheresis the secretion rate was unchanged, but the FCR was described as being increased. However, the non-steady-state conditions were not taken into consideration in interpreting the tracer data. If steady state is erroneously assumed, the FCR or secretion rate must necessarily change, if pool sizes changes.

By 1977 it had already been shown (22), using a different approach, that the LDL FCR does not change with plasmapheresis in patients with familial hypercholesterolemia: 7 days after reinjection of radiolabeled LDL a plasmapheresis was performed. This induced a shift but no change in the slope of the LDL decay curve. In another study Eriksson et al. (23) assumed that apoB metabolism remains unaffected by plasmapheresis and calculated metabolic parameters from the rebound of the plasma concentration after such a procedure. Because these parameters were close to those determined under steady state conditions using a tracer technique, this study gave indirect evidence that apoB metabolism is not affected by plasmapheresis or apheresis. On the other hand, in a previous study we have shown that LDL-apoB parameters established from the LDL-apoB rebound after apheresis and those established with tracer technology in the same patients are not concordant, the most likely explanation being that apheresis affects parameters of apoB metabolism (24). And, indeed, while plasma apoB secretion is constant, LDL-apoB production is decreased after apheresis because less VLDL is converted to LDL.

While we could not find any differences in rate constants established before and after apheresis therapy, we cannot exclude the possibility that differences in some parameters may become significant only if considerably larger groups of patients are studied. Furthermore, the methodology used in these studies may not be sensitive enough to detect small changes in the LDL FCR (25). Because LDL-apoB metabolism could be described only by a one-pool model (a more complex model was not supported by the data), subtle changes in LDL-apoB metabolism may also be missed. Furthermore, given the presence of noise in the data, it is necessary to use data obtained over a reasonable time period to estimate the LDL-apoB FCR with precision. Therefore, changes in parameter values occurring over short periods of time may not be detected. In addition, the removal pathways from the VLDL

and IDL fractions could also be nonlinear, however; this was not required to fit the experimental data.

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. Such a model has been used previously to describe apoB metabolism in patients undergoing apheresis therapy (2). The kinetic parameters established in this study are well within the range of previously published parameters of apoB metabolism in patients with hyperlipoproteinemia (2, 24, 26–28). The results of studies using endogenous and/or exogenous tracers indicate that in patients with heterozygous familial hypercholesterolemia the LDL-apoB FCR is consistently low (0.1–0.3 day^{-1} vs. 0.3–0.7 day^{-1} in normal subjects), while in patients with other forms of hyperlipoproteinemia the results of kinetic studies are not as clear. Overproduction and/or decreased catabolism of LDL usually characterize these patients. In the patients described here, apoB secretion is in the normal range while the LDL-apoB FCR is decreased, consistent with the diagnosis of heterozygous familial hypercholesterolemia or severe combined hyperlipoproteinemia.

The results of this study indicate that an acute reduction of LDL-apoB does not affect the apoB secretion rate and the VLDL- or LDL-apoB FCR. However, more VLDL are removed from plasma and fewer are converted to LDL compared with the preapheresis state, and thus the production rate of LDL-apoB is decreased.

In summary, our studies show that in most patients apheresis does not acutely alter secretion or catabolism of apoB-containing lipoproteins. In one patient the LDL-apoB FCR was temporarily increased after apheresis, probably reflecting an upregulation of LDL receptor activity, which may be one possible compensatory mechanism. This mechanism, however, may be activated only if intrahepatic mechanisms fail to compensate for the decreased net flux of lipid to the liver induced by apheresis.

We appreciate the help of our apheresis patients and the staff of the apheresis unit at Ludwig-Maximilians University Medical Department II. We are indebted to Ms. E. Berger for excellent technical assistance in preparing the samples for GC–MS and to Ms. G. Schwertfeger and A. Bedynek for the GC–MS analysis. This research was supported by grants from the Deutsche Forschungsgemeinschaft to K.G.P. (Pa 344/2-1) and T.D. (De 359/1-2) and from the National Institutes of Health to P.H.R.B. (RR12609).

Manuscript received 20 January 2000 and in revised form 30 May 2000.

REFERENCES

1. Kajinami, K., and H. Mabuchi. 1999. Therapeutic effects of LDL apheresis in the prevention of atherosclerosis. *Curr. Opin. Lipidol.* **10:** 401–406.
2. Parhofer, K. G., P. H. R. Barrett, T. Demant, W. O. Richter, and P. Schwandt. 1996. Effects of weekly LDL apheresis on metabolic parameters of apolipoprotein B in heterozygous familial hypercholesterolemia. *J. Lipid Res.* **37:** 2383–2393.
3. Streicher, J., P. Valent, H. Schmidt, G. Sengologe, O. Wagner, W. Strobl, W. H. Horl, and K. Derfler. 1999. Up-regulation of LDL-receptor expression by LDL-immunoapheresis in patients with familial hypercholesterolemia. *J. Invest. Med.* **47:** 378–387.
4. Parhofer, K. G., P. H. R. Barrett, D. M. Bier, and G. Schonfeld. 1991. Determination of kinetic parameters of apolipoprotein B metabolism using amino acids labeled with stable isotopes. *J. Lipid Res.* **32:** 1311–1323.
5. Stoffel, W., V. Greve, and H. Borberg. 1981. Application of specific extracorporeal removal of low density lipoprotein in familial hypercholesterolemia. *Lancet.* **2:** 1005–1007.
6. Seidel, D., V. W. Armstrong, and P. Schuff-Werner. 1991. The HELP LDL Apheresis Multicenter Study, an angiographically assessed trial on the role of LDL-apheresis in the secondary prevention of coronary heart disease. I. Evaluation of safety and cholesterol lowering effects during the first 12 months. *Eur. J. Clin. Invest.* **21:** 375–383.
7. Richter, W. O., B. G. Jacob, M. M. Ritter, K. Suhler, K. Vierneisel, and P. Schwandt. 1993. Three-year treatment of familial heterozygous hypercholesterolemia by extracorporeal low-density lipoprotein immunoadsorption with polyclonal apolipoprotein-B antibodies. *Metabolism.* **42:** 888–894.
8. Klein, R. L., and D. B. Zilversmit. 1984. Direct determination of human and rabbit apolipoprotein B selectively precipitated with butanol-isopropyl ether. *J. Lipid Res.* **25:** 1380–1386.
9. Demant, T., C. J. Packard, P. Stewart, A. Bedynek, A. G. Calder, J. Shepherd, and D. Seidel. 1994. Sensitive mass spectrometry techniques for measuring metabolism of human apolipoprotein B in vivo. *Clin. Chem.* **40:** 1825–1827.
10. Cobelli, C., G. Toffolo, and D. M. Foster 1992. Tracer to tracee ratio for analysis of stable isotope tracer data: link with radioactive kinetic formalism. *Am. J. Physiol.* **262:** E968–E975.
11. Beltz, W. F., Y. A. Kesaeniemi, B. V. Howard, and S. M. Grundy. 1985. Development of an integrated model for analysis of the kinetics of apolipoprotein B in plasma very low density lipoproteins, itermediate density lipoproteins, and low density lipoproteins. *J. Clin. Invest.* **76:** 575–585.
12. Demant, T., C. J. Packard, H. Demmelmair, P. Stewart, A. Bedynek, D. Bedford, D. Seidel, and J. Shepard 1996. Sensitive methods to study human apolipoprotein B metabolism using stable isotope-labeled amino acids. *Am. J. Physiol.* **270** (*Endocrinol. Metab.* **33**)**:** 1022–1036.
13. Kesaniemi, Y. A., G. L. Vega, and S. M. Grundy 1982. Kinetics of apolipoprotein B in normal and hyperlipidemic man: review of current data. *In* Lipoprotein Kinetics and Modeling. M. Berman, S. M. Grundy, and B. V. Howard, editors. Academic Press, New York. 181–205.
14. Phair, R. D., M. G. Hammond, J. A. Bowden, M. Fried, W. A. Fisher, and M. Berman. 1975. A preliminary model for human lipoprotein metabolism in hyperlipoproteinemia. *Fed. Proc.* **34:** 2263–2270.
15. Cobelli, C., and J. DiStefano III. 1980. Parameter and structural identifiability concepts and ambiguities: a critical review and analysis. *Am. J. Physiol.* **239:** R7–R24.
16. Parhofer, K. G., P. H. R. Barrett, J. Dunn, and G. Schonfeld. 1993. Effect of pravastatin on metabolic parameters of apolipoprotein-B in patients with mixed hyperlipoproteinemia. *Clin. Invest.* **71:** 939–946.
17. Pfohl, M., R. P. Naoumova, C. Klass, W. Knisel, B. Jakober, T. Risler, and G. R. Thompson. 1994. Acute and chronic effects on cholesterol biosynthesis of LDL-apheresis with or without concomitant HMG-CoA reductase inhibitor therapy. *J. Lipid Res.* **35:** 1946–1955.
18. Pfohl, M., R. P. Naoumova, C. Neuwirth, A. Sussekov, J. Smykowski, N. B. Rendell, G. W. Taylor, F. J. Seif, and G. R. Thompson. 1997. Upregulation of cholesterol synthesis after acute reduction of low density lipoprotein by apheresis in normocholesterolaemic subjects: evidence for a threshold effect. *Atherosclerosis.* **135:** 257–262.
19. Mamotte, C. D. S., M. Sturm, J. F. Foo, and R. R. Bockxmeer Taylor. 1999. Comparison of the LDL-receptor binding of VLDL and LDL from apoE4 and apoE3 homozygotes. *Am. J. Physiol.* **276:** E553–E557.
20. Dixon, J. L., and H. N. Ginsberg. 1993. Regulation of hepatic secretion of apolipoprotein B containing lipoproteins information obtained from cultured liver cells. *J. Lipid Res.* **34:** 167–179.

21. Arends, J., D. M. Bier, G. Schafer, V. W. Armstrong, J. Thiery, D. Seidel, and P. Schauder. 1993. No evidence for feedback inhibition of hepatic apolipoprotein-B (apo-B) production after extracorporeal low density lipoprotein precipitation as determined by [1-C-13]leucine infusion in normal volunteers. *Eur. J. Clin. Invest.* **23:** 602–614.
22. Thompson, G. R., T. Spinks, A. Ranicar, and N. B. Myant. 1977. Non-steady-state studies of low-density lipoprotein turnover in familial hypercholesterolemia. *Clin. Sci. Mol. Med.* **52:** 361–369.
23. Eriksson, M., L. Berglund, J. Gabrielsson, B. Lantz, and B. Angelin. 1993. Non-steady-state kinetics of low density lipoproteins in man—studies after plasma exchange in healthy subjects and patients with familial hypercholesterolaemia. *Eur. J. Clin. Invest.* **23:** 746–752.
24. Parhofer, K. G., P. H. R. Barrett, and P. Schwandt. 1999. LDL apolipoprotein B metabolism: comparison of two methods to establish kinetic parameters. *Atherosclerosis.* **144:** 159–166.
25. Barrett, P. H. R., and K. G. Parhofer. 1998. Low-density lipoprotein-apolipoprotein B metabolism following apheresis: simulation studies of mass changes and tracer kinetics. *Metabolism.* **47:** 478–483.
26. Aguilar-Salinas, C. A., P. H. R. Barrett, J. Pulai, X. L. Zhu, and G. Schonfeld. 1997. A familial combined hyperlipidemic kindred with impaired apolipoprotein B catabolism. Kinetics of apolipoprotein B during placebo and pravastatin therapy. *Arterioscler. Thromb. Vasc. Biol.* **17:** 72–82.
27. Vega, G. L., M. A. Denke, and S. M. Grundy. 1991. Metabolic basis of primary hypercholesterolemia. *Circulation.* **84:** 118–128.
28. Fisher, W. R., L. A. Zech, L. L. Kilgore, and P. W. Stacpoole. 1991. Metabolic pathways of apolipoprotein B in heterozygous familial hypercholesterolemia: studies with a [^{3}H]leucine tracer. *J. Lipid Res.* **32:** 1823–1836.