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The metabolism of lipoprotein(a) and other apolipoprotein B-containing lipoproteins: a kinetic study in humans

Thomas Demant *, Katja Seeberg, Andrea Bedynek, Dietrich Seidel

Institut für Klinische Chemie, Klinikum Grosshadern der LMU, Munich, Germany

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Abstract

Lipoprotein(a) is a risk factor for cardiovascular disease composed of an apolipoprotein B-containing lipoprotein to which a second protein, apolipoprotein(a), is attached. We investigated in seven subjects with Lp(a) levels of 39–85 mg/dl the metabolism of four apo B-containing lipoproteins ($VLDL_1$, $VLDL_2$, IDL and LDL) together with that of apo B and apo(a) isolated from Lp(a). Rates of secretion, catabolism and where appropriate, transfer were determined by intravenous administration of d_3-leucine, mass spectrometry for measurements of leucine tracer/tracee ratios and kinetic data analysis using multicompartmental metabolic modeling. Apo B in Lp(a) was secreted at a rate of 0.28 (0.17–0.40) mg/kg per day. It was found to originate from two sources — 53% (43–67) were derived from preformed lipoproteins, i.e. IDL and LDL, the remainder was accounted for by apo B, directly secreted by the liver. The fractional catabolic rates (FCRs) of apo B and of apo(a) prepared from Lp(a) were determined as 0.27 (0.16–0.38) and 0.24 (0.12–0.40) pools per day, respectively, which is less than half of the FCR observed for LDL. Our in vivo data from humans support the view that Lp(a) assembly is an extracellular process and that its two protein components, apo(a) and apo B, are cleared from the circulation at identical rates.

Keywords: Lipoprotein(a); Low density lipoprotein (LDL); Stable isotopes; Metabolic modeling; Apolipoprotein B; Apolipoprotein(a)

1. Introduction

Lipoprotein(a) (Lp(a)) contains apolipoprotein B-100, and as a second protein component, a highly glycosylated protein called apolipoprotein(a). The lipoprotein moiety of Lp(a) is similar to LDL, as has been shown by compositional analyses and metabolic studies [1]. Apo(a) is structurally homologous to plasminogen, a molecule which comprises five different so-called kringle loops (KI–KV) and a specific protease domain. Similarly, the apo(a) molecule consists of a plasminogen-like protease domain, one copy of kringle V and a variable number of kringle IV repeats [2]. The number of kringle IV copies is genetically determined and constitutes the basis of a marked size polymorphism of apo(a) ranging between 300 and 900 kDa. Apo(a) is covalently attached via a disulfide bond to apo B in a 1:1 molar ratio [3]. Thus, Lp(a) combines physically a cholesterol-rich particle with a molecule related to the blood clotting system. Lp(a) plasma concentrations and their relation to cardiovascular disease have been studied in a number of epidemiological trials. It has been demonstrated that at least in Caucasians, the apo(a) size polymorphism is inversely correlated with the Lp(a) plasma concentration and that Lp(a) is a risk factor for coronary heart disease and possibly for stroke [4–6].

The metabolism of Lp(a) has been investigated by different techniques. Early studies in humans using exogenously radiolabeled lipoprotein tracers suggested that Lp(a) is not derived from very low density lipoprotein (VLDL) precursors and that the fractional catabolic rate of Lp(a) is considerably lower than that of low density lipoprotein (LDL) [7,8]. Experiments with different cell lines as well as turnover studies in patients with familial hypercholesterolemia provided evidence that Lp(a) is a poor ligand for the LDL-recep-

* Corresponding author. Present address: Institut für Klinische Chemie und Labormedizin (IKL), Krankenhaus Dresden-Friedrichstadt, Friedrichstr. 41, 01067 Dresden, Germany. Tel.: +49-351-4803900; fax: +49-351-4803909.

E-mail address: demant-th@khdf.de (T. Demant).

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tor and that the LDL-receptor is not required for normal Lp(a) catabolism [9,10]. Tracer studies with exogenously radioiodinated Lp(a) also established that the plasma concentrations of Lp(a) are mainly determined by the rate of Lp(a) production rather than by its rate of catabolism [11,12].

In spite of intensive research in recent years, the mechanisms which govern Lp(a) secretion and assembly and notably the routes of its catabolism are still poorly understood. Studies in patients receiving a liver transplant made it clear that Lp(a) is secreted by the liver [13]. White et al. were able to show in primary cultures of baboon hepatocytes, using pulse chase experiments and immunoprecipitation techniques, that the rate of apo(a) secretion is inversely correlated to its molecular size. They also showed that the association between apo(a) and apo B occurs extracellularly following secretion rather than inside cellular compartments since co-precipitation of apo B and apo(a) was only observed in the culture medium and not in cell lysates [14,15]. Further evidence for the extracellular assembly of Lp(a) stems from experiments, where human LDL was infused into transgenic mice expressing human apo(a) resulting in the formation of Lp(a) particles [16]. Finally, studies both in vitro and in transgenic animals demonstrated that structural disruptions of the apo B molecule such as the $A_{3500} \rightarrow G$ mutation or the apo B-94 truncation interfere with apo(a) binding and Lp(a) formation [17,18]. However, it is not clear at present as to what extent the metabolism of apo B-containing lipoproteins and of Lp(a) are intertwined. Even less is known about the mechanisms involved in Lp(a) catabolism. As mentioned above, the LDL-receptor seems to play only a minor role in Lp(a) elimination. Recent observations suggest that the VLDL-receptor which is primarily expressed in skeletal muscle may be of significance for Lp(a) binding and degradation [19]. In line with the fact that chronic renal failure is associated with increased Lp(a) plasma levels, it was suggested that the kidneys may be important for Lp(a) catabolism [20]. In a recent paper, a renal arterio–venous Lp(a) concentration difference of 9% was reported but the mechanisms involved are at present entirely speculative [21].

In the present study we have investigated Lp(a) metabolism in humans using an endogenous labeling protocol based on the administration of a stable isotope substituted amino acid tracer. This approach allows the simultaneous study of the metabolism of the two protein constituents of Lp(a). In particular we sought to determine the rates of production, transfer and catabolism of apo(a) and of apo B. The latter was prepared not only from Lp(a) but also from LDL and its metabolic precursors IDL and two VLDL fractions, in order to uncover the interconnections between apo B and apo(a) metabolism under in vivo conditions. Since we used a stable isotope tracer protocol, any concerns about artefactual perturbations of apolipoprotein metabolism due to lipoprotein purification and exogenous radiolabeling can be ruled out. The metabolic data accumulated in this study enabled us to draw some conclusions about Lp(a) assembly and degradation in normolipidemic human subjects.

2. Methods

2.1. Subjects

Seven subjects — five males and two females — participating in this study were selected from medical students and laboratory staff. Their body weight was normal (body mass index (BMI) 20–26 kg/m^2), they were healthy, normolipidemic (total cholesterol < 250 mg/dl, triglyceride < 100 mg/dl) and they displayed a Lp(a) concentration of > 30 mg/dl. Metabolic data from turnover studies in two subjects (JD and GH) were also used in the control group of an earlier published study [22]. Throughout the study, participants were asked to continue with their normal diets. All subjects participating in the study gave informed consent. The study protocol was approved by the Ethics Committee of the Klinikum Grosshadern, LMU Munich.

2.2. Turnover protocol

The protocol employed has been described in detail elsewhere [23]. Subjects were fasted for 12 h overnight. At 08:00 h, either an intravenous bolus injection (6.0 mg/kg body weight (b.w.)) or a primed constant infusion (0.6 mg/kg; 0.6 mg/h for 10 h) of d_3-leucine (Cambridge Isotopes, Woburn, MA, USA) was given. Equivalence of the two modes of tracer application has been demonstrated earlier [23]. A light meal was offered 10 h after tracer injection. Plasma samples (10 ml blood collected in EDTA) were collected for apo B, apo(a) and plasma free leucine analysis immediately before and after tracer administration at the following time points; 5, 10, 15, 30, 45 min, 1, 1.5, 2, 3, 4, 6, 8, 10 h and in the fasting state — 1, 2, 3, 5, 7, 10 and 12 days.

2.3. Preparation of apo B from $VLDL_1$-, $VLDL_2$-, IDL- and LDL

$VLDL_1$ (S_f 60–400), $VLDL_2$ (S_f 20–60), IDL (S_f 12–20) and LDL (S_f 0–12) were prepared from 2 ml of EDTA plasma (final concentration 1.5 mg/ml) by cumulative gradient ultracentrifugation as earlier described [23]. Briefly, 2 ml plasma were adjusted to a density of $d = 1.118$ g/ml by addition of solid NaCl and built into a discontinuous six-step salt gradient ranging

from $d = 1.0988 - 1.0588$ g/ml. After the following centrifugational runs at 23°C in a Beckman SW40 rotor lipoprotein preparations were collected from the surface of the gradient: $VLDL_1$ (1.0 ml, replaced by 1.0 ml of $d = 1.0588$ g/ml solution); 39000 rpm, 1.63 h; $VLDL_2$ (0.5 ml); 18500 rpm, 15.68 h; IDL (0.5 ml); 39000 rpm, 2.58 h; LDL (1.0 ml); 30000 rpm, 21.17 h. Prior to delipidation, LDL preparations were tested for contamination by lipoprotein(a) which, when detectable, was removed quantitatively by affinity chromatography as described below. Apo B was precipitated from lipoprotein fractions by addition of an equal volume of isopropanol; the resulting pellet was delipidated with ethanol:ether (3:1), dried with ether and hydrolysed at 110°C for 20 h in the presence of 0.5–1.0 ml 6N HCl, which was subsequently removed by evaporation in a vacuum concentrator centrifuge. Apo B plasma pools were derived from the apo B content, which was calculated as the difference between total and isopropanol-soluble protein, and an estimate of the plasma volume (4% of the b.w.). The apo B masses of each of the lipoprotein fractions were corrected for experimental losses by comparing the total cholesterol recovered in the four fractions obtained by cumulative ultracentrifugation, with the non-HDL cholesterol value determined in native plasma. The fractional leucine content of apo B was taken as 0.1212 g/g.

2.4. Preparation of apo B and apo(a) from Lp(a)

Blood was collected into tubes containing EDTA and aprotinine (Trasylol, Bayer, FRG) at final concentrations of 1.5 mg/ml and 1000 IE/ml, respectively. Lp(a) was prepared using an affinity chromatography method with immobilised wheat germ agglutinine (WGA) published by Seman et al. [24]. Wheat germ agglutinine (Sigma, München, FRG) was attached to Sephacryl-1000 (Pharmacia, Freiburg, FRG) according to a protocol by Cuatrecasas [25]. This matrix was chosen because of its low unspecific retention of lipoproteins. About 2 ml of plasma was incubated with 1 ml of WGA-Sephacryl for 10 min at room temperature and transferred into a 5 ml column case. Unbound plasma proteins were eliminated with 20 ml PBS, 0.3 M NaCl, 0.2 M L-prolin [26]. Lp(a) was desorbed with 5 ml PBS, 0.2 M *N*-acetyl-D-glucosamine (Sigma, München, FRG). Fractions of the eluate containing cholesterol were pooled, adjusted to a density of $d = 1.150$ g/ml and ultracentrifuged at 95000 rpm and 15°C for 4 h in a Beckman TL-100.1 rotor. Lp(a) was recovered from the top of the tube and checked for purity by lipoprotein agarose gel electrophoresis [27] (Fig. 1). In order to dissociate by reductive cleavage the S–S bond between apo B and apo(a), the Lp(a) preparation was incubated with 1% β-mercaptoethanol for 1 h at 37°C [28]. The apo B-containing lipoprotein, Lp(−), and apo(a) were separated by a second ultracentrifugation at $d = 1.150$ g/ml as described above. Lp(−) was recovered from the top of the tube, whereas apo(a) formed a solid pellet. Apo B from Lp(−) was further processed as described for apo B prepared from VLDL, IDL or LDL (see above). The apo(a) pellet was resolubilised with 0.1 ml of 1% β-mercaptoethanol for 30 min at 50°C, dried in a vacuum concentrator centrifuge and hydrolysed as described above. All study participants expressed the apo(a) phenotype 18, 19 or 20. The second apo(a) isoform was either similar (phenotype 19 or 21) or of higher molecular weight (phenotypes 27, 28, 31 or 36). The latter, however, in accordance with the known inverse relationship between apo(a) plasma concentration and apo(a) molecular weight, were only weakly expressed as shown by the corresponding faint bands on the apo(a) phenotyping radiographs. Since direct protein measurements of apo(a) were perturbed by mercaptoethanol and the high carbohydrate content, estimates of the apo(a) pools were based on the results of Lp(a) phenotyping and the determinations of apo B derived from Lp(a) described above. The sequence derived molecular weight of apo B is 514 kDa [29], that of apo(a) phenotype 19 is 275 kDa [30], the fractional leucine contents are 0.1212 and 0.0350 g/g, respectively [2]. The mass ratio of leucine in apo B and in total apo(a) from Lp(a) was calculated as 6.5:1. This ratio was used to derive apo(a) leucine masses from apo B leucine mass determinations.

2.5. Free amino acid preparation from plasma

Proteins were precipitated from 1 ml plasma by adding 1 ml trichloroacetic acid (10%) and amino acids prepared from the supernatant by cation exchange chromatography using 2 ml columns filled with Dowex AG-50W-X8 resin (H^+-form, 50–100 mesh; Biorad, Richmond, CA, USA). The amino acids which bound

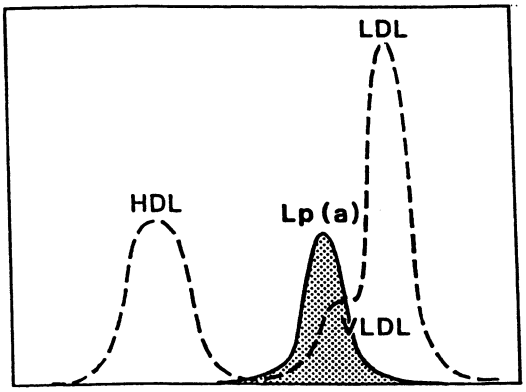

Fig. 1. Lipoprotein agarose gel electrophoresis of Lp(a) as prepared by wheat germ agglutinine affinity chromatography and ultracentrifugation at $d = 1.150$ g/ml (for details see text). Positions of LDL, VLDL and HDL are indicated by the broken line for reference.

to the resin were desorbed by 4 M NH_4OH which was subsequently removed by evaporation in a vacuum concentrator [31]. The samples were dissolved in a small volume of 1 M HCl transferred into microvials and dried again ready for derivatisation.

2.6. Other laboratory procedures

Plasma lipids and lipoproteins were analysed by standard laboratory procedures in line with the Lipid Research Clinics protocol [32]. Lp(a) was quantified as Lp(a) total mass by a nephelometric assay using a goat anti-human Lp(a) antibody (R. Greiner BioChemica, Flacht, Germany). Apo(a) isoform analysis was performed as described elsewhere, using agarose gel electrophoresis and chemiluminescence enhanced immunoblotting [33].

For compositional analyses, $VLDL_1$, $VLDL_2$ and IDL were prepared by the ultracentrifugational procedure described above. Additionally, LDL (S_f 0–12) was subfractionated into large LDL_1 (S_f 6–12) and small LDL_2 (S_f 0–6) by ultracentrifugation for 5.10 h at 36000 rpm followed by 12:15 h at 32000 rpm. Total and free cholesterol, triglyceride and phospholipids were determined by enzymatic routine laboratory tests (Boehringer Mannheim, Germany), protein was measured by the Lowry's method as described earlier.

2.7. Leucine tracer/tracee analysis by quadrupole GC-MS

Amino acids derived from apo B, from apo(a) or from plasma were transformed into *t*-butyl-dimethyl-silyl- (TBDMS-) derivatives by incubation with 50 μl of a freshly prepared 1:1 mixture of *N*-methyl-*N*-(*t*-butyl-dimethyl-silyl)-trifluoro-acetamide (MTBSTFA; Fluka, Buchs, Switzerland) and acetonitrile in crimped microvials at 80°C for 20 min. Enrichments were determined immediately by gas chromatography mass spectrometry using a quadruple GC-MS instrument (Trio 1000, Fisons, Manchester, UK).

A detailed description of the method used for GC-MS analysis has been published elsewhere [23]. The gas chromatograph was equipped with a DB1701 capillary column (J&W, Folsom, CA, USA) operated at 110°C for 1 min. after sample injection followed by an increase of temperature of 20°C per min up to 280°C. The mass spectrometer was used with electron impact ionisation (EI^+). Leucine ion mass fragments were monitored and quantified in the selective ion recording (SIR) mode at mass-to-charge ratios (m/z) 277, 276 and 274. From these measurements, the specific isotopic enrichment (E) and the leucine tracer/tracee ratio (Z) were calculated by the following formulae [34];

$$E = \frac{(R - R_N)}{[(1 + R)(1 + R_N)]} \quad (1)$$

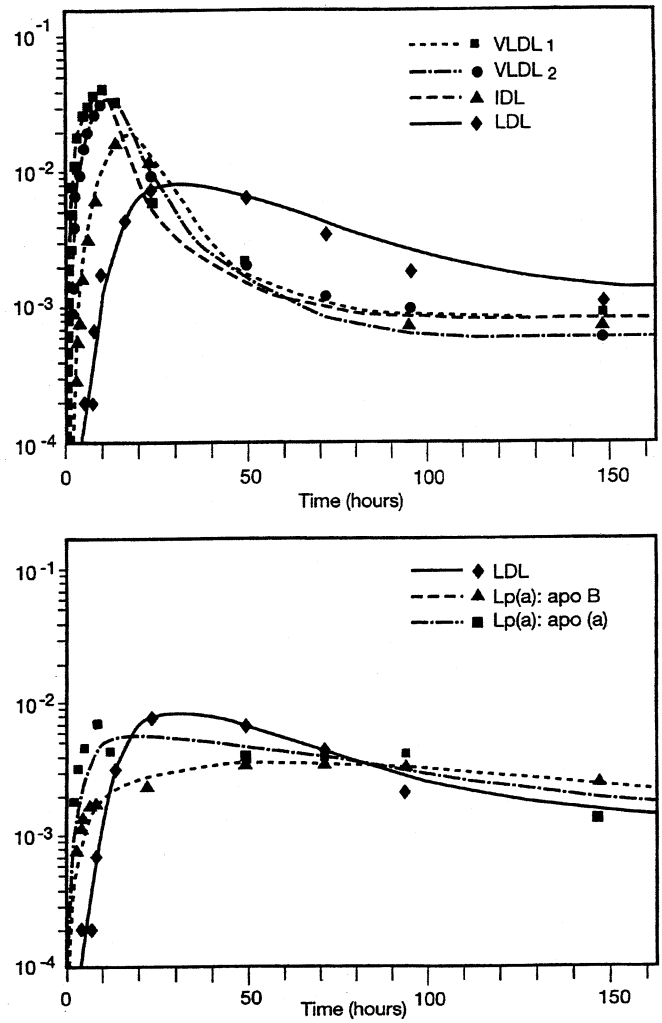

Fig. 2. Tracer/tracee ratios recorded after injection of a d_3-leucin tracer. Data are from a typical turnover study (JK). Top panel: apo B from $VLDL_1$, $VLDL_2$, IDL and LDL. Bottom panel: apo B from LDL and apo B and apo(a) isolated from Lp(a).

where, R is the m/z 277 to m/z 274 ratio for the enriched sample and R_N is the equivalent ratio for naturally occurring leucine ($R_N = 0.01697$; $n = 10$).

$$Z = \frac{E}{(E_I - E)} \quad (2)$$

where, E_I is the isotopic abundance of the infused tracer, which was determined to be 0.98.

2.8. Kinetic analysis and multicompartmental modeling

A typical example for the time courses of leucine tracer/tracee ratios measured in the four apo B-containing lipoproteins $VLDL_1$, $VLDL_2$, IDL and LDL and the two protein components of Lp(a), apo B and apo(a), is shown in Fig. 2. The tracer/tracee ratios for apo B and apo(a), both derived from Lp(a), from the seven subjects investigated, are depicted in Fig. 3. Tracer/tracee ratios

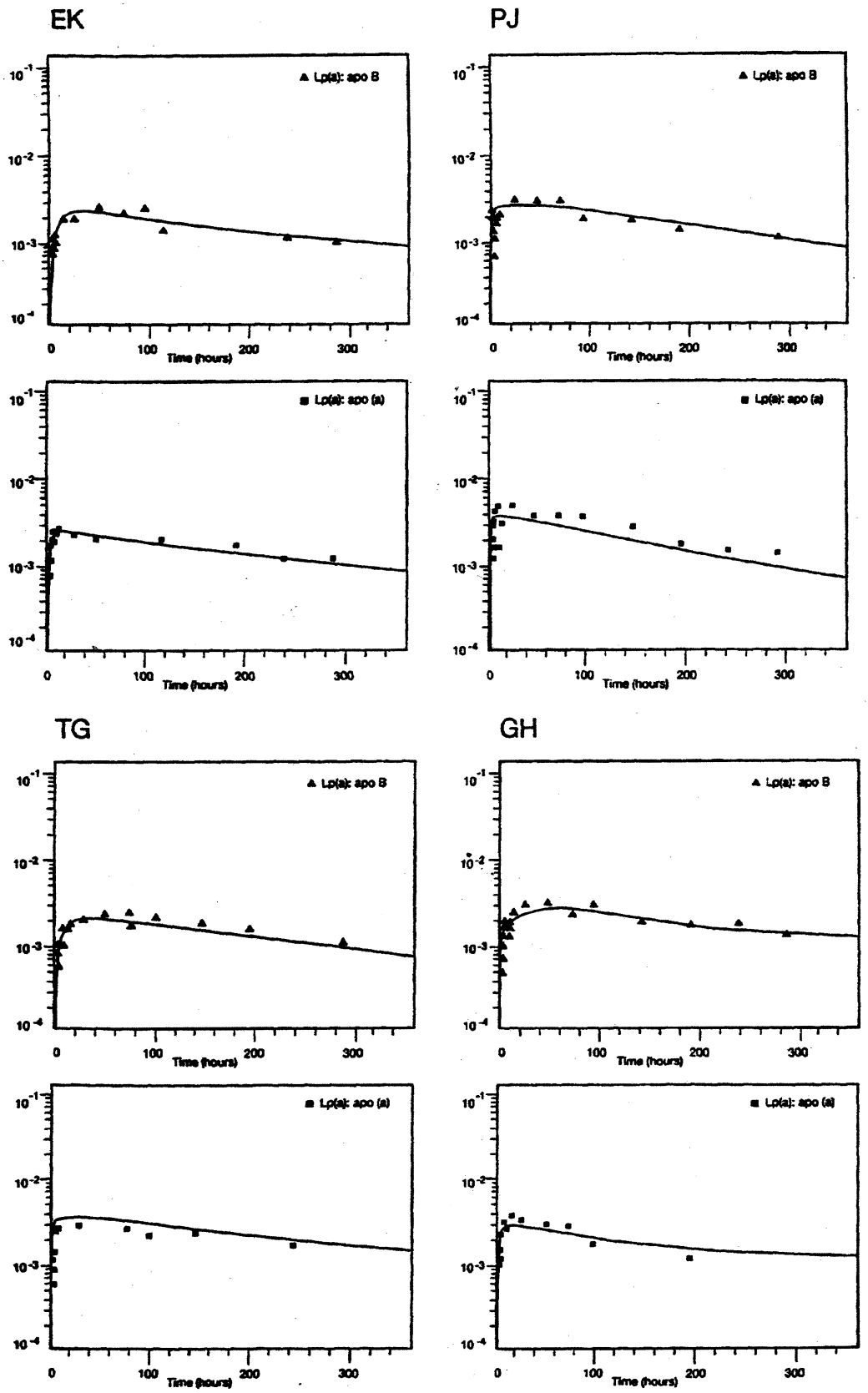

Fig. 3. Tracer/tracee ratios from seven subjects for apo B and apo(a) isolated from Lp(a).

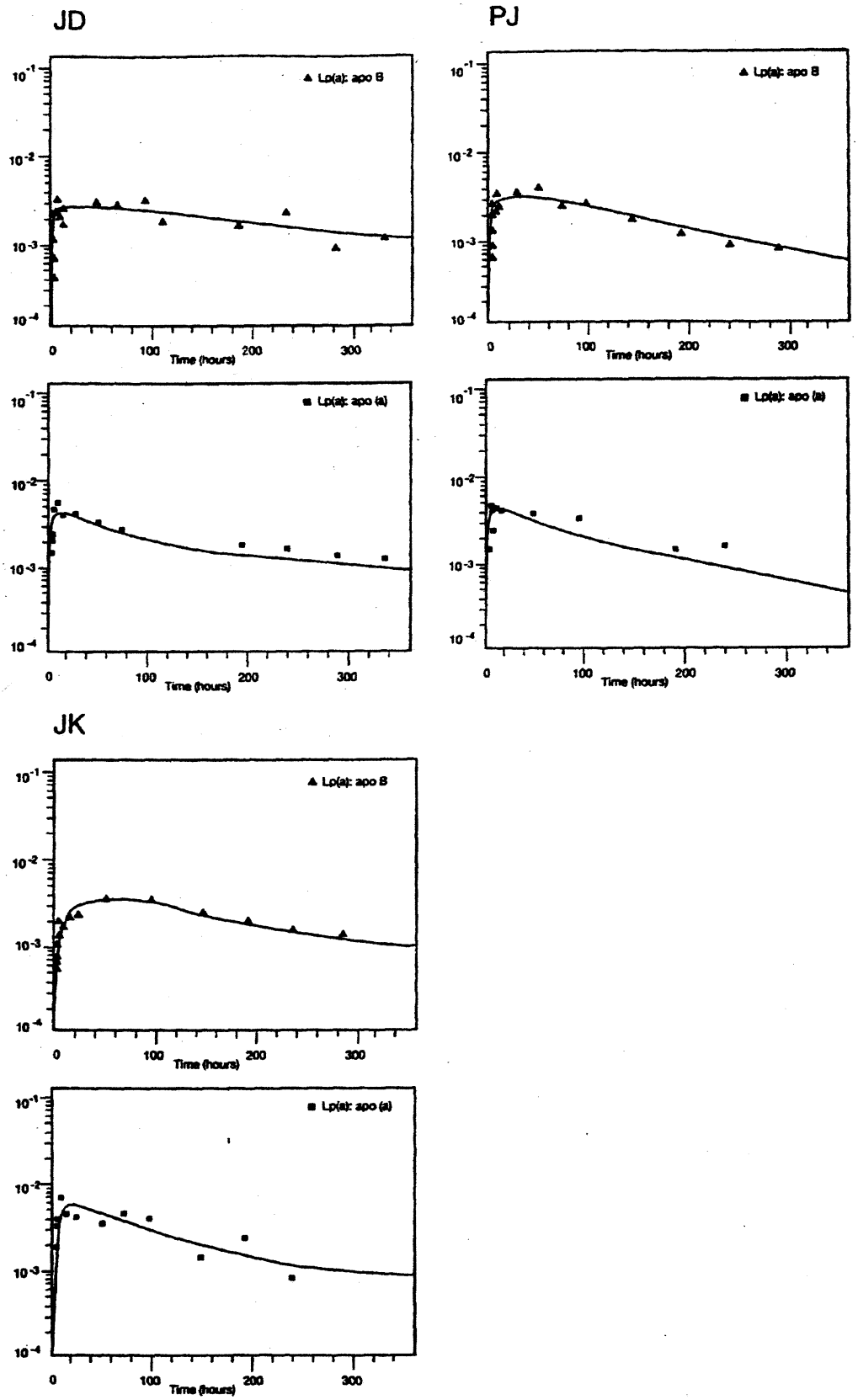

Fig. 3. (*Continued*)

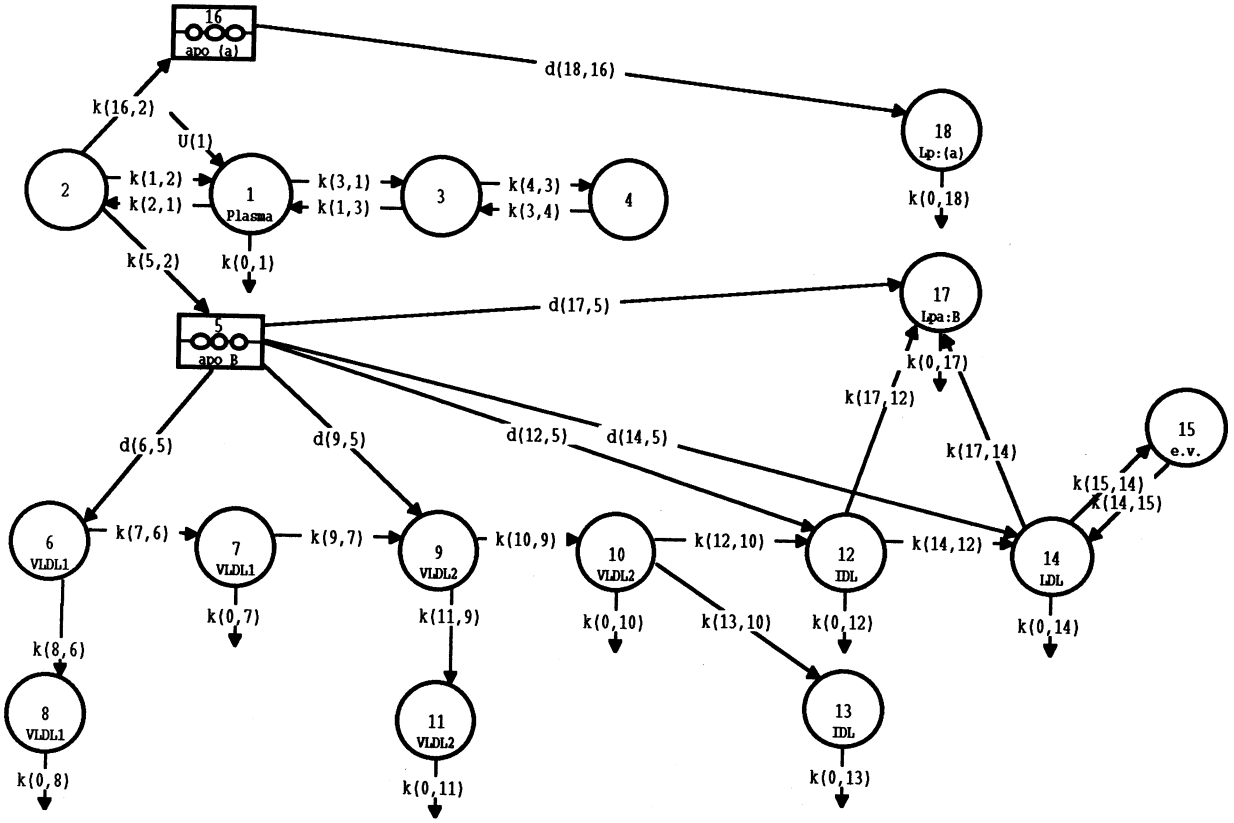

Fig. 4. The kinetic model of apo B and apo(a) metabolism as used in this study. q_i denotes compartments to which protein masses M_i are assigned (for M_i see Appendix A), $d_{i,j}$ denotes the distribution of transfer from the delay elements d_j to compartments q_i and $k_{i,j}$ kinetic rate constants, quantifying the fraction of M_j transferred from q_j to q_i per time. s_i signifies sets of compartments q which together represent measured lipoprotein or apolipoprotein fractions. For details see text.

were analysed using a computer program for simulation, analysis and modeling (SAAM II, SAAM Institute, Seattle, WA, USA). Kinetic analysis performed on the basis of a metabolic model resulted in the determination of transfer rate coefficients ($k_{i,j}$) and masses (M_i) for apo B or apo(a) compartments. Values for $k_{i,j}$ describe the transfer of material from compartment j to i as fraction of pool j per unit of time. M_i quantifies the mass content of compartment i. Individual transfer rate coefficients ($k_{i,j}$) were summarised to provide rates of protein secretion, transfer (where applicable) and catabolism. Kinetic parameters were usually determined with a fractional standard deviation (FSD) of less than 10%. For individual $k_{i,j}$ and M_i values and the appropriate FSDs see Appendix A.

The model used for quantitative kinetic analysis of tracer/tracee data from $VLDL_1$-, $VLDL_2$-, IDL- and LDL-apo B plus Lp(a) derived apo B and apo(a) is shown in Fig. 4 and has been described in detail elsewhere [23]. Its basic features are a four compartmental representation of free leucine kinetics (compartments Q1–Q4), a sequence of lipoprotein compartments accounting for the stepwise delipidation of $VLDL_1$ through $VLDL_2$ and IDL to LDL (compartments Q6–Q7, Q9–Q10, Q12, Q14) plus the three remnant compartments Q8, Q11 and Q13 for $VLDL_1$-, $VLDL_2$- and IDL-particles, which are removed directly from plasma. Free leucine and the apo B-containing compartments Q6, Q9, Q12 and Q14 in the $VLDL_1$-, $VLDL_2$-, IDL- and LDL-density range are linked via a delay compartment D5 which is set at 0.5 h accounting for the time required for apo B biosynthesis. Compartment Q15 allows for some intra-/extravascular exchange of LDL which is not observed for less dense lipoproteins. This metabolic model had to be extended in order to account, in addition, for the metabolism of apo B derived from Lp(a). Compartment Q17 represents the apo B moiety of Lp(a). Input into compartment Q17 originates from a precursor pool, either LDL ($k_{17,14}$) or IDL ($k_{17,12}$), or directly from denovo synthesis ($d_{17,5}$). Model simulation calculations were undertaken with either one of these input routes eliminated, which allowed to test different hypotheses about the metabolic origin of the apo B component of Lp(a). Requirements for a priori system identifiability in accordance with physiological considerations, led to the introduction of the following model constraints [24]: $k_{2,1} = k_{1,2}$; $k_{8,6} = k_{11,9}$; $k_{0,8} = k_{0,11}$; $k_{7,6} = k_{9,7} = k_{10,9}$; $k_{13,10} = k_{0,13}$; $k_{0,10} = ik_{0,12}$; $k_{14,15} = 2.5 \times k_{15,14}$. Since acceptable curve fits could be obtained without a provi-

Table 1
Lp(a)-study: lipoproteins and biometric data of study participants

	Sex	Age (years)	Weight (kg)	Height (cm)	Chol. (mg/dl)	Trig. (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	Lp(a) (mg/dl)	Phenotype (mg/dl)
EK	m	56	80	175	176	88	20	110	46	85	(19,19)
JL	m	25	67	178	130	70	14	69	48	45	(18,21)
TG	m	26	62	173	168	106	20	113	35	40	(18,36)
GH	f	26	57	168	220	40	9	132	79	39	(18,28)
JD	f	46	60	172	175	60	12	115	48	39	(19,28)
PJ	m	22	70	183	141	58	11	75	54	43	(20,27)
JK	m	24	65	180	188	210	41	108	39	51	(19,31)
Median		26	65	175	175	70	14	110	48	43	
Minimum		22	57	168	130	40	9	69	35	39	
Maximum		56	80	183	220	210	41	132	79	85	

sion for direct elimination of $VLDL_1$ from compartment Q7 the appropriate kinetic rate constant was eliminated from the model ($k_{0,7} = 0$). Input of native leucine was represented in the model by U_1. In order to simulate the specific tracer enrichment in the immediate precursor pool for apo B synthesis (compartment Q2) and to fit observed tracer/tracee curves for both plasma and lipoproteins, a dilutional factor P_1 had to be introduced. P_1 is proportional to an additional tracee input into compartment 2 and has been shown to be identifiable by multicompartmental model analysis [23].

The metabolism of apo(a) was modeled by a single compartment, compartment Q18, which was linked via a second delay compartment (compartment D16) to the same four-compartmental model for free leucine, was used for apo B kinetic analysis. Since biosynthesis of apo(a) takes at least 30 min, the delay time was set at 0.5 h [15]. Fractional catabolic rates (FCRs) for Lp(a)-derived apo B and apo(a), i.e. the kinetic parameters $k_{0,17}$ and $k_{0,18}$, were allowed to vary independently.

3. Results

Seven subjects, two females and five males, participated in this study. They were normolipidemic, with plasma cholesterol values ranging from 130 to 220 mg/dl and triglycerides from 40 to 210 mg/dl (Table 1). The Lp(a) concentrations in six subjects were quite similar, in the range of 39–51 mg/dl, but one individual (EK) displayed a higher value of 85 mg/dl. All subjects expressed the apo(a) phenotype 18, 19 or 20, whereas the second isoform was either similar (EK, JL) or of larger size (GH, JD, PJ, JK, TG). Radiographs from the phenotyping gels showed that these larger isoforms, in line with the known inverse relationship between molecular weight and plasma concentration, were expressed at a markedly lower level and, therefore, should not significantly influence the metabolic behaviour of the apo(a) fractions investigated.

The composition of lipoproteins analysed in this study is given in Table 2. In line with previous observations, $VLDL_1$ (S_f 60–400) was considerably more triglyceride-rich than small $VLDL_2$ (S_f 20–60), IDL (S_f 12–20) contained about twice as much cholesterol as triglyceride and total LDL (S_f 0–12) was composed of 40% cholesterol and 28% protein, more than 95% of which was apolipoprotein B. By modification of the gradient ultracentrifugation protocol, total LDL was divided into larger LDL_1 (S_f 6–12) and smaller LDL_2 (S_f 0–12), the latter being relatively protein rich at the expense of lipids. The protein content of Lp(a) was almost 40% due to the attached apo(a) molecule. However, Lp(−), the lipoprotein recovered after removal of apo(a), was compositionally very similar to small LDL_2.

Tracer/tracee ratios from a typical turnover study (JK) for apo B from $VLDL_1$, $VLDL_2$, IDL and LDL, and additionally for apo B and apo(a) isolated from Lp(a), are shown in Fig. 2. Curves for $VLDL_1$, $VLDL_2$, IDL and LDL are consistent with the precursor product relationship earlier reported [23]. Initially, the curves for apo B and apo(a) from Lp(a) rise more rapidly than the curve for LDL, indicating rapid appearance of tracer in the two Lp(a) protein constituents. The rise of the Lp(a) apo B curve, however, is diminished gradually towards a broad maximum reached 60–80 h after the start of the tracer infusion, whereas the apo(a) curve culminates at about 10 h. Thereafter, both curves fall off with a similar slope, being shallower than that observed for LDL-apo B. Tracer/tracee curves for apo B and apo(a) prepared from Lp(a) are compiled in Fig. 3 for all subjects investigated.

The metabolic model used to analyse tracer/tracee data from all apo B-containing lipoproteins, including Lp(a) with both its protein constituents, is shown in Fig. 4. It is an extended version of an earlier published metabolic model, which comprehensively describes the precursor-product relationship between VLDL, IDL and LDL in plasma [23]. Compartment Q17 represents apo B derived from Lp(a). It is connected to the basic

Table 2
Composition of $VLDL_1$, $VLDL_2$, IDL, total LDL, LDL_1 LDL_2, Lp(a) and Lp(−) in normolipidemic subjects ($n = 7$)[a]

	Free cholesterol (FC)	Cholesteryl ester (CE)	Triglyceride (TG)	Phospholipids (PL)	Proteins (PR)
$VLDL_1$	5.7 ± 1.2	6.2 ± 1.5	64.7 ± 2.6	16.2 ± 1.1	7.3 ± 1.0
$VLDL_2$	8.1 ± 0.7	13.3 ± 1.7	42.6 ± 3.7	21.7 ± 1.2	14.2 ± 1.9
IDL	10.3 ± 1.0	25.5 ± 2.0	16.8 ± 4.3	25.8 ± 1.2	21.3 ± 2.0
LDL	10.1 ± 1.2	29.3 ± 0.9	6.8 ± 1.5	26.0 ± 0.9	27.8 ± 1.0
LDL_1	10.4 ± 1.0	29.7 ± 0.8	6.7 ± 1.8	26.4 ± 0.7	26.6 ± 1.0
LDL_2	9.1 ± 1.0	28.8 ± 1.4	7.0 ± 1.2	24.7 ± 0.7	30.3 ± 1.6
Lp(a)	6.5 ± 1.2	23.5 ± 2.6	9.5 ± 4.2	21.3 ± 1.7	39.4 ± 4.5
Lp(−)	8.8 ± 1.6	26.3 ± 3.1	9.1 ± 3.0	25.0 ± 5.1	30.8 ± 4.6

[a] Figures are means ± S.D. given as g/100 g.

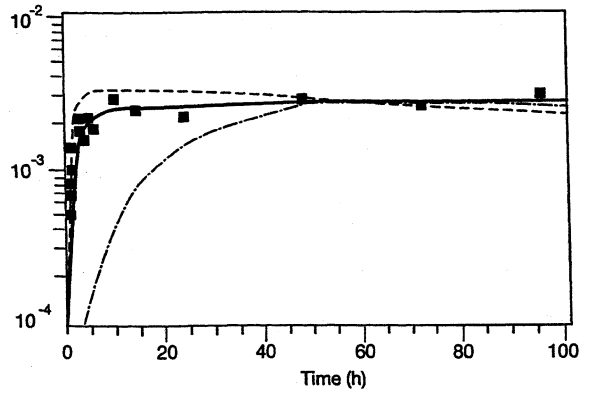

Fig. 5. Simulated and observed tracer/tracee ratios for apo B isolated from Lp(a). Neither exclusive de-novo synthesis, i.e. $k_{17,14} = k_{17,12} = 0$, (- - -), nor the elimination of this pathway, i.e. $d_{17,5} = 0$, (-.-.-.), from the model depicted in Fig. 3 allowed for a simulation of tracer/tracee curves compatible with observed data (■). These were only met by a curve generated by the mixed contributions from both sources of Lp(a):apo B (—).

model via links with IDL and LDL compartments (Q12 and Q14) and by a pathway providing for denovo synthesis of apo B from the precursor compartment Q2 via the common delay element D5. Simulation calculations, where either one of these metabolic routes was, eliminated, failed to generate the observed tracer/tracee curves as illustrated in Fig. 5. In the absence of $k_{17,12}$ and $k_{17,14}$, i.e. input of apo B from preformed IDL or LDL the simulated curve rose too quickly. However, without any denovo synthesis of apo B, the increase of tracer enrichment was far too slow. Only a hybrid approach, with contributions from both circulating lipoproteins and denovo synthesis, enabled the simulation of apo B tracer/tracee curves, which were in reasonable agreement with observed data. Apo(a) is also represented by a single compartment, Q18, which is connected to the hepatic precursor compartment Q2 through an independent delay element D16.

Global rates for protein synthesis, transfer and catabolism as derived from simulation calculations based on this model are given in Table 3. In line with the earlier findings the main apo B-containing lipoprotein secreted into the plasma compartment was $VLDL_1$, accounting for more than half of the total apo B production. Most of it was rapidly transferred to become $VLDL_2$, IDL and eventually LDL. On average, 85% of LDL apo B was derived from less dense lipoprotein precursors and only 1.7 mg/kg/dl were secreted directly into the LDL pool. LDL was cleared from plasma with a FCR of 0.64 (0.37–0.69) pools per day. As mentioned before, apo B in Lp(a) was found to originate from two independent sources: 0.14 (0.06–0.20) mg/kg per day were derived from plasma LDL or IDL and the same amount, 0.14 (0.09–0.24) mg/kg per day, was contributed by direct secretion of apo B into the plasma compartment. The percentage of apo B contributed by denovo synthesis was 53% (43–67). The rates of Lp(a):apo B secretion and of direct LDL secretion were found to be independent from each other. The rate of synthesis for apo(a) isolated from Lp(a) was determined as 0.16 (0.06–0.21) mg/kg per day, which is in proportion with the synthetic rate for the apo B moiety given the 1:1 molar ratio of both protein constituents and the molecular weights of 275 and 514 kDa. From the above rates of synthesis and influx for protein constituents and from the Lp(a) compositional data, an absolute rate of total Lp(a) production of approximately 1 mg/kg per day can be calculated. The FCRs for both apo B and apo(a) derived from Lp(a) were found to be very similar, 0.27 (0.16–0.38) and 0.24 (0.12–0.40) pools per day, respectively, suggesting that Lp(a) is cleared from plasma mainly as an integral particle with a FCR less than half of that observed for LDL.

4. Discussion

Using an endogenous stable isotope labelled amino acid tracer and a comprehensive multi-compartmental model for apolipoprotein B metabolism, we were able to investigate simultaneously the metabolism of two VLDL fractions, of IDL and LDL and, additionally, that of apo B and apo(a), the two protein components of Lp(a). Earlier, Lp(a) metabolism in humans was studied by use of exogenously radio-iodinated Lp(a), which was reinjected into donor subjects and monitored as a metabolic probe. Using this approach Krempler et al. [7] determined a fractional catabolic rate for Lp(a) in the range of 0.22–0.39 (median; 0.26) pools per day in good agreement with the values reported in this study. Lp(−), the LDL-like lipoprotein prepared from Lp(a) after reductive cleavage, was compositionally similar to smaller and denser LDL_2 (S_f 0–6) as opposed to larger, more buoyant LDL_1 (S_f 6–12). Both Lp(−) and LDL_2, due to their longer plasma residence time, showed a reduced lipid to protein ratio, compared with larger more readily catabolised LDL_1 (2.2 ± 0.4 and 2.3 ± 0.5 vs. 2.7 ± 0.3; $P < 0.05$).

Subsequent dual tracer studies, where both autologous radio-labelled Lp(a) and LDL were injected into patients with either heterozygous [35] or homozygous familial hypercholesterolemia [10], revealed that the LDL-receptor is not required for normal Lp(a) catabolism. By analysis of ^{125}I-Lp(a) decay curves it also became clear that Lp(a) plasma concentrations are largely independent of variations of the fractional catabolic rate but are determined primarily by the rate of Lp(a) production [11,12]. However, a more detailed picture of how Lp(a) enters the plasma compartment in vivo cannot be obtained by studying the elimination curves registered after the reinjection of preformed

exogenously labelled lipoprotein. This, in contrast, requires the separate analysis of the two distinct protein constituents of Lp(a) as carried out in the present study.

Both proteins, apo B and apo(a), are synthesised by hepatocytes but their association in the process of Lp(a) assembly seems to occur extracellularly. In vitro studies with primary cultures of baboon hepatocytes indicate that free apo(a) is secreted by these cells, bound to the cell surface by virtue of its kringle domains and then can associate with apo B-containing lipoproteins first by non-covalent protein-protein interactions and than by formation of a disulfide bond. Both VLDL and LDL could be added to the cultured hepatocytes in order to form Lp(a) [36]. In this in vivo study, we were able to show that the apo(a) moiety of Lp(a) appeared swiftly in plasma after a delay time of 0.5 h, accounting for protein biosynthesis in the liver. The apo B component, in contrast was not attributable to a single source. It was partly derived from preformed lipoproteins, either IDL or LDL, but an equally sized fraction seemed to be secreted directly by the liver. This fraction contributed an element of rapid tracer input into apo B recovered from Lp(a) required to account for the observed time course of specific tracer enrichment. The precise nature of these rapidly secreted apo B-contain-

Table 3
Summary of metabolic parameters

Subjects	Synthesis (mg/kg per day)	Flux (mg/kg per day)	Pool (mg)	Transfer (pools per day)	Catabolism (pools per day)
For $VLDL_1$, $VLDL_2$, IDL and LDL					
$VLDL_1$					
Median	9.4	–	29	19.2	2.2
Minimum	6.5	–	17	4.4	0.1
Maximum	15.5	–	159	32.5	4.3
$VLDL_2$					
Median	2.6	7.9	76	6.2	0.6
Minimum	0.0	6.5	47	5.0	0.0
Maximum	5.7	14.0	194	13.7	2.6
IDL					
Median	0.6	9.2	283	2.8	0.0
Minimum	0.0	6.5	99	1.9	0.0
Maximum	2.6	15.1	345	5.9	0.4
LDL					
Median	1.7	10.5	1508	–	0.64
Minimum	0.0	8.7	1044	–	0.37
Maximum	4.8	15.1	1669	–	0.69
For apo B and apo(a) derived from Lp(a)					
Lp(a): apo B					
EK	0.14	0.14	131		0.17
JL	0.12	0.06	60		0.19
TG	0.09	0.08	65		0.16
GH	0.14	0.14	63		0.27
JD	0.16	0.12	51		0.32
PJ	0.24	0.16	74		0.37
JK	0.15	0.20	73		0.31
Median	0.14	0.14	65		0.27
Minimum	0.09	0.06	51		0.16
Maximum	0.24	0.20	131		0.38
Lp(a): apo(a)					
EK	0.16		69		0.18
JL	0.10		32		0.21
TG	0.06		30		0.12
GH	0.14		33		0.24
JD	0.18		27		0.40
PJ	0.21		37		0.39
JK	0.17		35		0.32
Median	0.16		33		0.24
Minimum	0.06		27		0.12
Maximum	0.21		69		0.40

ing lipoprotein particles cannot be determined unequivocally by this approach. A model structure predicting input from one of the VLDL compartments (compartments Q7 or Q8, Q10 or Q11) did not provide simulated tracer/tracee kinetics in agreement with observed data (data not shown). This is in line with earlier results from VLDL turnover studies, where none of the radioactivity introduced with the VLDL tracer could be recovered from Lp(a) preparations [8]. Nevertheless, as in the case of directly secreted LDL, it can be argued that these lipoprotein particles are not secreted as such but rather generated from a rapidly turning over VLDL pool, which is not in equilibrium with the plasma compartment [37]. In summary, our findings in humans support the concept, initially developed from the above in vitro experiments, that apo(a) is secreted independently by the liver and then associates with apo B-containing lipoproteins of different origins to form native Lp(a). As mentioned before, plasma Lp(a) levels are mainly determined by the rate of apo(a) production [11,12]. In spite of the described links between LDL and Lp(a) production, reduction of LDL levels by lipid-lowering drug therapy, for instance by HMG-CoA-reductase inhibitors, does not result in a concomitant decrease of Lp(a) levels [38]. This indicates some flexibility in the choice of the apo B-carrying particles required to form Lp(a). In the present study, about equal amounts of apo B were contributed by preformed IDL/LDL and by direct secretion. Possibly, this ratio changes under LDL-lowering therapy towards a higher proportion of directly secreted apo B which before released into the circulation is bound to apo(a) present just outside hepatocytes. In any case, the amount of apo B required to establish even high levels of Lp(a) is small compared to the apo B pool size and, therefore, it seems plausible that the rate-limiting step in Lp(a) production is apo(a) secretion rather than apo B availability.

While the process of Lp(a) production has been elucidated to some extent, little is still known about the precise mechanisms involved in Lp(a) catabolism. As mentioned before, the LDL-receptor is probably of marginal, if any, significance [9,10,39]. However, the VLDL receptor, another member of the LDL receptor family, may play an important role in Lp(a) catabolism [19]. In end-stage renal disease (ESRD) the expression of the VLDL-receptor is markedly decreased and impaired clearance of Lp(a) may explain high Lp(a) levels often found in this condition [40]. Renovascular arteriovenous differences of Lp(a) concentrations of 1.5 mg/dl were reported from a carefully conducted angiographical trial in patients with normal renal function [21]. Given a usual renal plasma flow of 500 ml/min, an extraction rate of more than 140 mg/kg per day can be calculated. This is many times more than the absolute rates of Lp(a)synthesis of about 5 mg/kg per day reported from earlier turnover studies using ^{125}I-Lp(a) tracers, and of approximately 1 mg/kg per day as determined in the present study. Thus, metabolic studies, regardless of the tracer applied, do not provide evidence for a rapid Lp(a) turnover in plasma as stipulated by explaining the observed rate of renal Lp(a) extraction with renal Lp(a) degradation and catabolism. Alternative pathways such as renal extraction and bypass transport through lymphatic vessels are still awaiting experimental proof. The above difference between absolute rates of Lp(a) production determined either by radio-labelling or by stable isotopes is due to a previous lack of assay standardisation [41] and ill defined conversion rates for Lp(a) measurements based on whole particles or on individual constiuents.

Several groups have reported the detection of free apo(a) and apo(a) fragments in plasma and in urine [42–44]. Since the plasma concentrations of apo(a) fragments were correlated with Lp(a) plasma levels and with the amount of urinary apo(a)secretion, it is believed that these fragments originate from either denovo synthesised apo(a) or from Lp(a). The mechanism of Lp(a) assembly, explained in detail before, makes it conceivable that some apo(a) secreted by the liver does not associate with apo B but is degraded in plasma and secreted into urine by as yet unidentified mechanisms [42]. Likewise, some Lp(a) may be cleaved, possibly by polymorphonuclear cell elastase [45], thus providing free apo(a) and apo(a) fragments for renal elimination. However, the amount per day of apo(a) fragments eliminated via urine is small, accounting for less than 1% of the total FCR [42,44]. Our finding of similar rates of catabolism for the apo B and the apo(a) components of Lp(a) suggests that the bulk of Lp(a) is degraded as an integral particle without noticeable exchange of apo(a) between apo B containing lipoproteins. Some intravascular cleavage of Lp(a) into free apo(a) and a LDL-like lipoprotein cannot be ruled out since free apo(a) was not monitored in this study. In two earlier published ^{125}I-Lp(a) turnover studies, 10–25% of the initial radioactivity was recovered from the LDL fraction.

In conclusion, we have demonstrated in a turnover study in humans, using endogenous stable isotope labeling and multicompartmental kinetic analysis, that the apo B moiety of Lp(a) originates both from denovo synthesis and from preformed IDL and LDL, thus providing further evidence for in vitro observations, suggesting that Lp(a) assembly is an extracellular process. The fractional rates of catabolism for both apo B and apo(a) from Lp(a) were identical, and considerably lower than that observed for LDL, in line with the notion that the in vivo mechanisms of Lp(a) clearance are different from the LDL receptor and eliminate the bulk of Lp(a) as an integral particle from the circulation.

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Appendix A

Lp(a) study: kinetics of free leucine and leucine prepared from apolipoprotein B. Calculated values are given for $k_{i,j}$ and M_i, figures in italics denote coefficients of variance for $k_{i,j}$ and measured pools for M_i.

	EK		JL		TG		GH		JD		JK		PJ	
$k_{0,1}$[a]	0.566	*1.3*	2.022	*0.9*	1.283	*1.8*	1.632	*3.0*	0450	*2.1*	0.000	–	1.756	*0.6*
$k_{3,1}$	1.080	*1.2*	2.178	*1.1*	1.546	*1.3*	8.157	*2.4*	2.169	*2.0*	2.334	*0.3*	2.695	*0.8*
$k_{1,3}$	0.083	*1.4*	0.117	*0.8*	0.088	*2.7*	0.085	*1.2*	0.112	*1.3*	0.021	*0.3*	0.142	*0.6*
$k_{4,3}$	0.235	*1.5*	0.121	*0.7*	0.180	*2.2*	0.098	*0.5*	0.224	*1.3*	0.058	*0.4*	0.529	*1.8*
$k_{2,1}$	2.500	–	2.677	*1.6*	2.500	–	2.500	–	6.848	*11.5*	3.873	*0.9*	5.363	*1.2*
$k_{5,2}$	0.008	*0.2*	0.014	*0.2*	0.008	*1.2*	0.035	*2.4*	0.010	*1.7*	0.014	*0.3*	0.014	*0.5*
P_1[b]	0.877	*0.4*	1.000	–	1.000	–	1.000	–	1.000	–	0.558	*0.1*	0.780	*0.3*
$d_{9,5}$[c]	0.000	–	0.000	–	0.205	*2.7*	0.163	*2.6*	0.141	*4.3*	0.330	*0.3*	0.210	*1.0*
$d_{12,5}$	0.035	*4.1*	0.222	*0.5*	0.055	*4.1*	0.143	*1.2*	0.142	*1.5*	0.000	–	0.000	–
$d_{14,5}$	0.096	*1.1*	0.126	*0.9*	0.030	*1.3*	0.119	*1.8*	0.262	*0.7*	0.000	–	0.117	*0.5*
$d_{17,5}$	0.008	*1.1*	0.012	*2.0*	0.009	*1.2*	0.008	*0.8*	0.009	*4.1*	0.008	*1.2*	0.017	*1.1*
$k_{8,6}$	0.230	*1.7*	0.012	*1.5*	0.008	*8.4*	0.500	–	0.414	*4.4*	0.033	*2.1*	0.441	*1.7*
$k_{0,8}$	0.500	–	0.005	*1.3*	0.014	*7.6*	0.654	*2.0*	0.500	–	0.522	*9.6*	0.500	–
$k_{9,7}$	2.059	*0.3*	3.884	*0.5*	0.863	*0.8*	3.739	*1.0*	2.145	*2.3*	0.377	*0.2*	2.303	*0.6*
$k_{0,10}$	0.000	–	0.000	–	0.000	–	0.000	–	0.000	–	0.000	–	0.000	–
$k_{12,10}$	0.229	*0.4*	0.421	*0.2*	0.311	*1.1*	0.537	*0.4*	0.332	*0.9*	0.520	*0.4*	1.065	*0.5*
$k_{13,10}$	0.033	*1.5*	0.003	*1.9*	0.014	*8.3*	0.000	–	0.000	–	0.000	–	0.000	–
$k_{14,12}$	0.206	*0.8*	0.429	*0.3*	0.105	*1.1*	0.141	*0.3*	0.101	*0.6*	0.214	*0.3*	0.269	*0.3*
$k_{0,14}$	0.027	*0.4*	0.027	*0.2*	0.015	*0.8*	0.029	*0.3*	0.023	*0.5*	0.026	*0.2*	0.027	*0.2*
$k_{15,14}$	0.058	*5.6*	0.008	*2.7*	0.031	*14.8*	0.000	–	0.003	*7.6*	0.005	*2.0*	0.000	–
$k_{17,12}$	0.002	*2.0*	0.000	–	0.001	*2.5*	0.000	–	0.000	–	0.000	–	0.000	–
$k_{17,14}$	0.000	–	1.5e-4	*2.7*	0.000	–	2.6e-4	*1.8*	1.8e-4	*4.2*	3.5e-4	*1.4*	4.0e-4	*3.0*
$k_{0,17}$	0.007	*0.6*	0.008	*0.5*	0.006	*0.8*	0.011	*0.8*	0.013	–	0.013	*0.4*	0.016	*1.0*
$k_{16,2}$	1.9e-5	*1.1*	4.0e-5	*2.4*	1.3e-5	*1.3*	6.8e-5	*2.4*	2.8e-5	*2.2*	3.9e-5	*2.4*	5.8e-5	*2.1*
$k_{0,18}$	0.007	*0.3*	0.009	*0.2*	0.005	–	0.009	*0.2*	0.017	*0.6*	0.013	*1.0*	0.016	*0.2*
Q1[d]	945		247		395		159		562		422		370	
Q2	942		246		393		157		562		420		369	
Q3	12292		4612		7484		15309		10924		46482		7004	
Q4	122915		46119		74843		153088		109242		464826		70038	
U1[e]	542		503		485		265		259		5.79		655	
Q6	2.7		0.6		2.5		0.7		1.0		9.3		1.2	
Q7	2.7		0.6		2.5		0.7		1.0		9.3		1.2	
Q8	1.3		1.3		1.3		0.6		0.8		0.6		1.1	
$M(VLDL_1)$[f]	6.7	*7.5*	2.4	*3.2*	6.2	*6.5*	2.1	*2.3*	2.7	*3.0*	19.2	*19.4*	3.5	*3.9*
Q9	2.5		0.6		3.1		0.9		1.1		13.2		1.4	
Q10	19.3		5.2		8.3		6.0		7.2		9.5		3.0	
Q11	1.1		1.3		1.7		0.7		0.9		0.8		1.2	
$M(VLDL_2)$	22.9	*17.8*	7.0	*6.2*	13.1	*13.9*	7.6	*7.8*	9.2	*8.4*	23.5	*22.6*	5.8	*5.2*
Q12	22.5		6.7		25.9		28.7		31.2		23.2		12.0	

Q13	19.3		5.2		8.3		6.0		7.2		9.5		3.0	
M(IDL)	41.8	*44.5*	12.0	*14.3*	34.3	*32.0*	34.8	*34.4*	38.4	*36.6*	32.8	*34.0*	15.0	*15.5*
M(LDL) = Q14	194.3	*183.4*	126.5	*120.9*	182.8	*181.0*	162.2	*157.4*	202.3	*205.9*	185.0	*186.4*	138.9	*156.5*
Q15	77.7		50.6		73.1		64.9		80.9		74.0		55.6	
M(Lp-B) = Q17	15.9	*15.9*	7.3	*7.3*	7.9	*7.9*	7.6	*7.6*	6.2	*6.2*	8.9	*8.8*	9.0	*9.0*
M(apo(a)) = Q18	2.4	*2.4*	1.1	*1.1*	1.1	*1.2*	1.2	*1.2*	1.0	*1.0*	1.4	*1.4*	1.4	*1.4*

[a] $k_{i,j}$ describe the transfer of leucine from compartment j to i as fraction of pool j per h. Fractional standard deviations (F.S.D.) are given in parenthesis. $k_{i,j}$ values are transformed into kinetic transfer rates of apolipoprotein from compartment Q(j) to Q(i) per day by multiplication with a constant factor (that is times 24 h divided through 0.1212, the fractional leucine content of apo B or 0.035 for apo(a), respectively.). For model constraints see Section 2.

[b] P_1 is a dilutional factor calculated to determine the apo B precursor pool enrichment in Q2. For details see Section 2 and [23].

[c] $d_{i,5}$ describe the distribution of material leaving the delay compartment (compartment 5, Fig. 2) and entering the apo B compartments 6, 9, 12 and 14. $d_{6,5} + d_{9,5} + d_{12,5} + d_{14,5} = 1.00$.

[d] Q(i) is given as the leucine mass (mg) in compartment i. M_i can be transformed into corresponding apo B masses by division through 0.1212 (i.e. the fractional leucine content of apo B), M_{18} into the apo(a) mass by division through 0.035 (i.e. the fractional leucine content of apo(a)).

[e] U1 is the calculated tracee (i.e. native leucine) input into Q1.

[f] For *M*(VLDL-1), *M*(VLDL-2), *M*(IDL), *M*(LDL), *M*(Lp-B) and *M*(apo(a)) calculated and directly measured masses are given, the latter in italics.

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