European Journal of Clinical Investigation (1993) **23**, 602–614

No evidence for feedback inhibition of hepatic apolipoprotein B (apo B) production after extracorporeal low density lipoprotein precipitation as determined by [1-^{13}C]leucine infusion in normal volunteers

J. ARENDS*, D. M. BIER*, G. SCHÄFER†, V. W. ARMSTRONG‡, J. THIERY§, D. SEIDEL§ & P. SCHAUDER*, *Metabolism Division, Washington University School of Medicine, St. Louis, MO; †Abteilung für Gastroenterologie und Endokrinologie, Medizinische Universitätsklinik, Göttingen, Germany; ‡Abteilung für Klinische Chemie, Medizinische Universitätsklinik, D-3400 Göttingen, Germany and §present address: Institut für Klinische Chemie, Klinikum Großhadern, Universität München, München, Germany

Received 13 October 1992 and in revised form 10 May 1993; accepted 18 May 1993

Abstract. To determine the impact of an acute reduction of the circulating mass of apolipoprotein B (apo B) on apo B metabolism we studied six healthy male volunteers before (day 0), 1 day after (day 2), and 7 days after (day 8) an LDL apheresis treatment which reduced apo B mass by 59%. Appearance of newly synthesized apo B in plasma VLDL and LDL was studied using a primed-constant infusion of [1-^{13}C]-leucine. VLDL apo B pool size and fractional VLDL apo B production rate calculated using a one-compartment model were similar on all 3 study days. Absolute VLDL apo B production was not statistically different throughout the study (19·7 ± 12·3, 19·5 ± 7·5, 29·1 ± 17·7 mg kg^{-1} day^{-1}). LDL apo B fractional production rate was increased on day 2 (0·38 ± 0·17, 0·68 ± 0·08, 0·37 ± 0·06 pools day^{-1} on days 0, 2, and 8; $P < 0{\cdot}01$). Absolute LDL apo B production, however, remained constant throughout the study (10·8 ± 3·3, 11·0 ± 1·9, 10·8 ± 3·1 mg kg^{-1} day^{-1}). We conclude that in healthy male volunteers acute reduction of the circulating apo B mass by LDL apheresis does not affect apo B metabolism significantly.

Keywords. Apolipoprotein B, LDL-apheresis, [1-^{13}C]leucine, low density lipoproteins, very low density lipoproteins.

Introduction

Elevated plasma cholesterol levels are a major risk factor in the development of atherosclerosis and coronary heart disease. The greater part of this cholesterol is normally transported in the low-density lipoprotein (LDL) fraction and elevated LDL levels have been associated with an increase in coronary risk [1]. Regulation of the production of the apolipoprotein component is an important factor in determining LDL levels. The major apoprotein of LDL, apolipoprotein B-100 (apo B), is synthesized by the liver [2] and under normal circumstances enters the circulation almost exclusively as a component of very-low-density lipoproteins (VLDL) [3]. Conversion of VLDL to LDL and thus transfer of apo B to LDL takes place through lipolysis in the periphery, though it has been suggested that some LDL may be secreted directly by the liver [4]. LDL and its apolipoprotein are removed from the circulation by various cell types primarily by receptor-dependent mechanisms [5].

Apo B production has been studied under different physiological conditions by radioisotope techniques [6] and more recently by applying stable isotope technologies [7–12]. However, there are no *in vivo* data concerning the effects of altered circulating apolipoprotein B mass on hepatic apo B production. A feedback mechanism might be conceivable, similar to the regulation of hepatic cholesterol synthesis by receptor-mediated uptake of cholesterol [5,13]. Operation of an apo B feedback mechanism might counteract therapeutic measures such as plasmapheresis procedures designed for the treatment of severe hypercholesterolaemia [14]. Several selective LDL apheresis procedures are currently under clinical investigation [15–20]. These treatments remove large amounts of cholesterol and apo B from the body in a relatively short time, generally in the range of 2–4 h. Much remains to be clarified before these procedures can be assigned their proper place in therapy. One important aspect for evaluating the benefit of LDL apheresis is

Abbreviations: apo B, apolipoprotein B-100; EDTA, ethylenediaminetetraacetic acid; GCMS, gas chromatography-mass spectrometry.

Preliminary presentation: 55th Meeting of the European Atherosclerosis Society, Brugge, Belgium, May 16–19, 1990

Correspondence: J. Arends MD, Medizinische Universitätsklinik, Robert-Koch-Strasse 40, D-3400 Göttingen, Germany.

Table 1. Clinical and laboratory data of subjects

Subject	Age (years)	Weight (kg)	Height (cm)	BMI* (kg m^{-2})	CHOL† (mg dl^{-1})	TG† (mg dl^{-1})	LDL-chol‡ (mg dl^{-1})	VLDL-chol‡ (mg dl^{-1})	HDL-chol‡ (mg dl^{-1})
A	19	82	182	24·8	185	64	109	6·0	69·8
B	26	86	185	25·1	175	119	112	10·9	51·7
C	30	82	180	25·3	273	163	180	24·3	68·9
D	20	77	180	23·8	170	27	98	3·1	68·5
E	20	77	180	23·8	190	65	122	8·6	59·5
F	25	70	178	22·1	167	85	101	8·0	58·2
Mean	23·3	79·0	180·8	24·1	193·3	87·2	120·3	10·2	62·8
SD	4·4	5·6	2·4	1·2	40·0	47·8	30·4	7·4	7·4

* BMI, body mass index; † CHOL and TG, total cholesterol and total triglycerides; ‡ LDL-chol, VLDL-chol, and HDL-chol, LDL, VLDL, and HDL cholesterol.

the impact of this treatment on apo B synthesis and on the production of LDL.

To search for evidence of a feedback mechanism we observed the impact of an acute reduction of circulating apolipoprotein B mass on apo B metabolism in six healthy volunteers. We used stable isotope techniques and a primed tracer infusion of [1-^{13}C] leucine to measure production rates of VLDL apo B and LDL apo B before and after an LDL apheresis treatment. Similar methods have been applied earlier to measure fractional synthetic rates of VLDL apo B and LDL apo B in humans [7,8,9].

Subjects and methods

Subjects

The study protocol was reviewed by the Ethics Committee of the University of Göttingen. Six healthy non-obese male subjects participated in this study after giving informed consent. Subjects ranged in age from 19 to 30 years, they were of normal height (178–185 cm) and weight (70–86 kg), the mean body mass index was 24·1 ± 1·2 kg m^{-2} (mean ± SD). Five subjects had normal triglyceride (27–119 mg dl^{-1}) and cholesterol (170–185 mg dl^{-1}) levels, while subject C had LDL-cholesterol values in the upper 95th percentile of his age group (Table 1). None of the subjects was taking any medication and all maintained their normal diet throughout the study.

Experimental design

The studies were carried out in the Metabolic Research Unit at the Medical Center of the University of Göttingen. Subjects entered the Research Unit for the apheresis treatment, the tracer infusion studies and additional blood withdrawals, but left the Unit between procedures. They were counselled by a dietician to eat a weight-maintaining diet during the experimental period. Each subject underwent a single LDL apheresis treatment on Day 1 of the study to lower circulating LDL levels. One day before (day 0) and one (day 2) and 7 days after (day 8) the apheresis treatment tracer infusion studies with [1-^{13}C] leucine were carried out to determine fractional production rates of VLDL apo B and of LDL apo B. On days prior to tracer infusions the last meal was taken at 1800 h. All tracer infusion studies were then performed after an overnight fast in the postabsorptive state. No food was given during tracer infusions but subjects were allowed free access to drinking water. Two of the six subjects (C and D) were studied only twice with the leucine tracer (on days 0 and 2); in these two subjects blood was drawn at 0800 h on day 8 for determination of the LDL apo B pool size. On day 4 plasma was drawn in all subjects at 0800 h to determine the LDL apo B pool size.

LDL apheresis treatment

Details of the HELP (Heparin Extracorporeal LDL Precipitation) procedure have been published elsewhere [16]. Briefly, plasma, obtained by filtration of whole blood through a 0·2 μm filter, is continuously mixed with an equal volume of an acetate buffer containing heparin. After removal of the precipitated heparin complex by filtration, excess heparin is adsorbed to a specially developed filter and the clear plasma filtrate is subject to bicarbonate dialysis/ultrafiltration to restore physiologic pH and remove excess fluid. Each subject underwent a single apheresis in which 3 l of plasma were treated between 1400 and 1600 h on the day following the first tracer infusion. This apheresis procedure effectively eliminates LDL in the extracorporeal circuit leading to a 50–70% reduction in plasma LDL levels [16], the extent depending upon the plasma volume of the patient and the amount of plasma treated. Triglyceride levels are also reduced to a lesser degree, partly due to precipitation and partly due to increased lipolysis associated with the heparin anticoagulation therapy [16].

Tracer material

[1-^{13}C] Leucine was obtained from MSD Isotopes, Merck Chemical Division, St. Louis, MO, USA.

Isotopic purity and chemical purity as determined by conventional mass spectrometric methods were 98% [^{13}C] and 100% leucine. For infusion the tracer was dissolved in 0·15 M NaCl and sterile filtered (0·22 μm, Millipore Corporation, Milford, MA, USA). The tracer material was shown to be pyrogen-free by a licensed commercial firm (Scientific Associates Inc., St. Louis, MO, USA) using the rabbit body temperature assay.

Tracer infusion studies

Subjects remained recumbent throughout the infusion studies. On each study day at 0700 h two intravenous lines were placed in cubital veins of both arms, one served for tracer infusion and the other for sampling. The sampling line was kept open with a slow drip of 0·15 M NaCl. The tracer infusion was begun at 0800 h and involved a priming dose followed by a constant infusion of [1-^{13}C]leucine for 8 h. Thus the infusion study on day 2 was begun 16 h after the end of the LDL apheresis treatment. The tracer infusion rates were 5 μmol kg^{-1} h^{-1} and priming doses were 5 μmol kg^{-1} in subjects A to D. Tracer infusion rates were raised 4-fold in two subjects (E and F) to enhance the accuracy of low level enrichment measurements in LDL apo B in these two subjects. The corresponding priming doses and infusion rates were 20 μmol kg^{-1} and 20 μmol kg^{-1} h^{-1} of [1-^{13}C]leucine. Blood samples (10 ml) were collected at −30, 0, 30, 45, 60, 90, 120, 150, 180 min and hourly thereafter until 480 min. Samples were drawn into tubes containing EDTA (final concentration 0·1%) and plasma was separated by centrifugation at 4°C. Plasma was processed immediately for isolation of lipoproteins or stored at −20°C until further analysis.

Isolation of lipoproteins

Lipoproteins were isolated from plasma by sequential ultracentrifugation. VLDL were prepared from 4 ml of plasma after overlayering with 6 ml of 0·196 M NaCl (containing 2 mM EDTA) by a single ultracentrifugal spin at a density of 1·006 g ml^{-1} (84 000 × ***g***, 24 h, 12°C) in a Type 40 fixed angle rotor (Beckman Instruments, Inc., Palo Alto, CA, USA). After centrifugation VLDL were removed in the top 1·5 ml of each tube by tube slicing. Infranates were adjusted to a density of 1·063 g ml^{-1} with solid KBr, overlayered with 2 ml of a KBr solution of density 1·063, and centrifuged at 12°C for 16 h at 84 000 ***g***. LDL were removed in the top 1·5 ml of each tube by tube slicing. The concentrations of apo B in the VLDL and LDL fractions were determined by the isopropanol precipitation method of Egusa *et al.* [21]. The intra-assay coefficient of variation for replicate determinations of the same sample was 5·7.

Plasma and lipoprotein fractions were assayed for total cholesterol and triglycerides using commercially available enzymatic test kits (Boehringer Mannheim, Mannheim, Germany). Serum HDL-cholesterol concentrations were determined by the phosphotungstate precipitation method (Boehringer Mannheim) and LDL cholesterol by a precipitation technique based on dextran sulfate (Quantolip-LDL, Immuno GmbH, Heidelberg, Germany). Plasma glucose was measured using a Beckman glucose analyser (Beckman Instruments, Inc., Palo Alto, CA, USA) and plasma insulin levels were determined by radioimmunoassay (Pharmacia Diagnostics AB, Uppsala, Sweden).

Isolation of apo B and hydrolysis

Apolipoprotein B was isolated from VLDL or LDL preparations by isopropanol extraction as described by Egusa *et al.* This method has been reported to result in preparation of pure apo B in normocholesterolemic subjects [21]. In our hands when subjected to polyacrylamide gel electrophoresis and densitometric scanning isopropanol precipitates were free of apolipoproteins C and contained only minimal amounts (up to 5%) of apolipoprotein E. Contributions by apolipoprotein B-48 were undetectable as would be expected in the postabsorptive state. The precipitated protein was hydrolysed in 6N HCl for 16 h at 110°C [22], and the hydrochloric acid was evaporated at 110°C under a stream of nitrogen.

Measurement of [1-^{13}C]enrichment

This was performed as described previously [23]. Briefly, leucine was isolated from 0·5 ml aliquots of plasma by cation exchange chromatography (AG-50W-X8 resin, Bio-Rad Laboratories, Richmond, CA, USA); leucine obtained from plasma or apo B samples was derivatized to yield the N-acetyl-1-propanol ester and [^{13}C]enrichment was determined by gas chromatography-mass spectrometry (GCMS) using a Finnigan 3300 quadrupole mass spectrometer and methane positive chemical ionization. Calibration standards containing known amounts of [1-^{13}C]leucine in the range of 0–20% enrichment were analysed by GCMS. For each standard the observed ion current ratio (ICR) of ions 217/216 was plotted over the isotope mole ratio (R) of $^{13}C/^{12}C$. Standard curves were fitted by linear regression and were used to obtain isotope ratios from observed ICR 217/216 data of unknowns. Isotope ratios (R) were converted to enrichment values (E) by calculating fractional abundances as $q = R/(R+1)$, and subtracting the contribution of natural material (q_n) to yield $E = q - q_n$ [24]. All enrichment results were multiplied by 100 and expressed as mole percent excess. Assay precision was 1% relative standard deviation for measured ion current ratios; thus, the standard deviation for low enrichment levels was 0·14 mole % excess [23]. For enrichment in leucine from LDL apo B results were calculated as the average of at least 10 replicate GCMS injections; the precision of these data could thus be improved to S.E. = 0·04 mole % excess.

Calculation of plasma leucine flux

Plasma leucine flux was calculated according to Equation 1 [25]:

$$R_a = i \cdot (E_t/E_{pl} - 1) \qquad \text{(Eq. 1)}$$

where R_a is the endogenous plasma leucine flux, i is the [1-^{13}C] leucine infusion rate, E_t is the [^{13}C] enrichment of the infused tracer, and E_{pl} is the [^{13}C] enrichment of plasma leucine at plateau. For each experiment E_{pl} was taken to be the mean enrichment measured from 180 to 480 min. The relative standard deviation was less than 10% in all cases, and slopes after linear regression were not significantly different from zero.

Models for apo B metabolism

We used single-compartment models to describe the dynamic aspects of apo B metabolism both in VLDL and LDL. Input into the VLDL pool is from hepatic synthesis, output is catabolic conversion to LDL or receptor-mediated removal by the liver. Inflow into the LDL pool is from VLDL or from direct hepatic synthesis; outflow is receptor-mediated uptake by peripheral tissues or hepatocytes. These simplified models have the advantage of allowing an integrated quantitation of total apo B metabolism, while they sacrifice the resolution of the behaviour of subcompartments. If tracer material is introduced into an open one-compartment model then tracer enrichment is described by the basic equation [26]:

$$dE/dt = k_1 \cdot F - k_2 \cdot E \qquad \text{(Eq. 2)}$$

where E is the actual enrichment in the observed compartment (e.g. circulating VLDL apo B), F is the enrichment in the input material (e.g. newly synthesized VLDL apo B), and k_1 and k_2 are the fractional input and output rates per unit time (k_1 = input/pool size = fractional production rate, k_2 = output/pool size = fractional catabolic rate).

Under certain assumptions calculation of the model parameters may be simplified. *Case 1*: Assuming steady-state conditions for the pool size then k_1 and k_2 are identical and constant and may be replaced by the common turnover parameter k. This allows integration of Equation 2:

$$dE/dt = k \cdot (F - E) \rightarrow E = F \cdot (1 - e^{-k \cdot t}) \qquad \text{(Eq. 3)}$$

If it is further assumed that F, i.e. enrichment in the input material, is constant, then observing experimental data for E, i.e. pool enrichment, allows estimation of the fractional turnover parameter k by using a simple nonlinear model of asymptotic (first order reaction curve) regression. We applied this model to the metabolism of apo B in VLDL. *Case 2*: If, in a system with slow turnover, the compartment is observed shortly after beginning the tracer introduction, then enrichment in the compartment may be negligible compared to enrichment in the input material, i.e. $E \ll F$. Eq. 2 then reduces to:

$$dE/dt = k_1 \cdot F \qquad \text{(Eq. 4)}$$

If, again, enrichment in the input material, i.e. F, is constant then k_1 may be obtained from the linear rate of enrichment in the observed compartment in relation to enrichment in the input:

$$k_1 = (dE/dt)/F \qquad \text{(Eq. 5)}$$

In this case, however, it is important to note that the calculated parameter is k_1, i.e. the fractional production rate, and no assumption is made on the catabolic rate k_2. In fact, if the system is not in steady-state, i.e. $k_1 \neq k_2$, then an estimation of k_2 is only possible from additional data on the time course of the pool size. We applied this version of the model to the metabolism of apo B in LDL.

Fractional turnover rate of VLDL apo B

To calculate fractional turnover rates for VLDL apo B we assumed (1) that the purified apo B had been isolated from a homogeneous VLDL pool of constant size; (2) that apo B was synthesized at a constant rate; (3) that newly synthesized apo B was transported unidirectionally to the plasma membrane for secretion, and (4) that intrahepatic leucine enrichment was constant throughout the study. We then used the model described by Equation 3 above and applied nonlinear regression analysis to approximate the parameters of the model to fit leucine enrichment data in VLDL apo B (E_{VLDL}) observed from t = 30 to 480 min:

$$E_{VLDL} = E_p \cdot [1 - e^{-k \cdot (t-d)}] \qquad \text{(Eq. 6)}$$

where E_p is the VLDL apo B enrichment plateau, k is the fractional turnover rate for VLDL apo B (measured in pools day^{-1}) and d is the intra-hepatic delay time between the beginning of apo B synthesis and VLDL secretion. The enrichment plateau E_p is identical to the enrichment in newly synthesized apo B and should thus be identical to intrahepatic leucine enrichment. Adding a delay time d to the model was necessary, since enrichment in VLDL apo B was undetectable for some 20–30 min after beginning the tracer infusions. Nonlinear regression was performed on an IBM personal computer by an iterative least squares procedure using the Quasi-Newton method (Systat, Inc., Evanston, IL, USA). For each data set the procedure estimated the parameters k, E_p, and d; to indicate reliability, approximate standard errors were calculated for each estimated parameter [27] by estimating the Hessian matrix after the last iteration of each regression procedure.

Fractional production rate of LDL apo B

To calculate fractional production rates for LDL apo B we assumed: (1) that the purified apo B had been isolated from a homogeneous LDL pool; (2) that for

practical purposes the size of this pool was constant during the duration of each infusion experiment; that apo B entered this pool (3) at a constant rate; and (4) with constant enrichment. We further assumed (5) that during the duration of the infusion studies the enrichment in circulating LDL apo B was much lower than the enrichment of apo B newly entering the LDL pool. Tracer loss from the LDL pool could, therefore, be neglected. Since apo B may enter the LDL pool through either direct hepatic incorporation or through delipidation of VLDL, assumption (4) may have been compromised for the VLDL pathway during the early parts of each study when enrichment in VLDL apo B was changing rapidly. We, therefore, used only LDL apo B enrichment data between 180 and 480 min (six data points at 3, 4, 5, 6, 7 and 8 h). Since tracer incorporation into LDL apo B appeared to be constant during this period, we modelled the data by simple linear regression using the model described above in Equation 4:

$$dE_{LDL}/dt = k_1 \cdot F \quad \text{(Eq. 7)}$$

where k_1 is the fractional production rate of LDL apo B, and F is the enrichment of the apo B newly entering the LDL pool. To address the uncertainty of knowing the actual value of F, we used two linear models to calculate k_1 according to Equation 7, either assuming complete production of LDL from VLDL (LDL model 1: F equal to the mean enrichment in VLDL apo B between 180 and 480 min) or assuming exclusive hepatic LDL production (LDL model 2: F equal to the plateau enrichment in VLDL apo B, i.e. intrahepatic enrichment). In the case of exclusive production of LDL from VLDL, precursor enrichment, i.e. enrichment in VLDL, is not constant. To account for such a nonlinearity in precursor enrichment, we used a third model, in which the time course of VLDL apo B enrichment served as input function for LDL apo B [26] (LDL model 3, see appendix).

The smallest possible slope that could be detected with this method, was predicted by using the *t*-test $t = b \cdot (\sum x^2/MS_{res})^{0.5}$ with x denoting deviation from the mean X-value, $MS_{res} = SS_{res}/(n-2)$ denoting the residual mean square and n the number of points used for regression. The lowest detectable slope then was $b = t \cdot (MS_{res}/\sum x^2)^{0.5}$ with the minimum for the residual sum of squares SS_{res} depending on the precision of measuring enrichment in LDL apo B leucine. Since these values were measured with a variance of $s^2 = 0.04^2$ and an n of 6 points was used, SS_{res} could be estimated to be $s^2 \cdot (n-1) = 0.0080$. With $\sum x^2 = 17.5$ and $t_{0.05;1;4} = 2.78$ a minimum value at the $P = 0.05$ level for b thus was 0·03 mole % excess per hour.

Absolute apo B production rates

Absolute apo B production rates were calculated by multiplying VLDL and LDL apo B pool sizes by the corresponding fractional production rates; data were expressed relative to body weight (mg kg^{-1} day^{-1}). Pool sizes were determined as the products of measured apo B plasma concentration and plasma volume (0·045 l kg^{-1}).

Fractional catabolic rate of LDL apo B

A simplified model of LDL metabolism assumes that the input into the LDL pool is constant and independent of pool size and that the output is always a fraction of the pool size, i.e. production conforms to zero order, and removal to first order kinetics. If in this model the apo B pool is acutely depleted, then the subsequent rate of increase in pool size will be identical to the fractional catabolic rate [28]. This approach has been used previously to determine turnover of the LDL cholesterol pool [29]. We used this model and the data of the time course of LDL apo B pool size recovery after LDL apheresis to calculate apparent fractional catabolic rates for LDL apo B in our subjects. For each subject LDL apo B pool size was assumed to be 100% on Day 0, and the subsequent measurements were expressed relative to this baseline value. Data fitting was performed using the asymptotic model given in Equation 3 (assuming F = 100) and nonlinear regression analysis as described above [27].

Statistical analysis

All data are presented as means ± SD unless specified otherwise. To ascertain plateau levels for plasma leucine enrichment, plasma data of each study were tested for the absence of a significant slope using linear regression analysis and Student's *t*-test. Coefficients of correlation for regression lines describing LDL leucine enrichment obtained in subjects receiving low or high tracer infusion rates were compared using Student's *t*-test for independent comparisons. The effects of the LDL apheresis treatment on apo B metabolism were analysed by single factor analysis of variance for a repeated measures design [30]. Since two subjects did not complete the third study, ANOVA was restricted to the data of the four other subjects.

Results

Apo B pool sizes

The size of the VLDL apo B pool was determined in triplicate at the beginning of each leucine infusion study. Though for some subjects there was considerable variation between study days, mean VLDL pool size did not change significantly between days 0 and 8 (Table 2). The size of the LDL apo B pool was determined in all subjects on days 0, 1, 2, 4, and 8. Samples on days 0, 2, 4 and 8 were taken at 0800 h, while the sample on day 1 was taken directly after the apheresis treatment at 1600 h. The original pool size was reduced to 42 ± 7% at the end of the apheresis, and rose again to 54 ± 6, 77 ± 8, and 94 ± 10% on days 2, 4, and 8 (Fig. 1). During the leucine tracer infusion studies LDL apo B pool size was calculated from each

Table 2. Metabolic parameters for VLDL ApoB before (day 0) and after (days 2 and 8) LDL apheresis

Subject	Fractional production rate (pools day^{-1})			APO B pool size (mg)			Absolute production rate (mg kg^{-1} day^{-1})		
	Day 0	Day 2	Day 8	Day 0	Day 2	Day 8	Day 0	Day 2	Day 8
A	9·05	12·34	8·26	115·3	99·6	138·7	12·7	15·0	14·0
B	7·75	13·32	6·12	229·8	115·6	195·6	20·7	17·9	13·9
C	5·14	7·10	—	265·2	256·0	—	16·6	22·2	—
D	22·63	23·45	—	27·6	25·7	—	8·1	7·8	—
E	35·47	14·23	18·55	92·7	138·7	195·1	43·3	26·0	47·0
F	11·71	10·18	22·34	98·9	192·2	127·8	16·5	27·9	40·8
Mean	15·29	13·44	13·82	138·3	138·0	164·3	19·7	19·5	29·1
SD	11·60	5·53	7·86	90·4	79·3	36·1	12·3	7·5	17·7

Subjects C and D were not studied by tracer infusion on day 8.

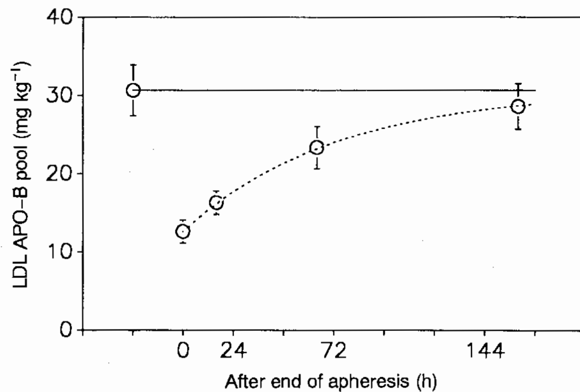

Figure 1. LDL apo B pool size before and after the apheresis treatment. All determinations were done from plasma samples drawn at 0800 h, except for the $t=0$ samples, which were drawn directly after the end of the apheresis procedure at 1600 h. Values represent means ± SE for six subjects. The solid line represents the mean original pool size (30·7 mg kg^{-1}), the dotted line represents the best fit for the observed mean data points of an asymptotic regression model ($Y = 30{\cdot}7 \cdot [1 - e^{-0{\cdot}331 \cdot t}]$, where t is in days).

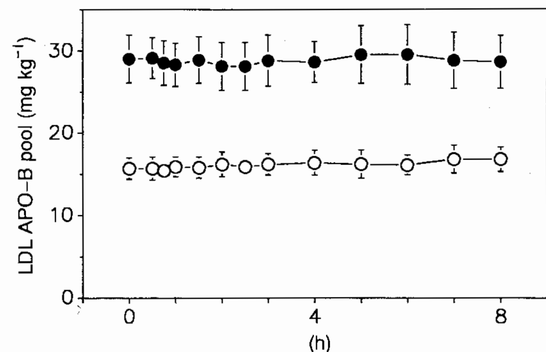

Figure 2. Time course of the LDL apo B pool size on day 2 (open circles, six subjects) and on day 8 (filled circles, four subjects). Values represent means ± SE. Mean LDL apo B pool size rose slightly but significantly during the study on day 2 (linear regression slope = 0·14 mg kg^{-1} h^{-1}; r = 0·886; $P < 0{\cdot}001$), but did not change on day 8.

plasma sample drawn. Pool size was constant during the first infusion study. The time course of the pool size during the experiments on day 2 (six subjects) and day 8 (4 subjects) is displayed in Fig. 2. The LDL apo B pool increased during the 8 h of infusion on day 2 by 7·4% from 15·7 ± 3·2 to 16·8 ± 3·6 mg kg^{-1}, the slope determined by linear regression analysis was different from zero ($r^2 = 0{\cdot}8865$, $P < 0{\cdot}001$). Pool size measured during the study on day 8, however, did not change significantly ($r^2 = 0{\cdot}054$, NS).

Isotopic enrichment data

Ion current ratios (m/z 217/216) of baseline samples taken before the start of tracer infusions were slightly higher on day 2 (some 1%) and day 8 (some 3%) when compared to day 0, indicating a low increase in the background abundance of [^{13}C]leucine caused by the preceding tracer infusions. Baseline ion current ratios on days 0, 2, and 8 were 0·1288 ± 0·0011, 0·1285 ± 0·0017, and 0·1356 ± 0·0096 for plasma, 0·1265 ± 0·0017, 0·1280 ± 0·0013, and 0·1302 ± 0·0033 for VLDL apo B, and 0·1265 ± 0·0009, 0·1278 ± 0·0017, and 0·1297 ± 0·0019 for LDL apo B. Enrichment values of subsequent samples during each infusion study were, however, calculated from the rise of ion current ratios above the current baseline level, and they were thus independent of the prevailing background abundance of [^{13}C]leucine.

[^{13}C]Enrichment in plasma leucine and leucine isolated from VLDL or LDL apo B on day 0 is shown for two subjects in Fig. 3. Absolute enrichment values were higher in subjects E and F due to the higher tracer infusion rate, but relative enrichment in the three compartments was similar in all subjects. To allow a meaningful graphic display of mean group data, all enrichment values of each subject were expressed relative to the corresponding plasma leucine enrichment plateau (Fig. 4). Plasma leucine enrichment remained stable throughout the infusions on all 3 days. After a short lag period of some 30 min [1-^{13}C] leucine appeared in VLDL apo B. Tracer enrichment in VLDL rose quickly at the beginning and levelled off towards

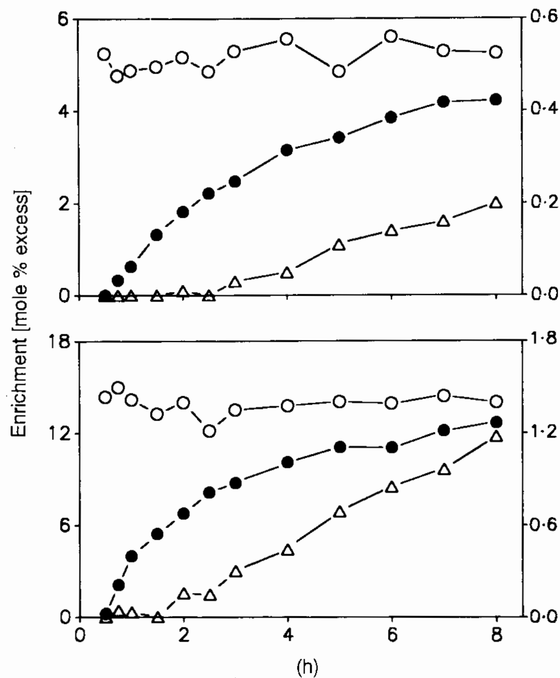

Figure 3. Enrichment in plasma free leucine (open circles), in leucine of VLDL apo B (filled circles) and in leucine of LDL apo B (open triangles) during [1-^{13}C]leucine infusion on Day 0. Data shown are for one subject receiving the low (top panel: subject A; 5 μmol kg^{-1} h^{-1}) and for one subject receiving the high (bottom: subject F; 20 μmol kg^{-1} h^{-1}) tracer infusion rate. Note: Left scales are for plasma and VLDL apo B only, while scales for LDL apo B are given on the right side.

the end of the infusion, approaching a plateau value. This asymptotic time course of tracer incorporation into VLDL apo B was similar on all three study days. Tracer incorporation into LDL apo B could only be detected after a longer lag period of 2 to 2·5 h; it then rose linearly throughout the remainder of the studies. In all studies LDL enrichment rose by more than 0·03 mole % excess/h and all individual slopes were different from zero ($P < 0·05$ or less). Tracer incorporation into LDL apo B was similar on days 0 and 8, but was markedly accelerated on day 2.

VLDL apo B turnover (Table 2)

Modelling of enrichment data in VLDL apo B using the asymptotic model yielded F values ($df_1 = 3$, $df_2 = 9$) between 175 and 4134 for individual curves corresponding to highly significant regression results with *P*-values less than 0·001 in all cases. Approximate standard errors for the parameter estimates, expressed as a fraction of the calculated parameters, were $3·4 \pm 1·2$% for the enrichment plateau, E_p, $12·3 \pm 6·8$% for the fractional turnover rate, k, and $15·8 \pm 9·3$% for the delay time, d. Enrichment at the end of the 8-h tracer infusion period had reached 99 ± 12, 100 ± 8, and 97 ± 11% of the calculated plateau values on Days 0, 2, and 8, respectively. The calculated fractional turnover rate, k, for VLDL apo B was not altered

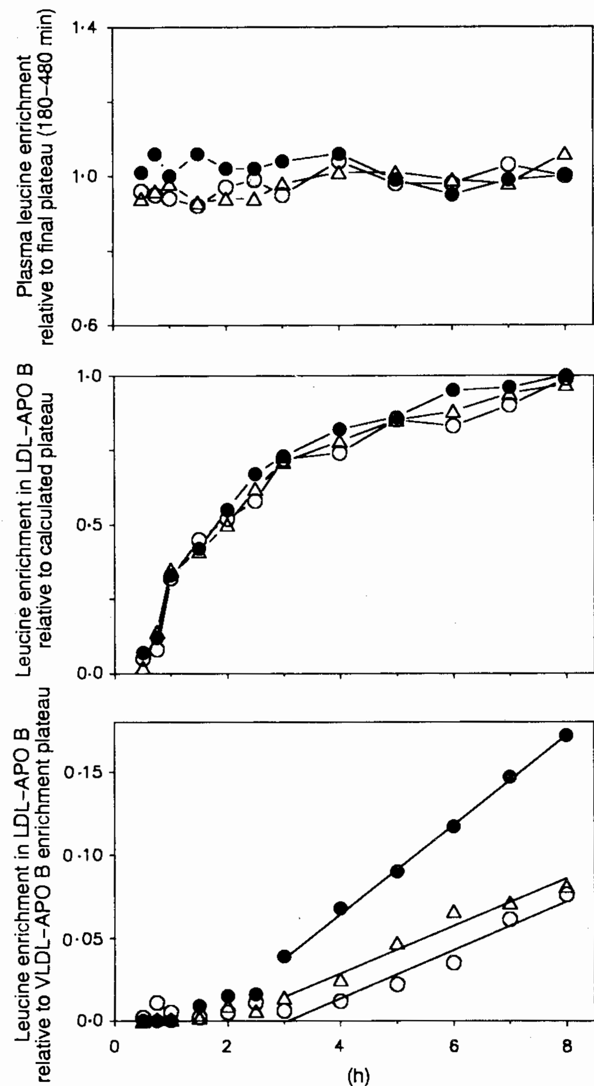

Figure 4. Relative [1-^{13}C]leucine enrichment in plasma (top panel), VLDL apo B (middle), and LDL apo B (bottom) during primed-constant infusion of [1-^{13}C]leucine in the subjects before (open circles, day 0), one day after (filled circles, day 2), and 7 days after (open triangles, day 8) LDL apheresis. Values of each infusion study were normalized to the corresponding enrichment plateau of plasma free leucine. Data shown represent means of six subjects (days 0 and 2) or of four subjects (day 8). Successive plasma and VLDL apo B data points are connected by lines. Lines shown in the bottom panel represent linear regression lines fitted to the mean LDL apo B data points between 3 and 8 h.

either 1 or 7 days after LDL-apheresis ($15·3 \pm 11·6$, $13·4 \pm 5·5$, $13·8 \pm 7·9$ day^{-1}; NS), however, there was considerable intra- (mean relative standard deviation 31%) and inter-subject variation (mean relative standard deviation 51%). Similarly, neither the plateau value, E_p, expressed as a fraction of the plasma leucine enrichment ($0·84 \pm 0·17$, $0·85 \pm 0·13$, $0·84 \pm 0·13$), nor the delay time, d ($0·42 \pm 0·05$, $0·43 \pm 0·09$, $0·48 \pm 0·06$ h) were affected by the apheresis treatment. Individual results for VLDL apo B pool size and fractional turnover rate are shown in Fig. 5 in comparison to

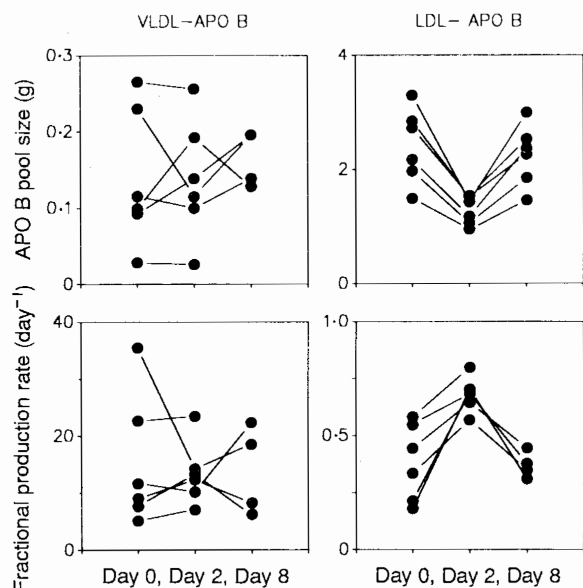

Figure 5. Individual results for pool size (top row) and fractional production rate (bottom row) of VLDL apo B (left side) and of LDL apo B (right side) in the subjects before (day 0), and one (day 2) or seven days (day 8) after LDL apheresis treatment. Each point represents a determination in one subject, measurements done in the same subjects are connected by lines. For two subjects (C and D) no determinations were done on day 8.

similar data for LDL apo B. Absolute VLDL apo B production, calculated as pool size multiplied by the fractional turnover rate did not differ significantly on the 3 study days (Table 2).

LDL apo B turnover (Table 3)

Fractional production rates calculated for LDL apo B were similar on days 0 and 8 but the rate was increased significantly on day 2. This result was independent of the model used. Using the average VLDL apo B enrichment between 180 and 480 min as the input enrichment into the LDL pool (LDL model 1), fractional production rates for LDL apo B were $0{\cdot}383 \pm 0{\cdot}169$, $0{\cdot}681 \pm 0{\cdot}075$, and $0{\cdot}370 \pm 0{\cdot}057$ day^{-1} on days 0, 2, and 8. Fractional production rates calculated using the plateau enrichment of VLDL apo B as precursor level (LDL model 2) were some 13% lower than the above rates ($0{\cdot}335 \pm 0{\cdot}170$, $0{\cdot}619 \pm 0{\cdot}099$, and $0{\cdot}318 \pm 0{\cdot}083$ day^{-1}). Using either model, values for day 2 were significantly higher than those of the other days ($P < 0{\cdot}01$). Similarly, data obtained with the nonlinear model (LDL model 3) showed a large increase of fractional production rates on day 2 ($0{\cdot}287 \pm 0{\cdot}130$, $0{\cdot}681 \pm 0{\cdot}125$, and $0{\cdot}349 \pm 0{\cdot}027$ day^{-1}). This increase in fractional turnover, however, was coinciding with the drop in LDL apo B pool size on day 2. Individual data for LDL apo B pool size and fractional production rate are shown in Fig. 5. As a consequence, absolute production rates of LDL apo B did not differ between the 3 study days (Table 3). In subject D production of LDL apo B exceeded production of VLDL apo B, indicating direct hepatic LDL production, in all other subjects LDL apo B production was a fraction of VLDL apo B production.

Our enrichment model measured fractional production of the LDL apo B pool but did not estimate the catabolic rate. Absolute production and catabolism of LDL apo B, however, can be assumed to have been nearly identical on days 0 and 8, since the LDL apo B pool size did not change on these days. In addition, our tracer data showed no change in LDL apo B production during the study period. Therefore, in our setting a simplified model of LDL metabolism may be proposed, which is characterized by a constant absolute inflow and a constant fractional outflow of apo B. In fact, pool size recovery conformed to an asymptotic curve in all subjects, as would be expected in such a model. Using the rate constant of LDL apo B pool size recovery after apheresis treatment as an estimate of the fractional catabolic rate, data agreed closely with fractional production rates calculated for days 0 and 8 (Table 4); fitting an asymptotic model to the pool recovery data of individual subjects gave an apparent fractional catabolic rate for the LDL apo B pool of $0{\cdot}367 \pm 0{\cdot}132$ day^{-1}.

Effect of tracer infusion rate

There was a slight drop in plasma glucose and insulin levels during the infusion studies and this pattern was similar on all 3 study days. However, there was no difference in glucose or insulin levels between subjects receiving the low or the high tracer infusion rates (Table 5). Plasma leucine flux did not vary between days 0, 2, and 8 ($103{\cdot}7 \pm 15{\cdot}7$, $93{\cdot}4 \pm 10{\cdot}0$, $91{\cdot}6 \pm 4{\cdot}5$ μmol kg^{-1} h^{-1}; n.s.). While plasma free leucine enrichment was higher in subjects E and F than in the other subjects, calculated leucine flux was similar in subjects receiving the low (mean of all study days; subjects A to D: $94{\cdot}0 \pm 11{\cdot}4$) or the high tracer infusion rate (subjects E, F: $101{\cdot}4 \pm 13{\cdot}3$ μmol kg^{-1} h^{-1}; NS). Similarly, plateau enrichment in VLDL apo B was higher in subjects E and F ($13{\cdot}4 \pm 0{\cdot}9$ vs. $4{\cdot}3 \pm 1{\cdot}0$ mole % excess). However, if plateau enrichment was expressed relative to the enrichment in plasma free leucine, there was no difference between subjects receiving different infusion rates ($0{\cdot}83 \pm 0{\cdot}11$ vs. $0{\cdot}85 \pm 0{\cdot}15$). There was no effect of the tracer infusion rate on estimates of other parameters of the VLDL model; means of all three study days for the fractional VLDL turnover rate were $11{\cdot}51 \pm 6{\cdot}59$ day^{-1} for subjects A to D and $18{\cdot}75 \pm 9{\cdot}34$ day^{-1} for subjects E and F (NS), analogous data for the delay time were $0{\cdot}45 \pm 0{\cdot}09$ h and $0{\cdot}41 \pm 0{\cdot}03$ h (NS). In addition, the higher infusion rate did not significantly alter the precision of VLDL apo B enrichment measurements as judged by the standard errors of the estimated model parameters. Means of the standard errors, expressed relative to the calculated parameters E_p, k, and d, were 4 ± 1, 13 ± 9, and 17 ± 11% for subjects A–D, and 3 ± 1, 11 ± 2, and 14 ± 5% for subjects E and F. Fractional

Table 3. Metabolic parameters for LDL apo B before (Day 0) and after (Days 2 and 8) LDL apheresis

	Fractional production rate (pools day^{-1})			APO B pool size (mg)			Absolute production rate (mg kg^{-1} day^{-1})		
Subject	Day 0	Day 2	Day 8	Day 0	Day 2	Day 8	Day 0	Day 2	Day 8
A	0·180	0·702	0·310	2841	1524	2259	6·2	13·0	8·5
B	0·548	0·683	0·376	1966	1063	1846	12·5	8·4	8·1
C	0·213	0·689	—	3295	1433	2988	8·6	12·0	—
D	0·582	0·798	—	1495	952	1458	11·3	9·9	—
E	0·444	0·644	0·445	2722	1536	2528	15·7	12·8	14·6
F	0·334	0·569	0·349	2167	1174	2364	10·3	9·5	11·8
Mean	0·383	0·681	0·370	2414	1280	2249	10·8	11·0	10·8
SD	0·169	0·075	0·057	657	251	291	3·3	1·9	3·1

Subjects C and D were not studied by tracer infusion on day 8. Fractional LDL apo B production was calculated using the mean VLDL apo B enrichment between 180 and 480 min as the input enrichment into the LDL pool.

Table 4. Fractional production and catabolic rates of LDL apo B

	Fractional production rate (pools day^{-1})		Fractional catabolic rate (pools day^{-1})
Subject	Day 0	Day 8	Days 0–8
A	0·180	0·310	0·153
B	0·548	0·376	0·391
C	0·213	—	0·337
D	0·582	—	0·560
E	0·444	0·445	0·348
F	0·334	0·349	0·414
Mean	0·383	0·370	0·367
SD	0·169	0·057	0·132

Subjects C and D were not studied by tracer infusion on day 8. Fractional LDL apo B production was calculated using the mean VLDL apo B enrichment between 180 and 480 min as the input enrichment into the LDL pool. Fractional catabolic rates were calculated as the rate constant of LDL apo B pool size recovery after apheresis treatment.

Table 5. Mean (±SD) plasma glucose and insulin levels on Day 0 in subjects receiving low or high tracer infusion rates

	Plasma glucose (mg dl^{-1})		Plasma insulin (mU l^{-1})	
Time (min)	Subjects A–D	Subjects E–F	Subjects A–D	Subjects E–F
−30	81·2±5·5	83·7	8·3±2·3	6·7
0	82·8±6·8	81·8	6·8±1·5	7·6
30	78·5±4·3	78·2	6·7±1·3	8·1
45	78·2±3·2	78·8	6·5±1·3	7·4
60	79·2±7·3	76·5	6·5±1·5	6·2
120	80·2±5·8	77·5	6·6±1·8	6·2
180	81·3±7·2	77·3	6·9±1·4	7·0
240	76·5±6·1	78·8	6·1±1·4	7·0
360	76·0±5·6	77·8	5·4±0·9	6·0
480	76·8±4·3	76·3	5·0±1·1	6·8

production rates calculated for LDL apo B were similar for subjects A–D and E–F. However, coefficients of determination for the fitted linear regression lines were significantly higher for the subjects receiving the high tracer infusion rate ($r^2 = 0{\cdot}979 \pm 0{\cdot}012$ for subjects E and F vs. $r^2 = 0{\cdot}913 \pm 0{\cdot}064$ for subjects A–D; $P < 0{\cdot}05$) indicating an increase in precision for these measurements.

Discussion

Investigation of *in vivo* protein synthesis in human subjects has recently gained new impetus with the introduction of several tracer infusion models using stable isotope methodology [7–12, 31,32, 33]. Regulation of apo B metabolism involves hepatic synthesis, conversion of VLDL to LDL particles, and receptor-mediated removal mechanisms, processes which may also interact with each other. Since apo B has been linked to atherogenesis, it appeared important to study the effect of circulating apo B on its hepatic production. If a feedback mechanism existed, it might lead to acutely increased apo B production after removal of circulating apo B; in addition, if such a mechanism were involved in maintaining normal apo B levels, a defect might result in apo B overproduction. Our results, however, show that in normal volunteers hepatic VLDL apo B production as well as LDL apo B production do not change after an acute reduction of the circulating apo B mass.

Estimation of *in vivo* protein synthesis by stable isotope tracer infusions is based on three basic procedures, infusion of an amino acid tracer, estimation of tracer enrichment at the site of protein synthesis, and determination of the time course of tracer enrichment in the protein of interest. Each of these steps needs to be addressed correctly to allow proper interpretation of the experiment.

The mode of tracer application, i.e. by bolus or constant infusion, is of major importance. We chose the well-established primed-constant infusion ap-

proach, since this model allows mathematical treatment in much simpler terms than is possible with a tracer bolus. Our tracer infusion was aimed at immediately labelling the protein precursor pool to a plateau level, so that precursor enrichment could be assumed to be constant for the duration of each experiment.

Interpretation of stable isotope studies has to assume that tracers are introduced in sufficiently small amounts that do not alter the metabolic properties of the observed system. Leucine infusion rates resulting in up to 10% enrichment in plasma leucine have been shown to not affect leucine metabolism, while infusions resulting in 15% enrichment may selectively increase leucine oxidation without changing protein synthesis or degradation [34]. An increase in whole body protein synthesis has been observed only during much higher leucine infusion rates [35]. To improve the accuracy of enrichment measurements in LDL apo B the leucine tracer was infused in two subjects at rates that labelled some 15% of plasma leucine molecules. However, calculated leucine flux rates in these subjects were not different from those in the subjects receiving less leucine tracer and they were also not different from flux rates observed previously in normal volunteers [36]. In addition, there was no effect of the tracer infusion rate on plasma glucose or insulin levels. We, therefore, considered metabolic effects of the infused leucine to have been negligible, and data of all six subjects were analysed together.

The rate of tracer incorporation into a protein depends both on the rate of protein synthesis and on tracer dilution at the site of protein synthesis. No information on the immediate protein precursor pool, i.e. hepatic leucyl-tRNA enrichment, was obtained. However, since analysis of our VLDL apo B model was based on the asymptotic time course of tracer enrichment, the corresponding precursor enrichment could be inferred from the final plateau enrichment in apo B—as derived from the mathematical solution of the model—and no separate data on precursor enrichment were necessary. Estimates of the precursor enrichment for direct hepatic synthesis of LDL apo B were based on the same assumption and plateau values of VLDL apo B enrichment were substituted for hepatic precursor enrichment. To judge the range of possible error introduced by this method, plasma free leucine enrichment may be taken to indicate the maximum enrichment possible for leucine bound to tRNA. Evidence supporting an actually close correlation between the behaviour of plasma and tRNA-leucine stems from studies indicating that leucine used for protein synthesis is taken directly from plasma without prior dilution in the cytosol [37,38,39]. In our study the ratio between the plateau enrichment in leucine of VLDL apo B and in plasma free leucine was 0·84.

Both VLDL and LDL have been shown to consist of heterogeneous particles [40]. To account for this heterogeneity both lipoprotein systems have been modelled extensively and radiolabel decay curve analysis has yielded multi-compartment models of differing degrees of complexity. It is, however, not always possible to assign physiological meaning to the compartments derived. We used simple one-compartment models for both the VLDL and the LDL apo B systems like others before us [8,9,10,12,41] assuming each lipoprotein pool to be composed of homogeneous particles. This analysis requires fewer data points, i.e. less blood, but reduces the complexity present in the system and sacrifices information on the internal structure within each lipoprotein class. However, simpler models have been shown to yield results that are very similar to those obtained with more complex models [11].

In our study, VLDL apo B concentrations did not change during the study period and the assumption of steady state conditions for the VLDL pool appear to be justified. In the case of VLDL it is necessary to assume predominantly hepatic production of VLDL apoliprotein B-100, even though production in the human intestine appears to be possible [42]. It is also necessary to assume uni-directional transport of newly synthesized apo B to the plasma membrane for secretion. This assumption does not preclude the existence of an intracellular path leading to degradation of newly synthesized apo B [43]. However, it is important to assume that newly synthesized apo B does not equilibrate in a non-accessible pool before being secreted into the plasma since this would complicate interpretation of plasma data significantly. *In vitro* data show that after intrahepatic apo B synthesis, sequential transcytosolic transport and secretion of assembled VLDL require about 30 min [43,44]. This has been considered in our single-pool VLDL model, and the observed delay time of some 27 min, together with the subsequent rapid rise in VLDL apo B enrichment are compatible with the assumed model. Further support for the lack of intrahepatic mixing of apo B may be seen in the similarity of our VLDL apo B turnover data to turnover rates obtained by injection of radiolabelled lipoproteins, which are not affected by intrahepatic processes [6].

The turnover of VLDL apo B has been calculated previously from stable isotope infusions using a linear model for the rise in apo B enrichment [8,9,10]. Cryer *et al.* infused a glycine tracer and used urinary hippurate enrichment as indicator of intrahepatic precursor enrichment [8], while Cohn *et al.* [9] and Lichtenstein *et al.* [10] used a leucine tracer and plateau enrichment in VLDL apo B to deduce the precursor enrichment level [9]. In these studies fractional turnover rates of VLDL apo B were calculated from a linear slope of enrichment in VLDL apo B relative to the precursor enrichment. Fitting a linear model to an asymptotic curve will, however, underestimate the true turnover rate, since loss of tracer from the VLDL pool is negligible only for a very brief period. Consequently, VLDL turnover rates reported by these authors are

significantly lower than the rates observed in our subjects.

Calculation of apo B production in the LDL pool requires knowledge of the tracer enrichment in the LDL precursor pool. Since apo B may enter LDL either through catabolism of VLDL particles or by direct hepatic synthesis [4] it was necessary to address possible differences in enrichment of these sources. Fractional synthesis rates of LDL apo B were, therefore, calculated using two different models. The first model assumed only indirect LDL production via VLDL catabolism and the average VLDL apo B enrichment between 3 and 8 h was used to represent the LDL precursor level. In the second model we used the VLDL apo B plateau enrichment as precursor equivalent to describe exclusive direct hepatic LDL production. However, since enrichment in VLDL apo B rose rapidly to approach hepatic leucine enrichment, both models gave very similar results.

The LDL apo B pool size was constant during the first and the third infusion experiments, but rose asymptotically during the early days after the apheresis treatment. However, the change in LDL pool size during the 8-h experiment on day 2 was less than 10%, thus introducing an error, that we felt was acceptable.

Our model for determination of LDL apo B production is similar to the method reported by Cohn *et al.* [9]. Since during each infusion study enrichment in LDL apo B was very low compared to that in the LDL precursor, loss of tracer from the LDL pool was minimal and could be ignored. This allowed the fractional LDL apo B production rate to be calculated as the linear increase in LDL apo B enrichment relative to the precursor enrichment. Results obtained in the baseline experiments are very similar to data reported for radiotracer studies of LDL apo B turnover [6].

The HELP apheresis procedure removes apo B-containing lipoproteins including VLDL. VLDL apo B levels, however, had returned to baseline 16 h after completion of the apheresis. This fast recovery is in keeping with the rapid turnover of VLDL apo B and agrees with the return of triglyceride [45] or VLDL [46] levels back to normal within 24 h after acute reduction by plasma exchange. Using the tracer infusion no systematic changes of fractional turnover rates could be observed either 1 or 7 days after the apheresis procedure. Thus hepatic production of VLDL apo B either did not change after the apheresis or had already returned to baseline on the following day. The observed intra- and inter-subject variations for the VLDL apo B production rates were considerable, i.e. from 12·7 to 47·0 mg kg^{-1} day^{-1} for VLDL apo B synthesis. Dietary irregularities might be responsible, since all subjects were investigated as outpatients and remained on their accustomed diets. Patients on more stringent dietary control have, however, displayed similar inter-subject variations in apo B fractional catabolic rates either when studied with radiotracers [6] or with stable isotopes [9]. In addition, no effect on VLDL apo B fractional synthetic rates was seen in subjects fasted for 48 h [7]. Therefore, the observed variations probably reflect true inter-subject differences in rates.

LDL apo B pool size was reduced to 40% directly after the apheresis and took much longer than VLDL to return to baseline concentrations. This return may have been brought about either by a transient increase in LDL production and/or a decrease in LDL removal. Our tracer infusion studies demonstrated a dramatic increase in fractional LDL apo B production on the day following the apheresis, but this increase could be explained completely by the reduction in apo B pool size, resulting in no significant change in the absolute production rate. This agrees well with results obtained by Soutar *et al.* [46] in four subjects with familial hypercholesterolaemia using reinjected radiolabelled LDL. These authors measured unaltered synthesis of LDL apo B 2 days after a plasma exchange and concluded that in hypercholesterolaemia apo B synthesis is independent of the apo B pool size. Our data extend these conclusions to normal subjects and exclude any significant direct or indirect feedback mechanism linking apo B plasma concentrations to hepatic apo B synthesis or apo B entering the LDL pool.

Our tracer data for LDL apo B allowed only the determination of LDL production rates and no actual catabolic rates were measured. However, since no change in LDL apo B production was observed, the recovery of the LDL apo B pool size after apheresis must have resulted from a temporary decrease in absolute LDL removal. A simple model to explain this behaviour would feature a constant fractional catabolic rate for LDL apo B [29]. After depletion of the LDL pool such a characteristic would lead to an asymptotic rise in pool size until the original steady state level was reached. In fact, the observed data points in our subjects fit a simple asymptotic model and regression yields a mean fractional catabolic rate of 0·367 day^{-1}, i.e. a value very similar to the fractional synthetic rates observed before and seven days after the apheresis. This may infer that also the fractional catabolic rate of LDL is independent of LDL pool size. A similar conclusion was drawn by Thompson *et al.* reporting on the recovery of LDL apo B levels after plasma exchange in four subjects with familial hypercholesterolaemia [47].

In conclusion, our study suggests that acute reduction of circulating apo B by LDL apheresis in healthy individuals affects neither the fractional removal rate nor the absolute production of either VLDL apo B or LDL apo B; i.e. basic characteristics of apo B metabolism appear to be not influenced by apo B pool size. Therefore, increased hepatic apo B release does not contribute significantly to the recovery of apo B pools after LDL apheresis. This finding is of importance when considering the therapeutic use of LDL apheresis in combination with cholesterol-lowering agents. At

the same time it appears that increases in plasma levels of apo B cannot result from a defect in a feedback mechanism linking plasma apo B mass to its production, since we could find no evidence that such a mechanism exists.

Acknowledgments

This work was supported by a grant from the National Institutes of Health (RR00954). J. Arends was supported by grants from the Deutsche Forschungsgemeinschaft (Ar 157/1-1) and the Deutsche Diabetesgesellschaft (Förderpreis 1985). We thank Drs T. Eisenhauer and P. Schuff-Werner and the HELP staff for their support in performing the apheresis treatments and Dr Hua Zhou for contributions to the mass spectrometry measurements.

References

1 Stamler J, Shekelle R. Dietary cholesterol and human coronary heart disease. The epidemiologic evidence. Arch Pathol Lab Med 1988;112:1032–40.
2 Havel RJ. Origin, metabolic fate and metabolic function of plasma lipoproteins. In: Steinberg D, Olefsky JM, eds. Contemporary issues in endocrinology and metabolism. Churchill Livingstone Inc., New York, 1986:117–141.
3 Sigurdsson G, Nicoll A, Lewis B. Conversion of very low density lipoprotein to low density lipoprotein. A metabolic study of apolipoprotein B kinetics in human subjects. J Clin Invest 1975;56:1481–90.
4 Beltz WF, Kesäniemi YA, Howard BV, Grundy SM. Development of an integrated model for analysis of the kinetics of apolipoprotein B in plasma very low density lipoproteins, intermediate density lipoproteins, and low density lipoproteins. J Clin Invest 1985;76:575–85.
5 Brown MS, Kovanen PT, Goldstein JL. Regulation of plasma cholesterol by lipoprotein receptors. Science 1981;212:628–35.
6 Kesäniemi YA, Vega GL, Grundy SM. Kinetics of apolipoprotein B in normal and hyperlipidemic man: review of current data. In: Berman M, Grundy SM, Howard BV, eds. Lipoprotein kinetics and modeling. New York: Academic Press 1982: 181–205.
7 Schauder P, Arends J, Schäfer G, Langer K, Bier DM. Incorporation of ^{15}N-glycine into VLDL and LDL: in vivo synthesis of apolipoprotein B in post-absorptive and fasting individuals. Klin Wochenschr 1989;67:280–5.
8 Cryer DR, Matsushima T, Marsh JB, Yudkoff M, Coates PM, Cortner JA. Direct measurement of apolipoprotein B synthesis in human very low density lipoprotein using stable isotopes and mass spectrometry. J Lipid Res 1986;27:508–16.
9 Cohn JS, Wagner DA, Cohn SD, Millar JS, Schaefer EJ. Measurement of very low density and low density lipoprotein apolipoprotein (Apo) B-100 and high density lipoprotein apo A-I production in human subjects using deuterated leucine. Effect of fasting and feeding. J Clin Invest 1990;85:804–11.
10 Lichtenstein AH, Cohn JS, Hachey DL, Millar JS, Ordovas JM. Schaefer EJ. Comparison of deuterated leucine, valine, and lysine in the measurement of human apolipoprotein-A-I and B-100 kinetics. J Lipid Res 1990;31:1693–701.
11 Parhofer KG, Barrett PHR, Bier DM, Schonfeld G. Determination of kinetic parameters of apolipoprotein B metabolism using amino acids labeled with stable isotopes. J Lipid Res 1991; 32:1311–23.
12 Walsh BW, Schiff I, Rosner B, Greenberg L, Ravnikar V, Sacks FM. Effects of postmenopausal estrogen replacement on the concentrations and metabolism of plasma lipoproteins. N Engl J Med 1991;325:1196–1204.
13 Russell DW, Yamamoto T, Schneider WJ, Slaughter CJ, Brown MS, Goldstein JL. cDNA cloning of the bovine low density lipoprotein receptor: Feedback regulation of a receptor mRNA. Proc Natl Acad Sci USA 1983;80:7501–05.
14 Thompson GR. Plasma exchange for hypercholesterolemia: a therapeutic mode and investigative tool. Plasma Ther Transf Technol 1980;1:5–15.
15 Stoffel W, Demant T. Selective removal of apolipoprotein B-containing serum lipoproteins from blood plasma. Proc Natl Acad Sci USA 1981;78:611–15.
16 Eisenhauer T, Armstrong VW, Wieland H, Fuchs C, Scheler F, Seidel D. Selective removal of low density lipoproteins (LDL) by precipitation at low pH: First clinical application of the HELP system. Klin Wochenschr 1987;65:161–8.
17 Mabuchi H, Michishita I, Takeda M *et al.* A new low density lipoprotein apheresis system using two dextran sulfate cellulose columns in an automated column regenerating unit (LDL continuous apheresis). Atherosclerosis 1987;68:19–25.
18 von Bayer H, Schwerdlfeger R, Schwartzkopf W *et al.* Selective removal of low density lipoproteins (LDL) by plasmapheresis combined with selective plasma-protein ultracentrifugation (SPU). Plasma Ther Transf Technol 1983;4:447–58.
19 Lupien PJ, Moojani S, Award J. A new approach to the management of familial hypercholesterolemia: removal of plasma cholesterol based on the principle of affinity chromatography. Lancet 1976;1:1261–5.
20 Nose Y, Usani M, Malchesky PS *et al.* Clinical thermofiltration: initial application. Artif Organs 1985;9:425–7.
21 Egusa G, Brady DW, Grundy SM, Howard BV. Isopropanol precipitation method for the determination of apolipoprotein B specific activity and plasma concentrations during metabolic studies of very low density lipoprotein and low density lipoprotein apolipoprotein B. J Lipid Res 1983;24:1261–7.
22 Roach D, Gehrke CW. The hydrolysis of proteins. J Chromatogr 1970;52:393–404.
23 Arends J, Bier DM. Labeled amino acid infusion studies of in vivo protein synthesis with stable isotope tracers and gas chromatography-mass spectrometry. Anal Chim Acta 1991; 247:255–63.
24 Cobelli C, Toffolo G, Bier DM, Nosadini R. Models to interpret kinetic data in stable isotope tracer studies. Am J Physiol 1987;253:E551–64.
25 Matthews DE, Motil KJ, Rohrbaugh DK, Burke JF, Young VR, Bier DM. Measurement of leucine metabolism in man from a primed, continuous infusion of L-[1-^{13}C]leucine. Am J Physiol 1980;238:E473–9.
26 Zak R, Martin AF, Blough R. Assessment of protein turnover by use of radioisotopic tracers. Physiol Rev 1979;59:407–47.
27 Snedecor GW, Cochran WG. Statistical methods, Iowa State University Press, 1989, Ames, Iowa.
28 Berlin CM, Schimke RT. Influence of turnover rates on the responses of enzymes to cortisone. Mol Pharmacol 1965;1: 149–56.
29 Apstein CS, Zilversmit DB, Lees RS, George PK. Effect of intensive plasmapheresis on the plasma cholesterol concentration with familial hypercholesterolemia. Atherosclerosis 1978; 31:105–15.
30 Hartung J, Elpelt B. Multivariate Statistik, R. Oldenbourg, Munchen 1989;221–68.
31 Thompson C, Blumenstock FA, Saba TM *et al.* Plasma fibronectin synthesis in normal and injured humans as determined by stable isotope incorporation. J Clin Invest 1989;84:1226–35.
32 Yudkoff M, Nissim I, McNellis W, Polin RA. Albumin synthesis in premature infants: determination of turnover with [^{15}N]glycine. Ped Res 1987;21:49–53.
33 Stein TP, Leskiw MJ, Wallace HW. Measurement of half-life of human plasma fibrinogen. Am J Physiol 1978;234:E504–10.
34 Tessari P, Tsalikian E, Schwenk WF, Nissen S, Haymond MW. Effects of [^{15}N]leucine infused at low rates on leucine metabolism in humans. Am J Physiol 1985;249:E121–30.
35 Schwenk WF, Haymond MW. Effects of leucine, isoleucine, or threonine infusion on leucine metabolism in humans. Am J Physiol 1987;253:E428–34.

36 Bier DM. Intrinsically difficult problems: The kinetics of body proteins and amino acids in man. Diabetes/Metabolism Reviews 1989;5:111–32.
37 Hider RC, Fern EB, London DR. Identification in skeletal muscle of a distinct extracellular pool of amino acids, and its role in protein synthesis. Biochem J 1971;121:817–827.
38 Flaim KE, Peavy DE, Everson WV, Jefferson LS. The role of amino acids in the regulation of protein synthesis in perfused rat liver. I. Reduction in rates of synthesis resulting from amino acid deprivation and recovery during flow-through perfusion. J Biol Chem 1982;257:2932–8.
39 Kelley J, Stirewalt WS, Chrin L. Protein synthesis in rat lung. Measurements in vivo based on leucyl-tRNA and rapidly turning-over procollagen I. Biochem J 1984;222:77–83.
40 Babiak J, Rudel LL. Lipoproteins and atherosclerosis. In: Shepherd J ed. Lipoprotein metabolism. London: Bailliere Tindall, 1987:515–50.
41 Fisher WR. Apoprotein B kinetics in man: concepts and questions. In: Berman M, Grudy SM, Howard BV eds. Lipoprotein kinetics and modeling. New York: Academic Press, 1982: 43–68.
42 Levy E, Rochette C, Londono I *et al.* Apolipoprotein B-100: immunolocalization and synthesis in human intestinal mucosa. J Lipid Res 1990;31:1937–46.
43 Borchardt RA, Davis RA. Intrahepatic assembly of very low density lipoproteins. Rate of transport out of the endoplasmic reticulum determines rate of secretion. J Biol Chem 1987; 262:16394–402.
44 Olofsson SO, Bjursell G, Boström K *et al.* Apolipoprotein B: structure, biosynthesis and role in the lipoprotein assembly process. Atherosclerosis 1987;68:1–17.
45 Kilpatrick D, Fleming J, Clyne C *et al.* Reduction of blood viscosity following plasma exchange. Atherosclerosis 1979; 32:301–06.
46 Soutar AK, Myant NB, Thompson GR. Metabolism of apolipoprotein B-containing lipoproteins in familial hypercholesterolaemia. Effects of plasma exchange. Atherosclerosis 1979;32:315–25.
47 Thompson GR, Spinks T, Ranicar A, Myant NB. Non-steady-state studies of low-density-lipoprotein turnover in familial hypercholesterolaemia. Clin Sci Mol Med 1977;52:361–9.
48 Swick RW. Measurements of protein turnover in rat liver. J Biol Chem 1958;231:751–64.
49 Waterlow JC, Stephen JML. The effect of low protein diets on the turnover rates of serum, liver and muscle proteins in the rat, measured by continuous infusion of ^{14}C-lysine. Clin Sci 1968; 35:287–305.
50 Garlick PJ, Millward DJ, James WPT. The diurnal response of muscle and liver protein synthesis in vivo in meal-fed rats. Biochem J 1973;136:935–45.

Appendix

It may be assumed that apo B enters the LDL pool exclusively through catabolism of VLDL. A continuously infused amino acid tracer will then first label the VLDL apo B pool before reaching LDL. After the start of the tracer infusion unlabelled apo B will decrease exponentially in the VLDL pool and the rise of enrichment in VLDL apo B will follow an asymptotic curve as described by Equation 3 above. Since the enrichment in VLDL apo B will be the source of label entering the LDL pool, it is necessary to use Equation 3 as an input function to describe the nonlinear time course of enrichment in LDL apo B. Assuming steady state conditions, i.e. constant pool size, we used the solution proposed by Zak *et al.* [26] to solve the system:

$$\frac{P}{F} = \frac{kF}{kF - kP} \cdot \frac{1 - e^{-kP \cdot t}}{1 - e^{-KF \cdot t}} - \frac{kP}{kF - kP}$$

where F and P are the measured enrichment levels in VLDL apo B (input, F) and LDL apo B (product, P), and kF and kP are the fractional turnover rates of the VLDL apo B and the LDL apo B pools. Similar models have been used previously by different authors [48,49,50]. Modelling was done by nonlinear regression analysis performed on a personal computer as described in the methods section.