In vivo metabolism of LDL subfractions in patients with heterozygous FH on statin therapy: rebound analysis of LDL subfractions after LDL apheresis

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Abstract **LDL can be subfractionated into buoyant (1.020–1.029 g/ml^{-1}), intermediate (1.030–1.040 g/ml^{-1}), and dense (1.041–1.066 g/ml^{-1}) LDLs. We studied the rebound of these LDL-subfractions after LDL apheresis in seven patients with heterozygous familial hypercholesterolemia (FH) regularly treated by apheresis (58 ± 9 years, LDL-cholesterol = 342 ± 87 mg/dl^{-1}, triglycerides = 109 ± 39 mg/dl^{-1}) and high-dose statins. Apolipoprotein B (apoB) concentrations were measured in LDL subfractions immediately after and on days 1, 2, 3, 5, and 7 after apheresis. Compartmental models were developed to test three hypotheses: *1)* that dense LDLs are derived from the delipidation of buoyant and intermediate LDLs (model A); *2)* that dense LDLs are generated directly from LDL-precursors (model B); or *3)* that a model combining both pathways (model C) is necessary to describe the metabolism of dense LDLs. In all models, it was assumed that apoB production and fractional catabolic rate (FCR) did not change with apheresis. Apheresis decreased buoyant, intermediate, and dense LDL-apoB by 60 ± 12%, 67 ± 5%, and 69 ± 11%, respectively. Models B and C, but not model A, described the rebound data. The model with the greatest biological plausibility (model C) was used to estimate metabolic parameters. FCR was 1.05 ± 0.86 d^{-1}, 0.48 ± 0.11 d^{-1}, and 0.69 ± 0.24 d^{-1} for buoyant, intermediate, and dense LDLs, respectively. Dense LDL production was 17.3 ± 0.2 mg/kg^{-1}/d^{-1}, 58% of which was derived directly from LDL precursors (VLDL, IDL, or direct secretion), while 42% was derived from buoyant and intermediate LDLs. Thus, our data indicate that in statin-treated patients with heterozygous FH dense LDLs originate from two sources. Whether this is also valid in other metabolic situations (with predominant small, dense LDLs) remains to be determined.**—Geiss, H. C., S. Bremer, P. H. R. Barrett, C. Otto, and K. G. Parhofer. **In vivo metabolism of LDL subfractions in patients with heterozygous FH on statin therapy: rebound analysis of LDL subfractions after LDL apheresis. *J. Lipid Res.* 2004.** 45: **1459–1467.**

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LDLs are a heterogeneous group of particles that can be separated into several subfractions by density gradient ultracentrifugation (1) or gradient gel electrophoresis (2). In general, LDL particles can be subfractionated into buoyant, intermediate, and dense LDLs. In vitro experiments (3–5) and epidemiologic studies (6–12) have shown that buoyant LDLs (12) and particularly dense LDLs (6–11) are more atherogenic than intermediate LDLs. Such findings may be partly explained by the association of dense LDLs with increased levels of plasma triglycerides and decreased levels of HDL-cholesterol (8). This association is also the basis of the ongoing controversy about the independent relationship of small, dense LDLs to atherosclerotic disease (13). On the other hand, there is also evidence that additional mechanisms [e.g., higher susceptibility to oxidation (3, 14) and higher capacity to bind to intimal proteoglycans (4)] contribute to the atherogenicity of small, dense LDLs.

The origin of LDL subfractions, especially the formation of proatherogenic dense LDLs, is not fully understood, but tracer studies indicate that different metabolic pathways may be involved (15–21). Campos et al. (17) suggest that dense LDLs are derived from precursor particles such as VLDLs or intermediate density lipoproteins (IDLs), whereas data from Aguilar-Salinas et al. (18) point to the production of dense LDLs from delipidation of

Abbreviations: AIC, Akaike information criterion; apoB, apolipoprotein B; DALI, direct absorption of lipoproteins; FCR, fractional catabolic rate; FH, familial hypercholesterolemia; HELP, heparin-induced extracorporeal LDL precipitation; IDL, intermediate density lipoprotein; PR, production rate.

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larger LDL subtypes. Other studies, however, suggest that dense LDLs may be derived from a combination of these metabolic pathways (19–21). As the results from tracer studies are discordant with regard to LDL subfraction metabolism, especially dense LDL formation, we used a different approach to determine the mechanism by which dense LDLs are formed: we observed the rebound of LDL subfractions after LDL apheresis. This approach is based upon the concept that a steady-state condition altered by a defined perturbation (e.g., LDL apheresis) will be ultimately restored and that the dynamics of the return to steady state will depend only on the fractional catabolic rate (FCR) (22–25), assuming no change in secretion rate.

The rationale for applying such a method comes from recent observations that each LDL subfraction can be removed effectively from plasma by LDL apheresis independent of the apheresis method (26, 27). Furthermore, LDL apheresis induces a shift in the LDL subfraction distribution with a relative increase of buoyant LDL and decrease of dense LDL immediately after treatment. This shift very likely reflects differences in the rate of rebound between dense and less dense LDL subfractions (26).

We therefore measured the rebound of LDL subfractions after LDL apheresis in seven patients with severe heterozygous familial hypercholesterolemia (FH) and coronary heart disease treated with statins and regular LDL apheresis. Compartment models were developed to describe apolipoprotein B (apoB) rebound data. The models included the formation of dense LDLs by delipidation of less dense LDL subfractions (model A); the direct production of buoyant, intermediate, and dense LDLs from LDL precursors (e.g., VLDL/IDL) (model B); and a model including both pathways (model C). The best fitting model provided metabolic parameters, including FCR and production rates (PRs), for buoyant, intermediate, and dense LDL subfractions.

METHODS

Design and patients

We examined seven patients with heterozygous FH and coronary heart disease (age 58 ± 9 years; four males and three females; concentrations before beginning apheresis therapy: cholesterol, 408 ± 102 mg/dl; LDL-cholesterol, 342 ± 87 mg/dl; triglycerides, 109 ± 39 mg/dl; HDL-cholesterol, 46 ± 12 mg/dl) regularly treated by LDL apheresis at weekly intervals for at least 2 years. All patients adhered to a lipid-lowering diet, and six of them were additionally treated by statins at maximal tolerable doses (atorvastatin, n = 5; simvastatin, n = 1). In all patients, apoB mutations were excluded and heterozygous FH was diagnosed on a clinical basis. The protocol was approved by the ethics committee of the university, and all patients gave written informed consent.

The rebound of seven LDL subfractions was determined from six serial measurements after LDL apheresis. Cholesterol and apo-B concentrations were determined in all seven LDL subfractions immediately after LDL apheresis and on days 1, 2, 3, 5, and 7 after apheresis.

Apheresis techniques

Two patients were treated by LDL hemoperfusion [direct absorption of lipoproteins (DALI) system; Fresenius, St. Wendel, Germany], which eliminates LDL particles from whole blood using a polyacrylate adsorber (28). In the remaining patients, plasma was separated from whole blood by a plasma filter or by centrifugation (29) and LDL elimination was done from plasma using immunoadsorption (n = 1; column with polyclonal anti-human apoB antibodies coupled to Sepharose; Plasmaselect, Teterow, Germany), heparin-induced extracorporeal LDL precipitation (HELP) apheresis (n = 2; LDL precipitation by acetic acid buffer and heparin; Braun, Melsungen, Germany), and dextran sulfate adsorption (n = 2; columns with cellulose-bound dextran sulfate; Kaneka, Osaka, Japan). In the patients treated by HELP, dextran sulfate adsorption and immunoadsorption anticoagulation were performed with heparin (1,000–5,500 IU as a bolus and up to 3,000 IU/h continuously); in the patients treated by the DALI system, citrate dextrose solution was given as recommended (28). The duration of LDL apheresis was stan-

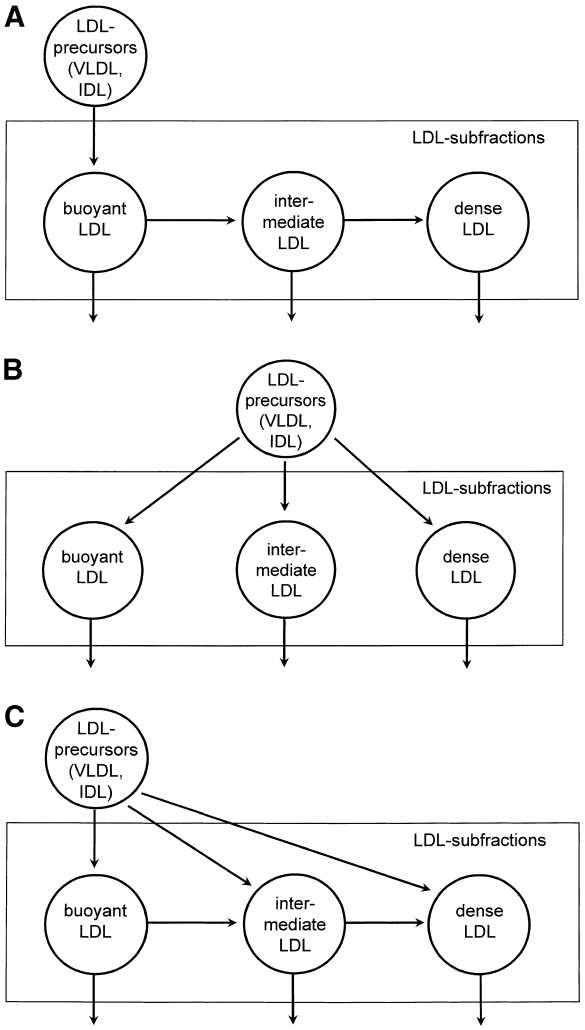

Fig. 1. Models to describe the rebound of apolipoprotein B (apoB) in different LDL subfractions. In model A (A), it is assumed that intermediate LDLs and dense LDLs are formed by the delipidation of buoyant LDLs. In model B (B), LDL subfractions are formed independently from each other from LDL precursors, such as VLDLs and/or intermediate density lipoprotein (IDLs). Model C (C) represents a combination of both models.

TABLE 1. Plasma lipid concentrations before and immediately after LDL apheresis

Patient	Cholesterol		LDL-Cholesterol		apoB		VLDL/IDL-apoB	
	Before	After	Before	After	Before	After	Before	After
	mg/dl							
A.W.	288	103	215	47	176	41	21	5.3
F.E.	275	103	213	63	167	49	41	11
K.K.	241	117	167	56	138	43	5.0	2.2
L.B.	254	101	176	55	130	36	32	14
S.G.	282	118	165	33	171	52	8	3.7
S.K.	206	117	137	56	124	59	24	11
S.P.	237	98	171	50	130	40	17	7
Mean ± SD	255 ± 29	108 ± 9	178 ± 28	51 ± 10	148 ± 22	45 ± 8	17 ± 12	5 ± 3

apoB, apolipoprotein B; IDL, intermediate density lipoprotein.

dardized such that a postapheresis LDL concentration of 50–60 mg/dl was reached corresponding to a treatment time between 1.15 and 3.20 h and a plasma volume between 2,500 and 6,000 ml.

Preparative and analytical methods

Fasting blood was taken in EDTA-containing tubes immediately before and after apheresis as well as at days 1, 2, 3, 5, and 7 after treatment. After centrifugation at 3,000 rpm for 10 min, plasma was stored at 4°C. Lipid analyses were performed within 48 h, and the determination of LDL subtypes was performed using frozen samples (−75°C).

Plasma lipids. Total plasma cholesterol and triglycerides were determined by enzymatic methods using an EPOS autoanalyzer (Eppendorf, Hamburg, Germany). HDL-cholesterol was measured after precipitation of apoB-containing particles by dextran sulfate and magnesium acetate. LDL-cholesterol was calculated by the formula of Friedewald, Levy, and Fredrickson (30) (all plasma triglyceride concentrations were <400 mg/dl). A lipoprotein fraction containing VLDL and IDL was isolated by preparative ultracentrifugation (d < 1.019 g/ml) before and after apheresis in each patient. ApoB and apoE was determined by nephelometry (Behring, Marburg, Germany) using antibodies against human apoB as well as apoE (anti-apoB/anti-apoE from rabbit; Behring).

LDL subfractionation. LDL subfractions were separated by isopycnic density gradient ultracentrifugation using the method described by Chapman et al. (1) with some modifications described previously (25). In brief, dry solid KBr was added to the plasma to increase its density to 1.21 g/ml. A discontinuous density gradient was constructed with 2 ml of a NaCl/KBr solution (d = 1.26 g/ml), 3 ml of plasma (d = 1.21 g/ml), 2 ml of a NaCl/KBr solution (d = 1.063 g/ml), 2.5 ml of another NaCl/KBr solution (d = 1.019 g/ml), and 2 ml of a NaCl solution (d = 1.006 g/ml). All solutions contained NaN_3 (0.1%) and EDTA (0.04%). Densities were measured by a precision density meter (DMA 38; Anton Paar, Graz, Austria). Ultracentrifugation was performed in a Beckmann SW40 Ti rotor at 40,000 rpm for 48 h at 15°C. Fifteen fractions were collected successively by aspiration of 0.5 ml with an Eppendorf pipette beginning at the top of each gradient. Seven LDL subfractions were isolated corresponding to fractions 5–11. They refer to following density intervals: LDL-1, 1.020–1.024 g/ml; LDL-2, 1.025–1.029 g/ml; LDL-3, 1.030–1.034 g/ml; LDL-4, 1.035–1.040 g/ml; LDL-5, 1.041–1.047 g/ml; LDL-6, 1.048–1.057 g/ml; and LDL-7, 1.058–1.066 g/ml.

Density limits were determined by a standard curve derived from control gradients constructed with a NaCl/KBr solution (d = 1.21 g/ml) instead of plasma. The densities were measured in 1 ml aliquots of the control gradient. Intraassay and interassay variability was <5%. Each run contained all six plasma samples from one individual patient to exclude interrun differences.

ApoB concentration was determined in each of the seven LDL subfractions. Buoyant LDLs were defined as LDL-1 and LDL-2 (d = 1.020–1.029 g/ml), intermediate LDLs were defined as LDL-3 and LDL-4 (d = 1.030–1.040 g/ml), and dense LDLs were defined as LDL-5, LDL-6, and LDL-7 (d = 1.041–1.066 g/ml). These three LDL subfraction groups were used to describe the rebound of apoB in the LDL subfractions and for the modeling. In addition, in four patients, the apoE content of buoyant, intermediate, and dense LDLs was determined before and after apheresis.

Modeling. Compartment models were developed to describe the LDL rebound data after apheresis. In model A (**Fig. 1A**), intermediate LDLs are the product of buoyant LDLs and the precursor of dense LDLs. In model B (Fig. 1B), buoyant, intermedi-

TABLE 2. apoB in buoyant, intermediate, and dense LDLs after LDL apheresis

Patient	Buoyant LDLs		Intermediate LDLs		Small LDLs	
	Before	After	Before	After	Before	After
	mg/dl (%)[a]					
A.W.	11.6 (7)	4.4 (11)	55.0 (35)	17.5 (43)	91.1 (58)	18.9 (46)
F.E.	12.0 (10)	4.6 (12)	61.6 (51)	22.4 (58)	47.4 (39)	11.5 (30)
K.K.	9.0 (7)	2.7 (6)	68.1 (52)	23.8 (55)	54.7 (42)	16.7 (39)
L.B.	10.6 (9)	3.8 (11)	57.8 (49)	15.0 (42)	48.7 (42)	17.3 (48)
S.G.	18.6 (10)	7.6 (16)	68.3 (37)	20.7 (43)	96.9 (53)	20.2 (42)
S.K.	8.6 (8)	5.8 (10)	46.7 (41)	20.4 (35)	58.1 (51)	32.4 (55)
S.P.	9.6 (7)	2.6 (6)	64.9 (50)	20.1 (50)	56.6 (43)	17.7 (44)
Mean ± SD	11.4 ± 3.1 (8 ± 1.4)	4.5 ± 1.6 (10 ± 3.2)	60.3 ± 7.2 (45 ± 7.0)	20.0 ± 2.7 (46 ± 8.3)	64.8 ± 18.9 (47 ± 7.1)	19.2 ± 5.9 (43 ± 7.9)

[a] Percentage of LDL-apoB.

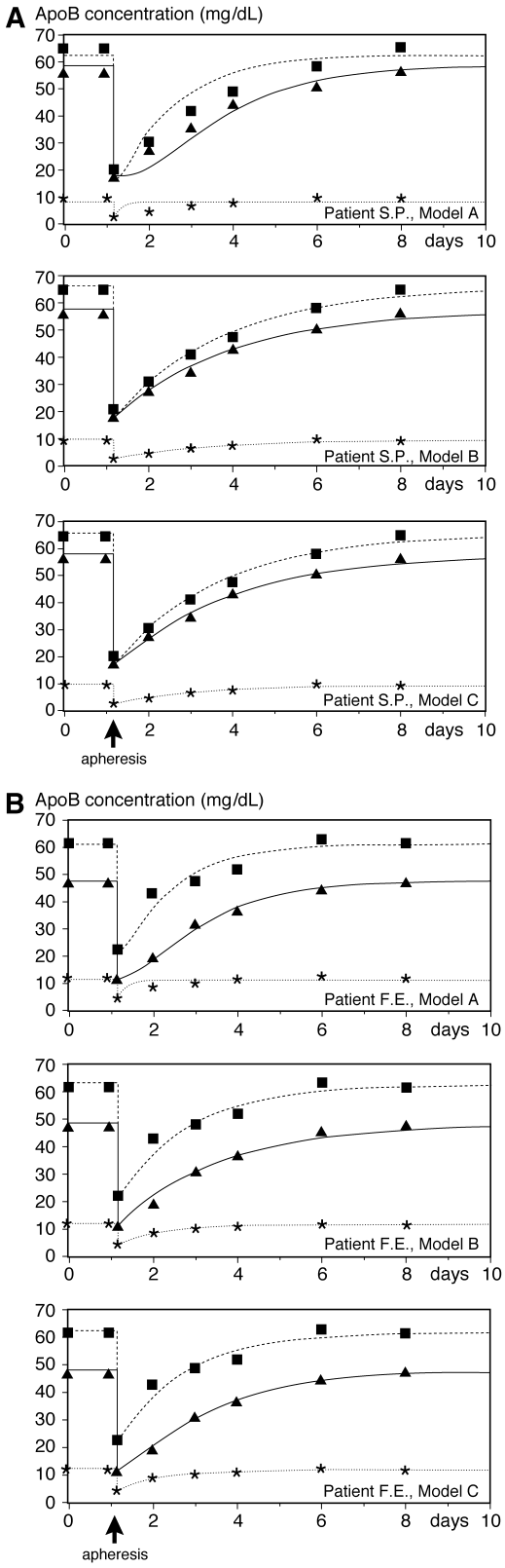

Fig. 2. Observed (symbols) and model-predicted (lines) data in two representative patients [S.P. (A) and F.E. (B)]. All panels show data for buoyant LDLs (stars, dotted lines), intermediate LDLs (squares, dashed lines), and dense LDLs (triangles, solid lines). The model-predicted lines are derived from model A, model B, or model C as outlined in Fig. 1. Please note that the agreement between observed and predicted data is much better for models B and C than for model A.

TABLE 3. Comparison of models with regard to the Akaike information criterion

Patient	Model A	Model B	Model C
A.W.	3.49	3.32	3.35
F.E.	2.81	2.78	2.82
K.K.	5.16	4.30	4.47
L.B.	2.93	2.85	2.85
S.G.	3.08	2.74	2.79
S.K.	7.15	5.65	5.53
S.P.	2.87	2.84	2.84
Mean ± SD	4.01 ± 2.59	3.61 ± 1.65	3.63 ± 1.55

$P = 0.018$ for model A vs. model B; $P = 0.028$ for model A vs. model C; $P = 0.39$ for model B vs. model C (Wilcoxon test); $P = 0.018$ for model A vs. model B vs. model C (Friedman test).

ate, and dense LDLs are directly produced from LDL precursors (e.g., VLDL/IDL) independently from each other. Model C (Fig. 1C) is the combination of models A and B and hypothesizes the formation of dense or intermediate LDLs from the delipidation of LDL precursors as well as from less dense LDL subfractions.

Each model assumed that the PR of apoB did not vary as a result of apheresis. To account for the apheresis-induced changes in VLDL and IDL concentrations, we used the preapheresis and postapheresis concentrations of VLDL/IDL-apoB and assumed that precursor concentrations rebound to preapheresis concentrations within 24 h, as previously shown (31). Because the VLDL/IDL concentrations rebound quickly, the model is not sensitive with respect to changes in VLDL/IDL concentration (data not shown).

Furthermore, it was assumed that all model rate constants remained constant during the course of the study, although tracer studies indicate that LDL FCR may increase in some patients immediately after apheresis and that LDL apheresis may affect the conversion of VLDL to LDL (30). However, when we tested a model in which LDL FCR was allowed to adjust, we did not observe a better fit of the model (data not shown). Furthermore, with additional adjustable parameters, the variance of the fitted parameters increased. Therefore, we assumed constant LDL FCRs in this study. The initial conditions (mass within each compartment at the start of the study) were set according to the mass measurements in each LDL subfraction. Modeling was performed using the SAAM II program (version 1.1; SAAM Institute, Seattle, WA). To differentiate between different model structures to a given data set, the program provides measures of model order, such as the Akaike information criterion (AIC). This parameter (a function of the number of adjustable parameters and the objective function, analogous to the weighted residual sum of squares) provides information that can be used to differentiate the fitting of different model structures to a set of experimental data. The lowest AIC was used to identify the model best describing the observed data. This model was subsequently used to estimate rates of production (PR) and catabolism (FCR).

Statistics

As the AIC, FCR, and PR rates were not normally distributed, nonparametric tests were used for statistical evaluation. The Wilcoxon test was used to compare pairs, and the Friedman test was used to compare more than two paired values.

RESULTS

LDL apheresis decreased cholesterol from 255 ± 29 to 108 ± 9 mg/dl (−57%), LDL-cholesterol from 178 ± 28

to 51 ± 10 mg/dl (−71%), apoB from 148 ± 22 to 45 ± 8 mg/dl (−69%), and HDL-cholesterol from 46 ± 10 to 39 ± 8 (−13%) (**Table 1**). Seven days later (day 7), LDL-cholesterol and apoB levels returned to preapheresis values (LDL-cholesterol, 171 ± 34 mg/dl; apoB, 137 ± 25 mg/dl). Similarly, cholesterol and HDL-cholesterol returned to preapheresis levels (251 ± 48 and 48 ± 12 mg/dl, respectively). The rate of rebound, however, was different among these lipid parameters, the highest being for HDL-cholesterol followed by total cholesterol, apoB, and LDL-cholesterol.

Influence of LDL apheresis on LDL subfractions

LDL apheresis decreased apoB in buoyant, intermediate, and dense LDLs by 60 ± 12, 67 ± 5, and 69 ± 11%, respectively (**Table 2**). After apheresis, apoB concentra-

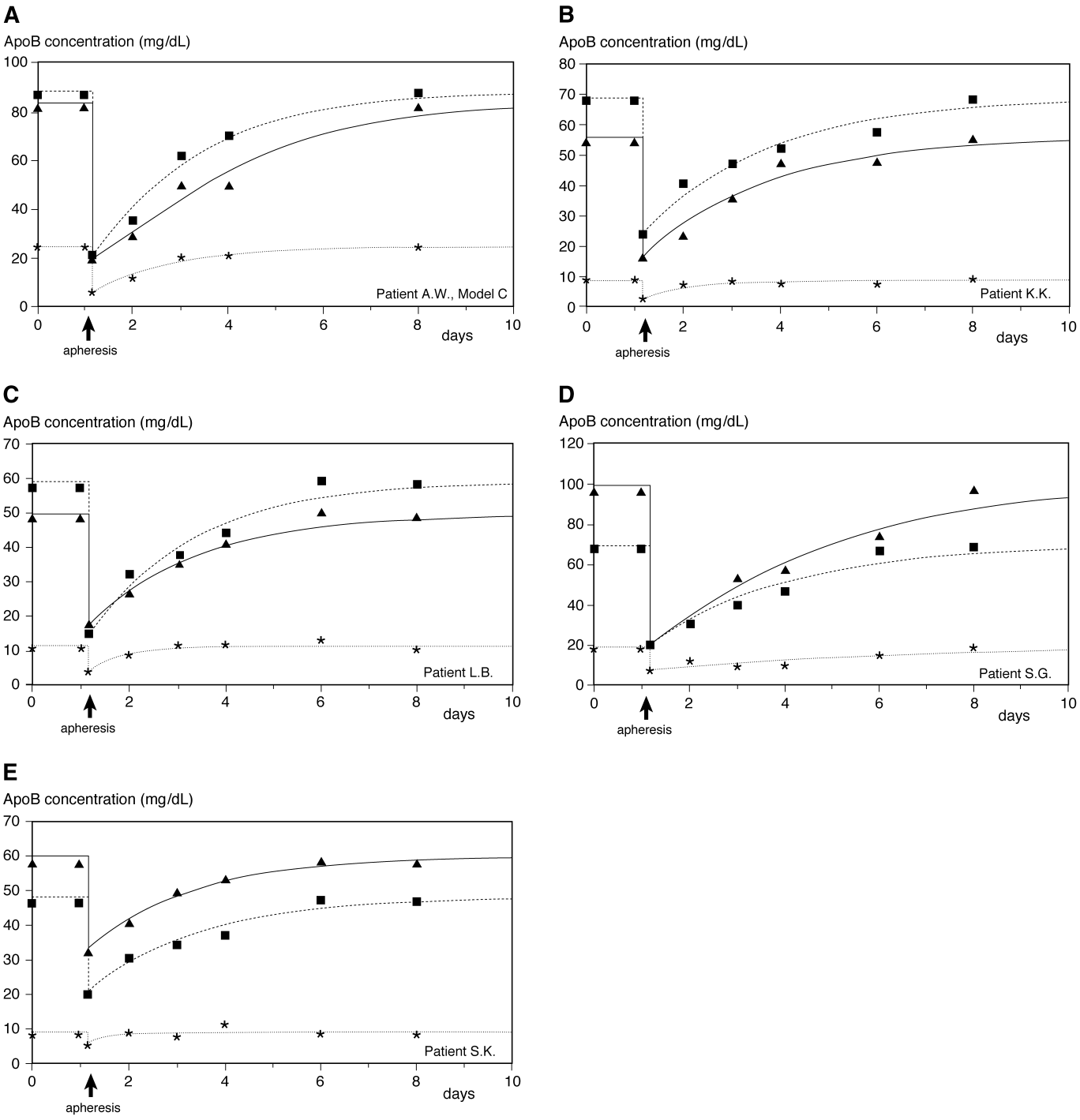

Fig. 3. Observed (symbols) and model C-predicted (lines) data in the other five patients [A.W. (A), K.K. (B), L.B. (C), S.G. (D), and S.K. (E)]. All panels show data for buoyant LDLs (stars, dotted lines), intermediate LDLs (squares, dashed lines), and dense LDLs (triangles, solid lines).

tions increased more rapidly in buoyant (percentage of preapheresis concentration on day 2, 85 ± 16%) than in intermediate (70 ± 7%) or dense (67 ± 9%) LDLs.

The composition of LDL subfractions was not affected by apheresis treatment, as the cholesterol/apoB ratio was not significantly different before or after LDL apheresis in buoyant (1.57 ± 0.18 vs. 1.35 ± 0.40; $P = 0.17$), intermediate (1.33 ± 0.07 vs. 1.26 ± 0.34; $P = 0.17$), and dense (1.13 ± 0.09 vs. 1.13 ± 0.29; $P = 0.90$ for differences, Wilcoxon test) LDL subfractions. In a subgroup of four patients, total apoE was reduced from 4.65 to 2.19 mg/dl (−53%) during LDL apheresis; however, none of the individual LDL subfractions (LDL-1 to LDL-7) contained a significant amount of apoE (<1 mg/dl) either before or after LDL apheresis.

Modeling of rebound data

Figure 2 shows the fit of the different compartment models to the apoB rebound data in two representative patients (patient S.P. in A and patient F.E. in B; identical results were seen in the other patients). Clearly, models B and C, but not model A, describe the apoB rebound data. This was confirmed by the AIC, which was significantly lower for models B (3.61 ± 1.64) and C (3.63 ± 1.55) than for model A (4.01 ± 2.59) (**Table 3**). ApoB data for the other five patients are shown in **Fig. 3**.

Estimation of metabolic parameters (according to model C)

Although models B and C can be used to describe the observed data, model C was used to estimate metabolic parameters, because previous studies using an endogenous tracer (21) demonstrated the conversion of buoyant LDLs to dense LDLs; thus, a model without such a pathway (i.e., model B) is physiologically not plausible. **Tables 4 and 5** and **Fig. 4** describe the FCRs and PRs for each patient as calculated from the rebound data using model C. In all patients, the FCRs of buoyant LDL-apoB (1.05 ± 0.86 day^{-1}) tended to be higher than those of intermediate LDL-apoB (0.48 ± 0.11 day^{-1}), and the FCR of intermediate LDL-apoB was significantly lower than the FCR of dense LDL-apoB (0.69 ± 0.24 day^{-1}). The total PR of LDL-apoB ranged between 20 and 33 mg/kg/day. The total flux of apoB through the dense LDL subfractions ranged between 9 and 23 mg/kg/day, corresponding to between 35% and 70% of total LDL-apoB production (Table 5).

On average, 58 ± 29% of dense LDLs was produced by direct delipidation of VLDL/IDL precursors (or direct secretion of LDL-apoB) and 42 ± 29% of dense LDLs was attributable to conversion of less dense LDL subfractions.

TABLE 4. FCRs of buoyant, intermediate, and dense LDL-apoB calculated from model C

Patient	Buoyant LDLs	Intermediate LDLs	Dense LDLs	LDLs
A.W.	0.63	0.60	0.69	0.35
F.E.	1.09	0.66	0.95	0.52
K.K.	0.69	0.39	0.42	0.42
L.B.	1.51	0.47	0.48	0.56
S.G.	0.48	0.46	0.85	0.38
S.K.	0.20	0.36	0.45	0.26
S.P.	2.75	0.45	0.97	0.71
Mean ± SD	1.05 ± 0.86	0.48 ± 0.11	0.69 ± 0.24	0.46 ± 0.18

Values shown are per day. FCR, fractional catabolic rate. $P = 0.06$ for buoyant vs. intermediate LDLs; $P = 0.40$ for buoyant vs. dense LDLs; $P = 0.02$ for dense vs. intermediate LDLs (Wilcoxon test); $P = 0.02$ for buoyant vs. intermediate vs. dense LDLs (Friedman test). The overall FCR of LDL-apoB may be lower than any of the individual FCRs because the model structure allows conversion between subfractions (Fig. 1).

TABLE 5. apoB production rates calculated using model C

Patient	Buoyant LDLs	Intermediate LDLs	Dense LDLs	LDLs
A.W.	6.3	21.1	22.9	27.5
F.E.	5.3	16.4	18.0	25.3
K.K.	2.4	10.7	9.3	22.5
L.B.	6.9	11.1	9.6	26.9
S.G.	1.9	12.3	19.7	20.5
S.K.	1.6	9.9	18.0	19.5
S.P.	10.0	8.6	23.2	33.3
Mean ± SD	4.9 ± 2.9	12.9 ± 4.1	17.3 ± 5.3	25.1 ± 4.4

Values shown are mg/kg/day. $P = 0.03$ for buoyant vs. intermediate LDLs; $P = 0.02$ for buoyant vs. dense LDLs; $P = 0.06$ for intermediate vs. dense LDLs (Wilcoxon test); $P = 0.02$ for buoyant vs. intermediate vs. small LDLs (Friedman test).

DISCUSSION

After LDL apheresis, apoB concentrations increased more rapidly in buoyant compared with intermediate and dense LDL subfractions in these patients with heterozygous FH on concomitant statin therapy. The rebound of apoB in different LDL subfractions was best explained by a model that allows the direct and independent production of buoyant, intermediate, and dense LDLs from precursors with simultaneous delipidation of less dense LDLs to dense LDLs. A model hypothesizing that dense LDLs are formed exclusively by delipidation of less dense LDL subfractions fails to explain the observed rebound data, whereas a model without such a pathway is physiologically not plausible.

The metabolism of LDL subfractions has been studied with exogenously (15, 19, 20) and endogenously (15, 16, 17, 20) labeled tracers. **Table 6** shows the key results of these studies with respect to the production of dense LDLs (15–20) to facilitate the interpretation of our data. In these studies, LDL subfractions were generally separated by density as "heavy" and "light" LDL subspecies. These studies revealed that heavy LDLs can be directly produced from precursors (e.g., VLDL/IDL) or by delipidation of light LDLs or by a combination of both pathways. The fraction of heavy LDLs that is produced from the delipidation of light LDLs varies between 8% (17) and 89% (20) depending on the methodology used and the underlying metabolic disorder (Table 6). Only one study (18) described the formation of heavy LDLs exclusively

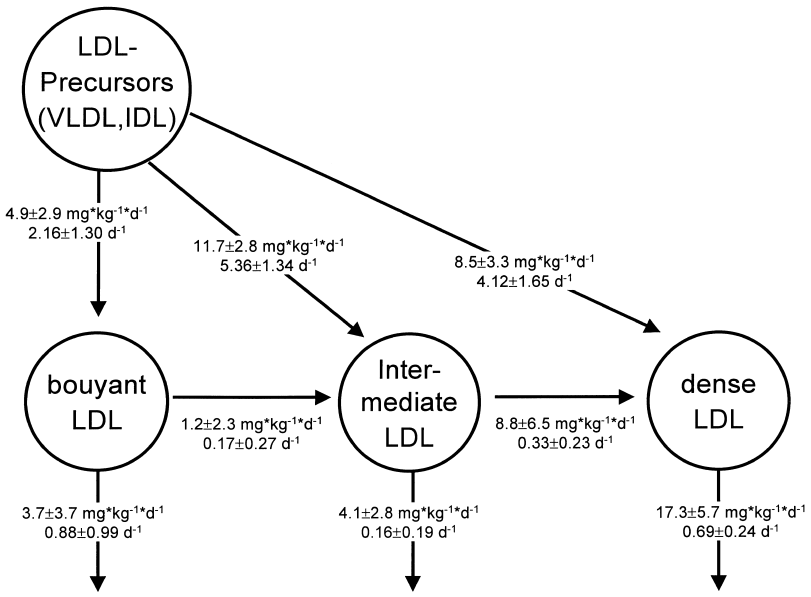

Fig. 4. Metabolic parameters (mean ± SD) as derived from the rebound analysis. Flux rates (mg/kg/day) and rate constants (per day) are shown.

from one source, such as the delipidation of light LDLs to heavy LDLs.

In patients with heterozygous FH, results are more ambiguous concerning the precursor-product relationship between different LDL subtypes (19–21). Radiolabeled buoyant LDLs did not significantly convert to dense LDLs when injected into patients with FH; thus, it was hypothesized that dense LDLs are largely produced independently from buoyant LDLs and are derived directly from less dense lipoprotein precursors (e.g., VLDL, IDL) (19, 20). This contrasts with the results of an endogenous labeling study (21), in which it was observed that 69% of heavy LDLs are derived from light LDLs in patients with FH. Thus, the different findings with respect to the production of heavy LDLs from light LDLs in patients with FH may relate to the methodology used.

Model C predicts an independent and direct production of small LDLs (58%) as well as a delipidation pathway from buoyant LDLs (42%). In interpreting our results, it is important to note that we report on rebound kinetics in patients with heterozygous FH receiving statin therapy who are regularly treated by LDL apheresis, factors that may affect the metabolism of individual subfractions. Although statins decrease all LDL subfractions in absolute terms, it has been shown by us and others that the LDL subtype distribution may change with such therapy (32–34). In normotriglyceridemic patients (FH and controls), a slight relative increase in small, dense LDLs is usually

TABLE 6. Summary of studies using exogenous or endogenous tracers to investigate LDL subtype metabolism

Study	Tracer	Patients	LDL Subfractions	Consistent with Model[a]	Conversion[b]	Mean FCR			Mean PR		
						Buoyant[c]	Intermediate[c]	Dense	Buoyant[c]	Intermediate[c]	Dense
					%	*day*$^{-1}$				*mg/kg/day*	
Ref. 20	Exogenous	FH (n = 2)	Light/heavy	B, C	44	0.23		0.26	9.1		14.1
Ref. 20	Exogenous	Controls (n = 2)		C	75	0.71		0.5	5.9		12.8
Ref. 19	Exogenous	Hyper-apoB (n = 2)		C	89	1.1		0.4	17.1		17.3
Ref. 17	Endogenous	Postmenopausal women (n = 8)	Light/heavy	B, C	8 (16)[d]	0.59		0.41	575[e]		434[e]
Ref. 18	Endogenous	Hyper-apoB (n = 5)	Light/heavy	A	100	0.82		0.22	11.9		9.8
Ref. 21	Endogenous	FH (n = 7)	Light/heavy	C	69	0.47		0.53	—		—
Ref. 16	Endogenous	Controls (n = 20)	LDL-phenotype A/I/B[f]			0.55	0.32	0.36	19.3	21.3	19.3
Present study	No tracer	FH (n = 7), statins, regular apheresis	Buoyant, intermediate, dense	C	42	1.05	0.48	0.69	4.9	12.9	17.3

FH, familial hypercholesterolemia; PR, production rate.
[a] Refers to models shown in Fig. 1.
[b] Refers to the percentage of the dense LDL pool size derived from buoyant LDLs.
[c] In some studies, buoyant and intermediate LDLs were not differentiated.
[d] Refers to women receiving hormone replacement therapy.
[e] This PR value is given in mg/day.
[f] A, I, and B refer to buoyant, intermediate, and dense LDLs as classified by gel electrophoresis.

observed (~5%). Whether this also affects the metabolic pathways determined in this study is unknown. It is also possible that LDL subtype metabolism is different when the underlying metabolic situation is different. This may be particularly true in insulin resistance, which is characterized by the predominance of small LDL subfractions. Further studies must determine whether the model is valid in such patients.

Our study is also limited by the assumptions associated with the modeling process. First, we assumed [on the basis of previous studies (31)] that overall apoB production was not affected by apheresis and that VLDL/IDL-apoB concentrations return to baseline within 24 h after apheresis (probably even faster). Although we do not know the precise kinetics of VLDL and IDL-apoB rebound, our model is not sensitive to changes in VLDL/IDL-apoB concentrations or metabolism as long as they are restricted to the first 24–36 h after apheresis (data not shown). In a previous study, we observed that LDL-apoB FCR increases transiently in some patients after apheresis (31). When this feature was included in our model, the fit of the model to the observed data did not improve, parameter values did not change, but the associated errors increased. We therefore assumed time-invariant FCRs after apheresis. In the same study (31), we observed that VLDL-apoB to LDL-apoB conversion decreased after apheresis. Although this was not significant, it is unclear whether apheresis disrupts the conversion pathway that could explain why in previous studies metabolic parameters derived from rebound analyses are similar but not identical to those derived from tracer studies (24). Although such an apheresis-induced alteration may affect the calculated parameters, it most likely will not affect the pathway itself (i.e., be responsible for the selection of model C over models A and B).

The FCRs of total, buoyant, intermediate, and dense LDL-apoB calculated in this study (Tables 4, 6, Fig. 4) are somewhat higher than those described by others (19–21, 24) in FH patients. This may relate to the fact that these patients are on statin therapy, which increases the FCR of LDL (15, 18, 35, 36). Furthermore, in the present study, there was a trend toward higher FCRs for buoyant LDLs compared with intermediate LDLs, a result that is consistent with the findings of Packard et al. (16), who described the highest FCR in controls with the LDL phenotype A (corresponding to an abundance of buoyant LDLs). To exclude the possibility that differences in the FCRs between buoyant, intermediate, and dense LDLs relate to a contamination with apoE, we determined apoE concentrations in all LDL subfractions in a subgroup of four patients: no significant levels of apoE were detectable in any LDL fraction before or after apheresis. Furthermore, LDL subtype composition was not affected by LDL apheresis treatment, as the preapheresis and postapheresis cholesterol/apoB ratio remained constant.

In agreement with previous studies (16, 20, 21), we did not observe a higher FCR of intermediate LDLs compared with dense LDLs. This contrasts to tracer studies in postmenopausal women (17) and in patients with mixed hyperlipoproteinemia (18, 20) as well as with results from in vivo experiments (5) in which a higher FCR of intermediate/light LDLs compared with dense/heavy LDLs was observed. The basis of the observed difference remains to be determined, but it may relate to several factors (underlying metabolic disease, methodology used, influences by lipid-lowering drugs or other modalities such as LDL apheresis).

Our model C allows dense LDLs to be produced directly from LDL precursors and by delipidation of less dense LDL subtypes. The direct production of dense LDLs may relate to several pathways, such as the delipidation of VLDL and IDL particles through a shunt pathway, thus without the formation of more buoyant LDLs or "true" direct secretion from the liver (37). Our methodology does not allow us to distinguish between these pathways. However, data from moderately hypercholesterolemic subjects (15) show that buoyant combined with intermediate LDLs (LDL-I + LDL-II) but not dense LDLs (LDL-III; range 1.044–1.060 g/ml) strongly correlate with direct hepatic LDL secretion. Thus, this pathway also might be of minor relevance for the direct production of dense LDLs in our FH patients.

In summary, analysis of rebound apoB concentrations after apheresis indicates that in heterozygous FH patients on statin therapy, dense LDLs are derived from direct production from precursors as well as from the delipidation of more buoyant LDLs.

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