Apolipoprotein B metabolism and the distribution of VLDL and LDL subfractions

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Abstract **Apolipoprotein B (apoB) metabolism was investigated in 20 men with plasma triglyceride 0.66–2.40 mmol/l and plasma cholesterol 3.95–6.95 mmol/l. Kinetics of $VLDL_1$ (S_f 60–400), $VLDL_2$ (S_f 20–60), IDL (S_f 12–20), and LDL (S_f 0–12) apoB were analyzed using a trideuterated leucine tracer and a multicompartmental model which allowed input into each fraction. $VLDL_1$ apoB production varied widely (from 5.4 to 26.6 mg/kg/d) as did $VLDL_2$ apoB production (from 0.18 to 8.4 mg/kg/d) but the two were not correlated. IDL plus LDL apoB direct production accounted for up to half of total apoB production and was inversely related to plasma triglyceride ($r = -0.54$, $P = 0.009$). Percent of direct apoB production into the IDL/LDL density range ($r = 0.50$, $P < 0.02$) was positively related to the LDL apoB fractional catabolic rate (FCR). Plasma triglyceride in these subjects was determined principally by $VLDL_1$ and $VLDL_2$ apoB fractional transfer rates (FTR), i.e., lipolysis. IDL apoB concentration was regulated mainly by the IDL to LDL FTR ($r = -0.71$, $P < 0.0001$). LDL apoB concentration correlated with $VLDL_2$ apoB production ($r = 0.48$, $P = 0.018$) and the LDL FCR ($r = -0.77$, $P < 0.001$) but not with $VLDL_1$, IDL, or LDL apoB production. Subjects with predominantly small, dense LDL (pattern B) had lower $VLDL_1$ and $VLDL_2$ apoB FTRs, higher $VLDL_2$ apoB production, and a lower LDL apoB FCR than those with large LDL (pattern A). Thus, the metabolic conditions that favored appearance of small, dense LDL were diminished lipolysis of VLDL, resulting in a raised plasma triglyceride above the putative threshold of 1.5 mmol/l, and a prolonged residence time for LDL. This latter condition presumably permitted sufficient time for the processes of lipid exchange and lipolysis to generate small LDL particles.**—Packard, C. J., T. Demant, J. P. Stewart, D. Bedford, M. J. Caslake, G. Schwertfeger, A. Bedynek, J. Shepherd, and D. Seidel. **Apolipoprotein B metabolism and the distribution of VLDL and LDL subfractions.** ***J. Lipid Res.*** **2000.** 41: **305–317.**

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ApoB-100 is the major protein component of very low, intermediate, and low density lipoproteins (VLDL, IDL, LDL). These particles are linked in a delipidation cascade in which triglyceride-rich VLDL, released from the liver, is converted to cholesterol-rich LDL which is in turn catabolized by specific cell-membrane receptors throughout the body. Abnormalities in the metabolism of apolipoprotein B (apoB)-containing lipoproteins are responsible for the generation of hyperlipidemia and the associated increased risk of developing coronary heart disease. Risk has been known for a long time to be strongly related to elevated LDL levels but recently a syndrome, the atherogenic lipoprotein phenotype (ALP), has been identified as a common dyslipidemia in patients with CHD (1–3). It is characterized by a normal cholesterol concentration with a moderate increase in plasma triglyceride, a decrease in high density lipoprotein (HDL) cholesterol, and a shift in the size profile of LDL towards small dense species. All three features may contribute to risk; moderately raised plasma triglyceride (i.e., VLDL) promotes thrombosis and can affect endothelial function (4), low HDL compromises reverse cholesterol transport, while small dense LDL has properties that enhance its atherogenicity (5, 6). Recently, we described a quantitative model for the development of ALP (7). Based on epidemiological data from others and ourselves (1, 7–9) it was proposed that the appearance of small dense LDL (a pattern B phenotype on gel electrophoresis (1, 6) or a high LDL-III concentration on density gradient centrifugation (3)) was associated with a plasma triglyceride in excess of 1.5 mmol/l and a hepatic lipase level in the range seen in humans. The aim of the present report was to examine, using stable isotope methods, how apoB metabolism differs in men with a preponderance of small dense LDL in order to gain further insight into the factors that give rise to this atherogenic state. Earlier studies by Teng et al. (10) into the metabolic abnormalities seen in subjects with dense LDL in hyper-

Abbreviations: apo, apolipoprotein; FCR, fractional catabolic rate; FTR, fractional transfer rate; d_3-leucine, tri-deuterated leucine; VLDL, very low density lipoprotein; IDL, intermediate density lipoprotein; LDL, low density lipoprotein; RT, residence time in plasma.

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apobetalipoproteinemia revealed that subjects with this disorder had a reduced clearance rate and higher production of VLDL. Comparison of the kinetics of "heavy" versus "light" LDL in controls and hyperapobetalipoproteinemic subjects demonstrated also that heavy LDL had a slower clearance rate than its lighter counterpart.

In general, VLDL apoB production rates measured by the incorporation of amino acids into apoprotein (11–14) have agreed well with those obtained using radioiodinated tracers (12, 13, 15). Furthermore, results appear to be independent of the amino acid given, the protocol of administration, and the dose of tracer. There are drawbacks to the method however: in particular, tracer recycling and the low level of incorporation of tracer into slowly turning over lipoproteins such as LDL. We have developed sensitive detection methods that allow quantification of $VLDL_1$ (S_f 60–400), $VLDL_2$ (S_f 20–60), IDL, and LDL-apoB production and catabolic rates (15, 16). The metabolic behavior of these fractions is again very similar to that observed with radioiodinated tracers. The objectives of the study were *i*) to evaluate how $VLDL_1$ and $VLDL_2$ production and clearance rates varied across a range of lipid levels and so to understand the metabolic factors that regulate plasma triglyceride concentration, *ii*) to examine the consequences for IDL and LDL apoB metabolism of variation in precursor VLDL kinetics, and *iii*) to understand further the metabolic environment in which formation of small, dense LDL is favored.

METHODS

Subjects were healthy men, laboratory staff and medical students in Glasgow and Munich. They were screened for disorders of thyroid, liver and kidney function and hematological abnormalities. Their characteristics and plasma lipid levels are given in **Table 1**. Lipid and lipoprotein levels were measured according to the Lipid Research Clinic's protocol using an enzymatic assay for cholesterol and triglyceride (17). Apolipoprotein E phenotyping was performed on plasma essentially as described by Havekes et al. (18). LDL subfraction profile was determined by electrophoretic separation on 2–16% polyacrylamide gradient gels of d 1.019–1.063 g/ml LDL isolated from plasma stored at −70°C (19). Previous studies have documented the preservation of the LDL electrophoretic pattern in frozen plasma (20). Subjects were classified as phenotypes A, I, or B following the divisions used by Krauss and Dreon (21). Phenotype A was assigned when the LDL peak diameter on calibrated gradient gels was in excess

TABLE 1. Plasma lipid and lipoprotein concentrations in subjects of differing LDL size phenotype

Subject	Age	Body Weight	Plasma Cholesterol	Plasma Triglyceride	Cholesterol in VLDL	Cholesterol in LDL	Cholesterol in HDL	ApoE Phenotype	LDL Phenotype
	yr	*kg*	*mmol/l*	*mmol/l*		*mmol/l*			
1	21	65	4.01	0.66	0.28	2.33	1.42	3/3	A
2	27	68	3.95	0.75	0.31	2.22	1.42	3/3	A
3	24	84	4.52	0.81	0.41	2.45	1.65	3/3	A
4	30	81	5.55	0.90	0.70	3.05	1.80	3/2	A
5	23	73	4.32	0.92	0.47	2.92	0.96	4/3	A
6	30	83	4.50	1.00	0.50	2.70	1.35	3/3	A
7	26	67	4.94	1.07	0.44	3.28	1.21	4/4	A
8	33	60	6.00	1.10	0.40	4.15	1.45	3/3	A
9	42	83	5.65	1.16	0.49	3.67	1.50	3/3	A
Mean	28	74	4.83	0.93	0.44	2.97	1.42		
SD	6	9	0.75	0.17	0.12	0.64	0.24		
10	40	75	5.55	0.75	0.35	4.00	1.20	3/4	I
11	29	61	5.36	1.07	0.54	3.59	1.24	3/3	I
12	53	78	6.36	1.39	0.57	4.50	1.29	3/3	I
13	33	104	6.95	1.60	0.75	5.30	0.90	3/3	I
14	54	58	6.28	1.95	0.80	4.16	1.32	nd	I
Mean	42[a]	75	6.10[a]	1.35[a]	0.60	4.31[a]	1.19		
SD	11	18	0.65	0.46	0.18	0.64	0.17		
15	28	75	4.00	1.38	0.59	2.00	1.40	3/3	B
16	57	90	6.95	1.55	0.85	5.00	1.10	3/2	B
17	31	85	5.50	1.70	0.90	3.65	0.85	3/3	B
18	47	111	6.20	2.10	1.25	4.10	0.85	3/3	B
19	34	93	6.30	2.10	0.90	4.45	0.95	3/3	B
20	59	89	6.25	2.40	0.95	3.55	0.95	3/4	B
Mean	43[b]	88	5.87[b]	1.87[b,c]	0.91[b,c]	3.79	1.02[b]		
SD	14	13	1.02	0.39	0.21	1.03	0.21		
ANOVA	0.026	NS	0.02	<0.001	<0.001	0.018	0.009		
A vs. B[d]	0.053		0.065	<0.001	0.002	0.13	0.006		
A vs. I	0.060		0.009	0.12	0.13	0.006	0.063		
I vs. B	0.91		0.66	0.10	0.032	0.34	0.17		

Subjects 4, 6, 8, 9, 11, 14, and 15 appeared as controls in reference 48. Subjects 6, 7, 12, 14, 19, and 20 were used in reference 25; nd, not determined.

[a,b,c] Groups significantly different by one-way ANOVA; Fishers pairwise comparison significant for: [a] A vs. I; [b] A vs. B; [c] I vs. B.

[d] *P* values for these comparisons were generated by *t*-test.

of 26.0 nm. Phenotype B was assigned when the LDL peak diameter was less than 25.0 nm. Individuals who did not fall into A or B were typed as I (intermediate) in accordance with the revised nomenclature of Krauss and Dreon (21).

Turnover protocol

Two protocols for administration of tracer were used: bolus and primed constant infusion. Subjects fasted from 8.00 pm the previous night and were given l-[5,5,5,$^{-2}H_3$]leucine (d_3-leucine, Cambridge Isotope Laboratory, Woburn, MA) starting at 8.00 am either in the form of a large bolus (6.0 mg d_3-leucine/kg body weight; subjects 1, 2, 3, 5, 7, 11, 12, 14) or a small bolus (0.6 mg d_3-leucine/kg body weight) followed by a continuous infusion (0.6 mg d_3-leucine/kg; subjects 4, 6, 8, 9, 10, 13, 15–20) for 10 h. During the initial 10-h period they continued to fast but were allowed non-caloric drinks and remained ambulatory. At 6:00 pm they were given a light snack and allowed to go home. Blood samples (10 ml), collected in EDTA, were taken before administration of tracer (0 h) and then frequently (at 0.16, 0.33, 0.5, 0.75, 1, 2, 4, 6, 8, 10, 12, 15, 24 h) over the next 24 h.

Further fasting samples were obtained at approximately 8.00 am, daily for the next 14 days. A complete description of the procedures for conducting the turnover is given in an earlier publication (15).

Lipoprotein isolation and preparation of apoB

The preparation of VLDL subfractions $VLDL_1$ (S_f 60–400) and $VLDL_2$ (S_f 20–60), IDL (S_f 12–20,) and LDL (S_f 0–12) has been described in a previous publication (22) and is based on the procedure of Lindgren, Jensen, and Hatch (23). In this method, which depends on flotation rate, there is little contamination of LDL with lipoprotein[a] (M. J. Caslake and C. J. Packard, unpublished observations), however, any Lp[a] protein present would co-precipitate with apoB in LDL and potentially contaminate the fraction. To each fraction was added an equal volume of isopropanol at room temperature (24). This led to the precipitation of apoB and lipids while other apoproteins remained in solution. The soluble proteins were removed and the pellicle was delipidated thoroughly with repeated ethanol/ether washes at 4°C until apoB remained as a fine white protein pellet. It was important that delipidation was exhaustive prior to subsequent analysis.

Preparation and analysis of leucine in apoB and plasma

ApoB was hydrolyzed in glass tubes (Labco, High Wycombe, UK) in the presence of 0.5–1.0 ml 6 N HC1 at 110°C for 24 h and the resultant amino acid mixture was concentrated in a vacuum concentrator centrifuge (Gyrovap, VA Howe, Banbury, UK) and aliquotted into microvials (Chromacol, Herts, UK). After complete removal of HCl, samples were ready for derivatization and mass spectrometric analysis.

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; Bio-Rad, Richmond, CA).

The amino acids that bound to the resin were desorbed by 4 m NH_4OH which was subsequently removed by evaporation in a vacuum concentrator (Gyrovap, VA Howe, Banbury, UK), transferred into microvials, and dried again ready for derivatization.

The method used for the analysis of d_3-leucine enrichment in protein hydrolysates and plasma amino acids is presented in detail elsewhere (15, 16). Ion mass fragments at *m/z* 277, *m/z* 276, and *m/z* 274 were monitored by selective ion recording (SIR mode). The advantages in terms of enhanced sensitivity and precision of monitoring higher mass fragments have been described (15, 16). The *m/z* 277:*m/z* 276 ratio showed a linear relationship with isotopic enrichment over the range 0.0–10.0% atom percent excess (APE). The ratios of *m/z* 277:*m/z* 276 were multiplied by an average value for the constant ratio of *m/z* 276:*m/z* 274 (determined repeatedly throughout the analytical run, it shows no change over the range 0.0–10% APE) and the resulting *m/z* 277:*m/z* 274 values were used to calculate specific isotopic enrichments and tracer/tracee ratios (15, 25).

Measurement of apoB masses in lipoprotein fractions

Compositions of $VLDL_1$, $VLDL_2$, IDL, and LDL were determined by measurements of total and free cholesterol, triglycerides, phospholipids, and total protein (22). The apoB content was calculated as the difference between total and isopropanol-soluble protein (24). ApoB plasma pools were determined for each lipoprotein fraction from apoB concentrations (in mg/ml of plasma) and an estimate of the plasma volume (taken as 4% of the body weight). These were corrected for centrifugal losses (26) and then the leucine content of the apoB pool was calculated from the apoB amino acid composition (27).

Kinetic analysis and multicompartmental modelling

The change in tracer/tracee ratios with time in plasma and in apoB in the four lipoprotein fractions, together with the measured apoB pool size for $VLDL_1$, $VLDL_2$, IDL, and LDL, were used as the data set for the derivation of kinetic parameters. A multicompartmental model was constructed using the SAAM II modelling program (SAAM Institute, Seattle, WA), the development of which has been described (15). Briefly, the model (**Fig. 1**) has the following features: plasma leucine is represented by a compartment (compartment 1) in rapid equilibrium with an intracellular compartment (compartment 2) which is the immediate source of leucine for apoB synthesis. Compartments 3 and 4 are required to account for the uptake and subsequent slow release of leucine by slowly turning over body protein pools. Continuous measurement of plasma leucine tracer/tracee ratios

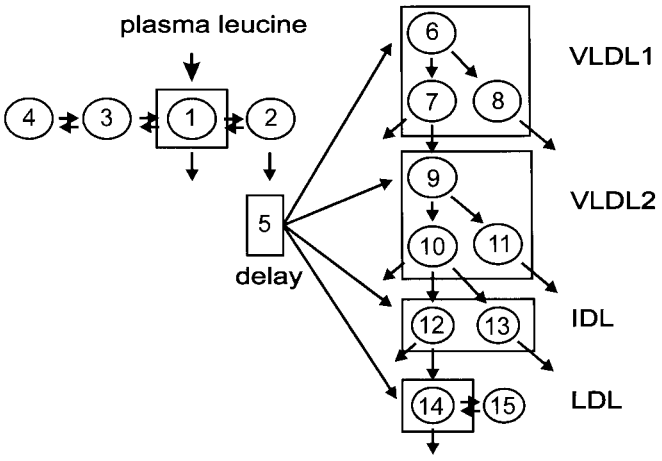

Fig. 1. Multicompartmental model for apoB metabolism. Both bolus and primed constant infusion data were analyzed with the same model. Plasma leucine was represented by a compartment (comp. 1) which received the tracer and distributed it to body protein pools (comps. 3 and 4) and an intracellular compartment (comp. 2) which was the precursor to apoB synthesis. After a delay (comp. 5), tracer appeared throughout the delipidation chain, i.e., in $VLDL_1$ ($k_{6,5}$), $VLDL_2$ ($k_{9,5}$), in IDL ($k_{12,5}$), and in LDL ($k_{14,5}$). Parameter dependencies were $k_{2,1} = k_{1,2}$; $k_{8,6} = k_{11,9}$; $k_{0,11} = k_{0,8}$; $k_{0,13} = k_{13,10}$; $k_{3,4} = 0.1 \times k_{4,3}$; $k_{14,15} = 2.5 \times k_{15,14}$. A fuller description of the model is given in reference 15.

throughout the 14-day turnover permitted modelling of this recycling process and hence its impact on IDL and LDL apoB metabolism could be taken into account when deriving kinetic parameters. Compartment 5 is a delay component which was usually set at 0.5 h but could be adjusted if required. Direct input of apoB occurs into $VLDL_1$ (compartment 6), $VLDL_2$ (compartment 9), IDL (compartment 12), and LDL (compartment 14). Compartments 6, 7, 9, 10, 12, and 14 form a delipidation chain, a feature found in most models of apoB metabolism (28–30) while compartments 8, 11, and 13 represent "remnant populations" of particles which are cleared relatively slowly from each density interval (28–30). The need for remnant pools is demonstrated in **Fig. 2**. In panel A the lack of a remnant pool as represented by compartment 8 led to an inability to fit the tail of the $VLDL_1$ apoB curve (i.e., from 10 to 24 h post tracer administration) even though the rise, peak, and initial decay portion of the curve fitted well. A similar situation was seen for $VLDL_2$ (not shown). The requirement for a delipidation chain within $VLDL_2$ is seen in panel B. Omission of compartment 10 and use of a single compartment resulted in an unsatisfactory fit. The rise portion of the $VLDL_2$ apoB curve fitted well but the peak was not "broad" enough to fit the observed data until compartment 10 was included.

It was this need to generate a broad peak in VLDL that led to the formulation of the delipidation chain in earlier studies (28–30). In nearly all the normal subjects reported here, a delipidation chain was optional for $VLDL_1$, but when the current model is applied to patients with elevated plasma triglyceride levels, the same phenomenon is seen for $VLDL_1$ as for $VLDL_2$ in panel B (data not shown). For consistency and to allow comparison of results, we used a short delipidation chain in $VLDL_1$ and $VLDL_2$ in all types of subjects. IDL apoB, which is largely restricted to the plasma space, is modelled as two intravascular compartments, one of which is delipidated to LDL (compartment 12). In panel C, it can be seen that if a single IDL compartment is included in the model, the rise, peak, and initial down slope can be accounted for satisfactorily but again the model cannot fit the terminal exponential (seen after 50 h) until a further compartment (compartment 13) with a slower decay rate is added. LDL apoB, on the other hand, is represented by a single plasma compartment and the bi-exponential nature of the d_3-leucine decay curve is accounted for by intra-extravascular exchange. The effect of omitting the extravascular compartment 15 can be seen in Fig. 2 panel D. With only compartment 14 present, LDL decay is monoexponential whereas there are clearly two exponential decays present in the observed data. A satisfactory fit is obtained with the inclusion of an extravascular LDL compartment. In other studies using radioiodinated lipoprotein tracers, it has been possible to resolve the LDL apoB decay further with the inclusion of two plasma compartments, one with a more rapid catabolic rate than the other. This required either injection of dual tracers (30) or collection of excreted radioactivity (7). Administration of an amino acid labels all body proteins and it is not possible to distinguish the metabolic fate of individual LDL sub-species within a density interval.

In many subjects, apoB input was mainly into the $VLDL_1$ density interval. However, some had an early rapid rise in tracer/tracee ratio in $VLDL_2$, IDL, or LDL apoB. In the model presented in Fig. 1, this latter phenomenon is accounted for by permitting direct input from the delay compartment, 5, into compartments 9, 12, and 14. This gives a satisfactory fit in all subjects.

An alternative model, examined in our earlier evaluation of the methodology, had a rapidly turning over pool V_T (see panel F, Fig. 2) in $VLDL_1$ which had outputs into $VLDL_2$, IDL, and LDL. This arrangement is favored by some investigators (29) who prefer the concept of rapid lipolysis to that of secretion of smaller lipoproteins from the liver. In the 6 subjects studied in reference 15, the majority (about 70%) of apoB appeared in $VLDL_1$ and there was limited need for input of apoB into $VLDL_2$, IDL, or LDL. However, the present series includes subjects in whom $VLDL_2$, IDL, and LDL account for over half of total apoB input. To illustrate this, data are presented for subject 16 in panel E (Fig. 2). Using the model in Fig. 1, a satisfactory fit was obtained with approximately equal input of apoB into $VLDL_1$ and $VLDL_2$ (see Appendix 1). In the alternate model, no input to $VLDL_2$ was allowed (i.e., no transfer 5→9) but a rapidly turning over compartment was included in $VLDL_1$ (V_T, panel F in Fig. 2). If a lipolysis rate for V_T of up to 10 times the lipolysis rate of the rest of $VLDL_1$ was permitted, a poor fit was obtained; the $VLDL_1$ apoB calculated curve was displaced above the observed data while the $VLDL_2$ apoB calculated points fell below the observed data during the first 2–3 hours. Allowing V_T to turn over at a rate in excess of 100 pools/d did permit the data from subject 16 and others to fit such a model. However, it is difficult to conceive how one $VLDL_1$ particle can be delipidated at 1–2 pools/d and another at 100 pools/d in the same person. Conceptually we prefer a system in which the liver secretes a range of particles varying in size from $VLDL_1$ to LDL. HepG2 cells (a human-derived liver cell (31)) and perfused rabbit livers in recent studies from our laboratory (32) secrete particles the size of small VLDL, IDL, and LDL, thus the model is physiologically plausible. Further, new evidence has emerged that human cardiac muscle can secrete LDL directly (33).

The model was constrained by introducing parameter dependencies to reduce the number of unknowns to within the limit necessary for system identifiability (see legend to Fig. 1). The model was tested and found to be a priori globally identifiable (15), i.e., all rate constants could be determined (34) if the data were of sufficient precision.

Steady state production rates (in mg/d) and transport rates of apoB (in mg/d) from one lipoprotein fraction to another were obtained by the application of the model to the observed data. Fractional transfer rates (FTR) were calculated as follows: for $VLDL_1$ to $VLDL_2$ the transport rate from compartments 7 to 9 was divided by the $VLDL_1$ apoB mass (combined masses of compartments 6 + 7 + 8); for $VLDL_2$ to IDL transport rates from compartment 10 to 12 and 10 to 13 were combined and divided by the $VLDL_2$ apoB mass (compartments 9 + 10 + 11); for IDL to LDL the transport rate from compartment 12 to 14 was divided by IDL apoB mass (compartments 12 + 13). Fractional rates of direct catabolism were derived similarly for $VLDL_1$ by dividing the sum of outputs from compartments 7 and 8 (in mg/day) by the $VLDL_1$ apoB mass, for $VLDL_2$ by dividing the sum of outputs from compartments 10 and 11 by the $VLDL_2$ mass, and for IDL by dividing the sum of outputs from compartments 12 and 13 by the IDL apoB mass. The overall fractional catabolic rate (FCR) for $VLDL_1$, $VLDL_2$, and IDL-apoB was the sum of the FTR and the fractional rate of direct catabolism for each lipoprotein. The LDL apoB fractional catabolic rate was calculated as the output from compartment 14 divided by its mass. The residence time (RT) of a lipoprotein was the reciprocal of the FCR for each lipoprotein class.

Ethical considerations

All subjects participating in this study gave written, informed consent. The study met the requirements of the Ethics Committees of the Klinikum Grosshadern in Munich and of Glasgow Royal Infirmary University NHS Trust.

Statistical analysis

Relationships between kinetic variables and plasma lipid, lipoprotein and apolipoprotein B concentrations were tested by regression analysis using Minitab Version 10 (Minitab Inc., State College, PA).

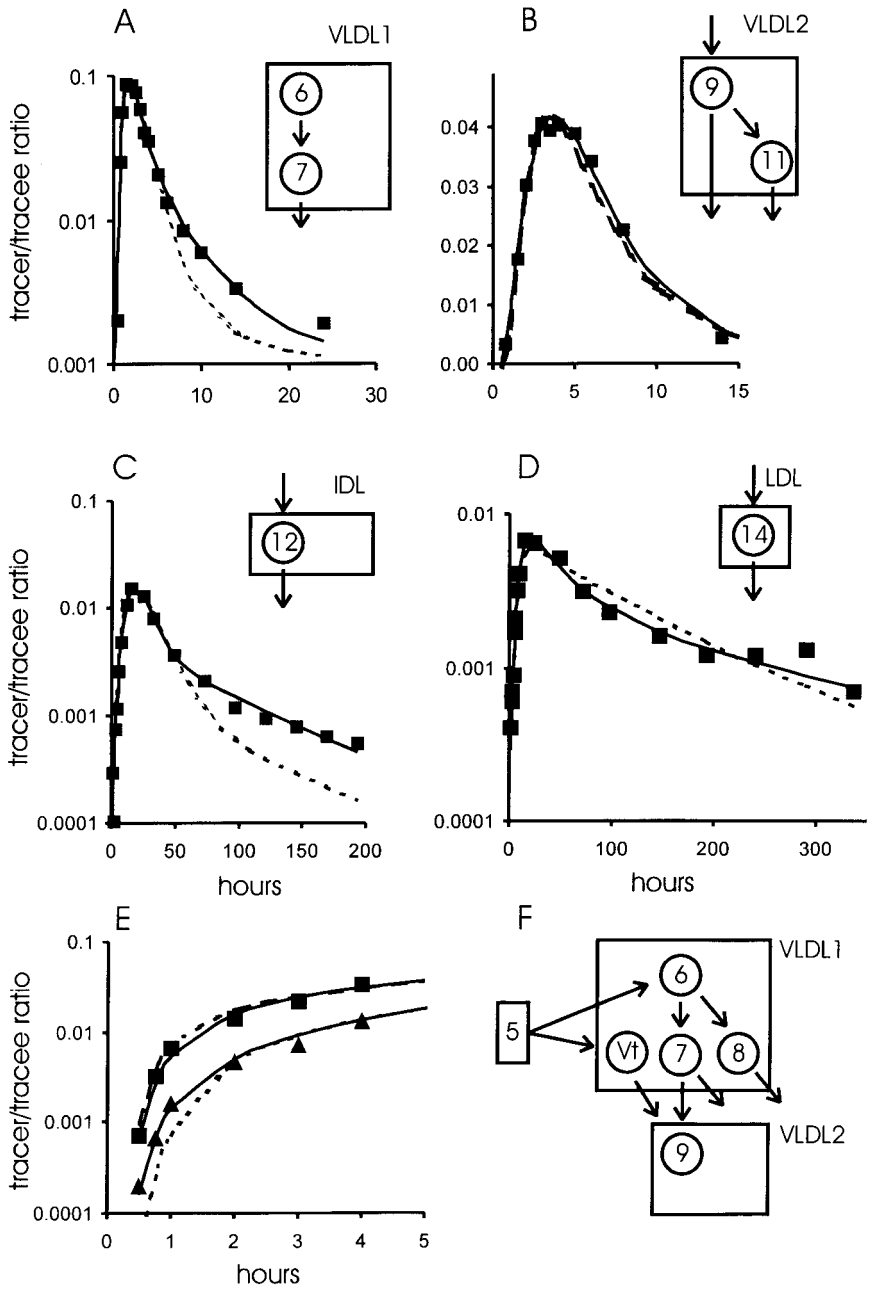

Fig. 2. Characteristics of the multicompartmental model of apoB metabolism. The model in Fig. 1 appears complex. It was constructed as detailed in reference 15. The need for multiple mathematical compartments in each of the lipoprotein density ranges is illustrated in panels A–D (data from subject 12 is shown in panels A and B, data from subject 17 in panel C and from subject 15 in panel D). In panel A, compartment 8 representing $VLDL_1$ 'remnants' was omitted from the model. While the rise and initial decay in the tracer/tracee ratio in $VLDL_1$ apoB could be fitted using compartments 6 and 7, the terminal portion of the curve did not fit (as indicated by the dashed line); inclusion of compartment 8 gave a satisfactory fit as shown by the solid line. In panel B a remnant compartment was included in $VLDL_2$ but the second compartment in the short, internal delipidation chain (compartment 10 in Fig. 1) was omitted. As shown by the dashed line (note: linear y axis) the reduced model generated a fit to the rise portion of the curve but not to the decay portion (5–10 hours). The breadth of the peak could only be fitted using the chain of compartments 9 and 10 (solid line). Panel C demonstrates that omission of the second compartment in IDL which represents slowly metabolized particles (compartment 13) gave an unsatisfactory fit (dashed line). Its inclusion allowed the terminal exponential (after 50 h) to be fitted satisfactorily. Panel D shows that omission of an extravascular LDL compartment (compartment 15 in Fig. 1) restricted the LDL decay curve to a mono-exponential function (dashed line) while its inclusion gave a satisfactory fit to the observed bi-exponential decay (solid line). Panels E and F show the effect of including a rapid lipolysis compartment (V_t) in $VLDL_1$ rather than having direct $VLDL_2$ secretion from the liver, i.e., omitting the transfer from compartment 5 to compartment 9 in Fig. 1. If the fractional transfer rate of $V_t \rightarrow$compartment 9 ($VLDL_2$) was permitted to rise to up to 10 times the fractional transfer rate of $VLDL_1$ to $VLDL_2$ (compartment 7$\rightarrow$compartment 9) no satisfactory fit was obtained (dashed line). Application of direct $VLDL_2$ production as in the Fig. 1 model did give a good fit to observed data (solid line) (data from subject 16).

Concentrations rather than pool sizes were used to identify possible associations between production and transport rates (which were expressed per kg body weight) and the plasma level of an apoB-containing lipoprotein. This overcomes possible exaggeration of the strength of a relationship due to the inclusion of body weight as a factor in both response and predictor variables. Variables that were not normally distributed (as tested by generating normality plots in Minitab) were transformed prior to use in regression analysis. Specifically, plasma triglyceride, $VLDL_1$ FTR, $VLDL_2$ FTR, and the fractional rates of $VLDL_1$, $VLDL_2$ direct catabolism, and the fraction of IDL + LDL apoB direct production were normalized by taking their logarithm. Best subset and stepwise regression were performed to ascertain the potential metabolic determinants of plasma triglyceride, IDL apoB- and LDL apoB-plasma concentrations. Analysis of variance (ANOVA) was used to compare kinetic parameters in subjects in the three LDL-size pattern groups (A, I, B). Fisher's pairwise comparisons were made if the ANOVA was significant ($P < 0.05$) and a *t*-test was used to generate *P* values for the comparison.

RESULTS

The subjects of this study were 20 males aged 21 to 59 years. They were chosen initially not by LDL size phenotype but to represent a wide range of plasma lipid levels: 0.66 to 2.40 mmol/l for plasma triglyceride and 3.95 to 6.95 mmol/l for plasma cholesterol (Table 1), in order to uncover the principal kinetic parameters that governed VLDL metabolism across the putative threshold of a plasma triglyceride of 1.5 mmol/l and so influenced the appearance of small dense LDL. Body weight varied from 58 to 111 kg and was related positively to plasma triglyceride ($r = 0.46$, $P = 0.023$), negatively to HDL cholesterol ($r = -0.45$, $P = 0.028$) but not to plasma or LDL cholesterol.

$VLDL_1$ and $VLDL_2$ apoB kinetics and the regulation of plasma triglyceride

Across the plasma triglyceride range seen in these subjects, $VLDL_1$ and $VLDL_2$ apoB concentration and pool size varied about 10-fold (**Table 2**, Appendix 1). The levels of the two lipoproteins were strongly correlated, $r = 0.70$, $P < 0.0001$, but as described below, they had distinct kinetic properties. Typical tracer/tracee curves for apoB in the four lipoprotein fractions are given in **Fig. 3** for subjects with pattern A versus pattern B LDL. In $VLDL_1$ and $VLDL_2$ the initial rate of rise in tracer/tracee ratio was more rapid for the subject with pattern A than for pattern B subject, indicating a higher turnover rate (i.e., FCR). Similarly, in IDL apoB and LDL apoB, the rate of clearance of tracer from these fractions was faster in the pattern A than pattern B subjects. Multicompartmental modeling generated kinetic rate constants which are summarized for subjects with pattern A, I, and B in Table 2 and **Table 3** and presented individually in Appendices 1 and 2.

$VLDL_1$ apoB production rate in the whole group of subjects varied from 5.4 to 26.6 mg/kg/d (Table 2; 452 to 2074 mg/day, Appendix 1). There was no significant correlation between $VLDL_1$ apoB concentration and the rate of $VLDL_1$ apoB production, but there was a positive association between the residence time of the particle and its concentration ($r = 0.80$, $P < 0.0001$). As the disappearance of tracer from $VLDL_1$ was followed over a prolonged period, it was possible to distinguish two fates for the apoB of this fraction. The first was transfer to $VLDL_2$, the second, direct catabolism of the lipoprotein. The fractional rate of the former diminished as plasma triglyceride increased (log $VLDL_1$ FTR vs. log plasma triglyceride, $r = -0.83$, $P < 0.0001$). The rate of the latter was highly variable and showed no univariate association with plasma triglyceride or $VLDL_1$ apoB concentration.

TABLE 2. VLDL apolipoprotein B kinetics in subjects with LDL size phenotypes A, I, and B

	$VLDL_1$ (S_f 60–400)				$VLDL_2$ (S_f 20–60)				
			Fractional Rate of					Fractional Rate of	
LDL Phenotype	Production	Concentration	Direct Catabolism	Transfer to $VLDL_2$	Direct Production	Tranfer from $VLDL_1$	Concentration	Direct Catabolism	Transfer to IDL
	mg/kg/d	*mg/dl*	*pools/d*	*pools/d*	*mg/kg/d*	*mg/kg/d*	*mg/dl*	*pools/d*	*pools/d*
A									
Mean	12.7	1.4	8.0	16.8	2.2	8.3	3.3	0.70	8.7
SD	5.4	0.6	9.6	9.9	2.0	3.4	1.2	0.92	5.1
I									
Mean	15.4	3.2[a]	8.1	6.8[a]	2.6	7.0	5.1[a]	0.37	3.9[a]
SD	7.6	1.9	5.1	2.8	1.5	1.9	1.4	0.33	1.2
B									
Mean	11.6	3.4[b]	1.9	6.7[b]	5.0[b,c]	8.5	8.2[b,c]	0.7	3.2[b]
SD	3.4	1.1	2.5	2.2	2.0	2.5	1.7	1.1	1.1
ANOVA	NS	0.009	NS	0.004	0.032	NS	<0.001	NS	0.005
A vs. B[d]		0.006		0.004	0.021		<0.001		0.003
A vs. I		0.11		0.013	0.66		0.040		0.018
I vs. B		0.83		0.98	0.053		0.011		0.35

[a,b,c] Groups significantly different by one-way ANOVA. Fisher's pairwise comparison significant for: [a] A vs. I; [b] A vs. B; [c] I vs. B.
[d] *P* values derived by *t*-test for comparison.

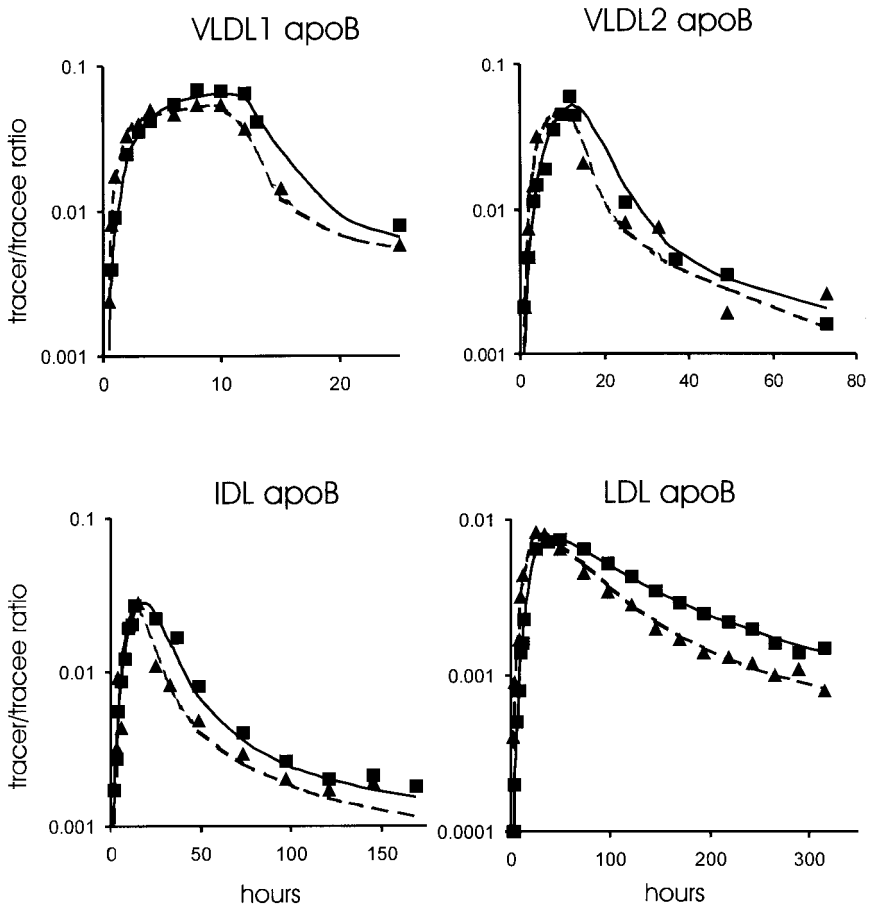

Fig. 3. Apolipoprotein B metabolism in pattern A and pattern B subjects. Tracer/tracee curves are given for subjects with a pattern A LDL size phenotype (subject 6, triangles with dashed line) and a pattern B (subject 20, squares with solid line).

Direct production of apoB in the $VLDL_2$ fraction showed substantial variation, from 0.2 mg/kg/d to 8.4 mg/kg/d (Table 2, 15 to 782 mg/d, Appendix 1). This variable tended to increase with increasing plasma triglyceride; the association was not significant when production was expressed as mg/kg/d but was in mg/d ($VLDL_2$ apoB production vs. log plasma triglyceride, $r = 0.50$, $P = 0.015$). $VLDL_1$ apoB and $VLDL_2$ apoB production rates were not related to one another ($r = 0.0$, NS). There were correlations between the $VLDL_2$ apoB concentration in plasma and the $VLDL_2$ apoB direct production rate ($r = 0.51$, $P = 0.013$) as well as the $VLDL_2$ FTR ($r = -0.74$, $P < 0.0001$) but not between $VLDL_2$ apoB concentration and the $VLDL_1$ to $VLDL_2$ apoB transport rate ($r = 0.0$, NS) or the fractional rate of $VLDL_2$ apoB direct catabolism ($r = -0.32$, $P = 0.093$).

Plasma triglyceride was strongly correlated with variation in both $VLDL_1$ and $VLDL_2$ apoB concentrations ($r = 0.84$ and $r = 0.92$, respectively, both $P < 0.0001$). Best subset and stepwise regression analysis revealed that the most important predictors of plasma triglyceride were $VLDL_1$ apoB FTR (explaining 71% of the variation) followed by the fractional rate of $VLDL_1$ apoB direct catabolism (explaining an additional 7%) and $VLDL_2$ apoB production (an additional 3%). $VLDL_1$ and $VLDL_2$ apoB FTR were highly correlated ($r = 0.76$, $P < 0.0001$) and could be used interchangeably in the regression analysis. Together the above parameters of $VLDL_1$ and $VLDL_2$ apoB metabolism explained 81% of the observed variation in plasma triglyceride levels. Most subjects were homozygotes for the E_3 allele of apoE and there was no relationship between apoE phenotype and $VLDL_1$– or $VLDL_2$–apoB kinetics in this group.

Apolipoprotein B kinetics in IDL and LDL

D_3-leucine enrichment in IDL and LDL apoB rose over the first 10 h of the turnover (Fig. 3) and these lipoprotein fractions exhibited peak incorporation of tracer many hours after its administration, e.g., in LDL apoB at 30–50 h. The general validity of the stable isotope technique can be seen by comparing the results for the LDL apoB FCR obtained in the present study (**Fig. 4**) with those derived using a ^{125}I-labeled-LDL tracer in normolipemic subjects in an earlier publication (35). The values obtained for the LDL FCR and the relationship to plasma triglyceride were similar using both methods.

IDL apoB concentration (Table 3; Appendix 2) correlated with the $VLDL_2$ apoB FTR (log $VLDL_2$ FTR vs. IDL

TABLE 3. IDL and LDL apolipoprotein B kinetics in subjects with LDL size phenotypes A, I, and B

	IDL (S_f 12–20)					LDL (S_f 0–12)				
				Fractional Rate of						
LDL Phenotype	Direct Production	Transfer from $VLDL_2$	Concentration	Direct Catabolism	Transfer to LDL	Direct Production	Transfer from IDL	Concentration	Fractional Catabolic Rate	Total apoB Production
	mg/kg/d	*mg/kg/d*	*mg/dl*	*pools/d*	*pools/d*	*mg/kg/d*	*mg/kg/d*	*mg/dl*	*pools/d*	*mg/kg/d*
A										
Mean	2.7	9.7	10.7	0.60	3.04	1.9	10.5	59	0.55	19.3
SD	2.2	3.4	3.8	0.63	2.20	1.5	2.7	15	0.20	6.0
I										
Mean	1.4	7.8	12.9	0.47	1.56	1.9	8.0	78	0.32[a]	21.3
SD	1.0	2.7	2.7	0.27	0.37	2.2	2.2	10	0.11	9.2
B										
Mean	1.6	10.2	12.6	0.91	1.85	1.1	9.0	76	0.36[b]	19.3
SD	1.7	3.0	3.0	0.67	0.58	1.3	2.9	19	0.14	4.4
ANOVA	NS	NS	NS	NS	NS	NS	NS	NS	0.03	NS
A vs. B[c]									0.045	
A vs. I									0.017	
A vs. B									0.62	

[a,b] Groups significantly different by one-way ANOVA; Fisher's pairwise comparison significant for: [a] A vs. I; [b] A vs. B.
[c] *P* values derived by *t*-test for comparisons.

apoB concentration, $r = 0.69$, $P < 0.001$) but not the amount of $VLDL_2$ converted to IDL apoB ($r = 0.0$, NS) or the IDL apoB production rate ($r = 0.0$, NS). Variation in IDL apoB concentration was also related to IDL apoB FTR ($r = -0.71$, $P < 0.0001$). In regression analyses, IDL apoB FTR was the most important determinant of IDL apoB concentration (accounting for 53% of the variation), followed by IDL apoB fractional rate of direct catabolism (explaining a further 17% of the variation) and $VLDL_2$ apoB FTR (explaining a further 8%).

Variation in LDL apoB concentration was related in univariate regression to LDL apoB FCR ($r = -0.77$, $P < 0.001$) and $VLDL_2$ apoB production ($r = 0.48$, $P = 0.018$) but not significantly to $VLDL_1$ apoB production ($r = 0.0$, NS), IDL apoB production ($r = 0.38$, $P = 0.058$), or LDL apoB production ($r = 0.0$, NS). In multivariate analysis, LDL apoB concentration was best explained by two variables, LDL apoB FCR (accounting for 61% of the variation) and $VLDL_2$ apoB production (explaining a further 7%). LDL apoB FCR was related reciprocally to plasma triglyceride ($r = -0.60$, $P = 0.002$, Fig. 4) but positively to both the amount of IDL + LDL apoB production ($r = 0.59$, $P = 0.004$) and the fraction of total apoB input into the IDL/LDL density range due to direct production ($r = 0.65$, $P = 0.001$, **Fig. 5A**).

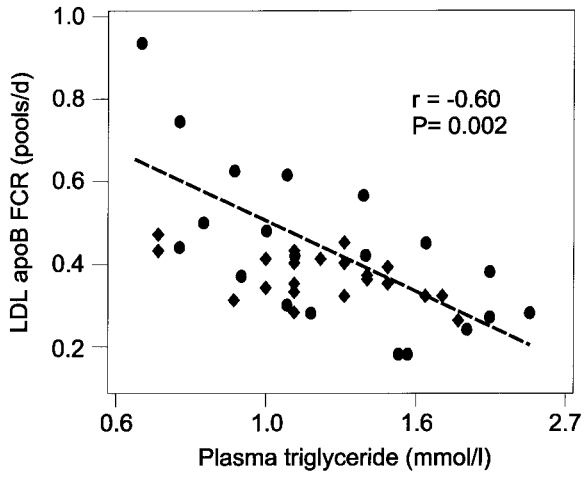

Fig. 4. Relationship between plasma triglyceride and LDL apoB FCR. LDL apoB FCR values in the present study were compared to those seen in an earlier group of subjects in whom the apoLDL FCR was determined using a tracer of ^{125}I-labeled-LDL (35). The correlation for the previous data set was $r = -0.54$, $P < 0.01$ (35); (◆)-previous study; (●)-present subjects.

In both the IDL and LDL density intervals it was necessary using the model in Fig. 1 to invoke apoB direct production in order to generate a satisfactory fit to the observed data. The amount of apoB entering the metabolic cascade by these routes was unrelated to the circulating mass of IDL and LDL but the percent of total apoB production that was directly released into these density ranges was inversely associated with the plasma triglyceride level ($r = -0.54$, $P = 0.009$, Fig. 5B), and positively related to HDL cholesterol ($r = 0.59$, $P = 0.004$) and to the $VLDL_1$ to $VLDL_2$ apoB FTR ($r = 0.48$, $P = 0.018$) but not to the $VLDL_2$ to IDL apoB FTR ($r = 0.18$, NS).

ApoB metabolism in subjects with LDL size pattern A vs. pattern B

As might be predicted from the above findings and the known association of LDL subfraction distribution with plasma triglyceride, LDL apoB kinetic parameters differed in individuals with differing LDL size phenotypes (Tables 1, 2, 3). Pattern B subjects on average had a low mean LDL apoB FCR (0.36 ± 0.14) and a low percent IDL/LDL derived by direct production (13 ± 8%) (Fig. 5B). In contrast, pattern A subjects had a higher mean LDL apoB FCR (0.55 ± 0.20) and a tendency to derive a

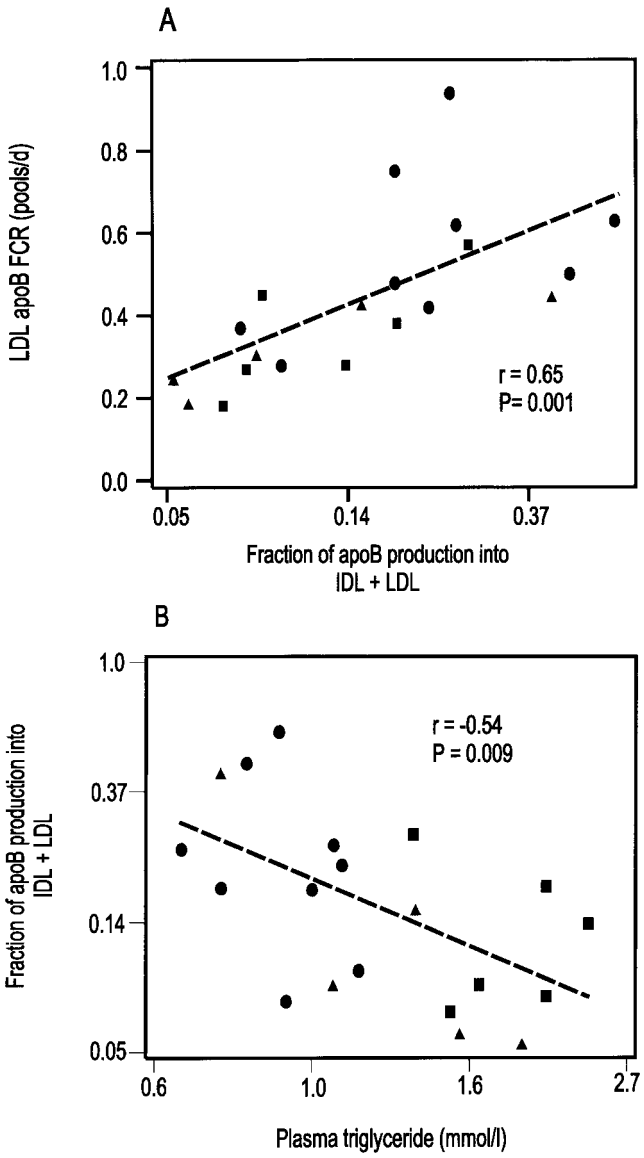

Fig. 5. Relationship between IDL/LDL apoB direct production, LDL apoB FCR, and plasma triglyceride. In panels A and B, the fraction of direct production was calculated from the data in Appendices 1 and 2. It is the amount of IDL + LDL apoB direct production divided by the total apoB input. Plasma triglyceride and the fraction of IDL/LDL apoB direct production were transformed to $\log_e$ values while LDL apoB FCR was used untransformed in the regression analyses. Separate symbols are used for subjects of differing LDL size phenotype: (●)-phenotype A; (▲)-intermediate phenotype; (■)-phenotype B. Regression lines were generated using all subjects.

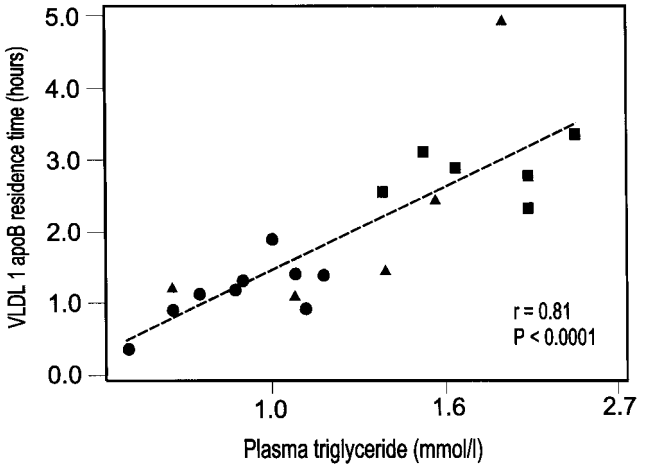

Fig. 6. $VLDL_1$ residence time in subjects of differing LDL size phenotype. Relationship between residence time (RT = reciprocal of the sum of the $VLDL_1$ apoB FTR plus the fractional rate of $VLDL_1$ apoB direct catabolism) and plasma triglyceride. Symbols as in Fig. 5. Regression line was derived using all subjects.

higher proportion of their LDL from IDL/LDL direct production (25 ± 16%). Further significant differences were seen in the VLDL density range with higher $VLDL_1$ and $VLDL_2$ apoB concentrations in pattern B due principally to reduced FTRs for these lipoproteins (Table 2). $VLDL_2$ apoB production was significantly higher in pattern B compared to pattern A subjects. Plasma triglyceride levels in the pattern B group were above 1.5 mmol/l principally because of a prolonged residence time for VLDL particles (**Fig. 6**) in these subjects. In general those with an intermediate LDL size (pattern I) gave results intermediate between patterns A and B.

DISCUSSION

Most subjects who suffer a coronary event have plasma lipid levels that fall within the range of values commonly seen in the population. Hence the present study examined the differences in apoB metabolism in men with normal to moderately elevated cholesterol and triglyceride levels. More particularly, we attempted to understand the transport of apoB-containing lipoproteins in subjects with ALP who were at particularly elevated CHD risk. Two protocols were used for the administration of deuterated leucine, bolus and infusion, and the resultant kinetic data merged in the present report. Theoretically, using a multicompartmental modeling approach to data analysis, derived kinetic parameters should not be influenced by tracer administration protocol. In confirmation of this Parhofer et al. (13, 36) in two separate studies directly compared the results obtained by bolus versus infusion protocols in four normal and five hyperlipidemic subjects. The latter were studied on two occasions (on and off drug). In all instances the same derived rate constants fitted both sets of experimental data. In an earlier publication we reproduced these findings for $VLDL_1$, $VLDL_2$, and IDL apoB in a single individual given bolus versus infused amino acid. There was a discordancy in the LDL apoB FCR with the two protocols but this we attributed to measurement difficulties rather than an inherent systematic difference.

Before focussing on ALP, a number of general observations can be made about apoB kinetics in the men studied. First, $VLDL_1$ and $VLDL_2$ apoB production rates, although varying widely, were not correlated. This finding indicates that the liver can regulate independently the as-

sembly and secretion of these subclasses of VLDL, a suggestion that receives further support from recent in vivo observations in which *a*) estrogen administration was shown to stimulate $VLDL_1$ apoB but not $VLDL_2$ apoB production (37) and *b*) the acute infusion of insulin in a hyperinsulinemic, euglycemic clamp in normal subjects was shown to suppress the production of $VLDL_1$ apoB but not affect that of $VLDL_2$ apoB (38). The mechanism by which the liver is able to vary the amount of large versus small VLDL secreted is unknown.

Recent experiments in cell culture (31, 39) suggest that VLDL assembly is more complex than was originally thought, involving a two-step process in which a small lipoprotein particle containing at first little triglyceride is formed in the rough endoplasmic reticulum (ER), and then the bulk of the triglyceride core is added to this at the junction of the rough and smooth ER. It is possible that the release of small VLDL follows the addition of a relatively small quantity of triglyceride (or of cholesteryl ester) to the nascent particle while large VLDL is formed by the addition of a substantial triglyceride core in a second, quantum step. Second, input of apoB into IDL and LDL was substantial and inversely related to plasma triglyceride. The nature of 'direct' LDL production has been controversial with some investigators suggesting that it is the consequence of a very fast lipolytic pathway (29, 40). However, on the basis of the arguments rehearsed in Methods, we favor the concept that direct IDL/LDL production occurs; cultured hepatic cells (HepG2 [31]), perfused rabbit livers (32), and cardiac tissue (33) all appear capable of producing apoB-containing particles of the size of IDL/LDL. The link with plasma triglyceride concentration suggests that in subjects with a low circulating concentration of this lipid, the liver has insufficient triglyceride to assemble even a $VLDL_2$-sized particle and IDL/LDL are secreted. Third, in a population survey conducted previously in normolipemic subjects, 75% of the variation in plasma triglyceride was determined by the $VLDL_1$ concentration (9). The present study shows that the level of this lipoprotein subclass is regulated mainly by its fractional transfer rate to $VLDL_2$. The substantial decrease in clearance rates of both $VLDL_1$ and $VLDL_2$ as plasma triglyceride rises in the normal population is in good agreement with the findings of Cortner et al. (41) who observed a fall in total VLDL (i.e., S_f 20–400) apoB FCR from 27 pools/d at a plasma triglyceride of 0.4 mmol/l to 4.6 pools/d at 2.2 mmol/l. This fall-off in FTR is likely to reflect the rates of lipolysis and could be due to alteration in lipoprotein lipase (LpL) activity; however, in a separate survey in normal subjects we found, in confirmation of the results of Lamarche et al. (42), only a weak, negative correlation between post-heparin plasma LpL and plasma triglyceride ($r = -0.21$, $P = 0.007$, n = 137; C. J. Packard, C. E. Tan, and J. Shepherd, unpublished observations). Thus, we suggest that other factors such as the apoC-II content or the apoCII/CIII ratio in VLDL (43) may determine the rate of VLDL lipolysis in our subjects.

The atherogenic lipoprotein phenotype which is characterized by moderately elevated plasma triglyceride (generally >1.5 mmol/l), low HDL cholesterol, and the presence of small, dense LDL is associated with a 3- to 7-fold risk of CHD (1–3). Of the present group, six subjects had pattern B LDL (i.e., small dense particles) and most of these had associated alterations in plasma triglyceride and HDL cholesterol. These were compared to nine pattern A subjects and five in whom the LDL size was intermediate. ApoB metabolism was altered throughout the VLDL–LDL cascade in the group with pattern B versus pattern A LDL. Specifically, in the former there was an accumulation of $VLDL_1$ and $VLDL_2$ due to a reduced FTR (i.e., lipolysis rate) for these lipoproteins (Fig. 6), and a prolonged residence time for LDL. The findings here are in concordance with the earlier characterization of apoB kinetics in hyperapobetalipoproteinemics who exhibit a preponderance of dense LDL (10). The relationship between LDL apoB FCR and plasma triglyceride is complex. We have shown that, compared to subjects with low plasma triglyceride levels, those with high normal triglyceride or moderate hypertriglyceridemia have a reduced LDL FCR (35). On the other hand, patients with more severe hypertriglyceridemia exhibit higher than normal LDL clearance rates, possibly due to accelerated, receptor-independent catabolism (7). A full discussion of this topic is presented in a recent review (7).

The metabolic conditions described for pattern B subjects in Tables 2 and 3, we suggest, provide the environment needed for the formation of small, dense LDL (**Fig. 7**). The key step in reducing LDL size in this model is the removal of core triglyceride and surface phospholipid by hepatic lipase (HL). This enzyme has been shown to have an important role in determining small dense LDL levels in particular in men, who have substantially higher levels of activity than women (7, 9). Because HL cannot act on cholesteryl ester, this lipid must be exchanged out of LDL and replaced with triglyceride for a particle to be susceptible to the enzyme's action. Cholesteryl ester transfer protein (CETP) is the agent which facilitates this core lipid exchange. From the results of the present investigation it is proposed that LDL has to have a residence time in excess of 3 days and be exposed to a high level of VLDL triglyceride (generated either by over-production of VLDL as in diabetics (44) or inefficient lipolysis as presented here) before enough triglyceride is present in the LDL particle so that the next time it is exposed to HL, a size reduction is effected. The model suggests that the cholesteryl ester content of the most abundant, intermediate-sized LDL has to be reduced (by CETP-mediated transfer) to that seen in small LDL before the conversion step can occur. In pattern A subjects there is insufficient time for a LDL particle to lose this cholesteryl ester and acquire triglyceride before it is catabolized by receptors. Further, in these subjects VLDL triglyceride availability is limited while HDL cholesteryl ester is relatively abundant and available via CETP to transfer to LDL to maintain its core composition. In pattern I subjects the residence time for LDL is prolonged and VLDL triglyceride

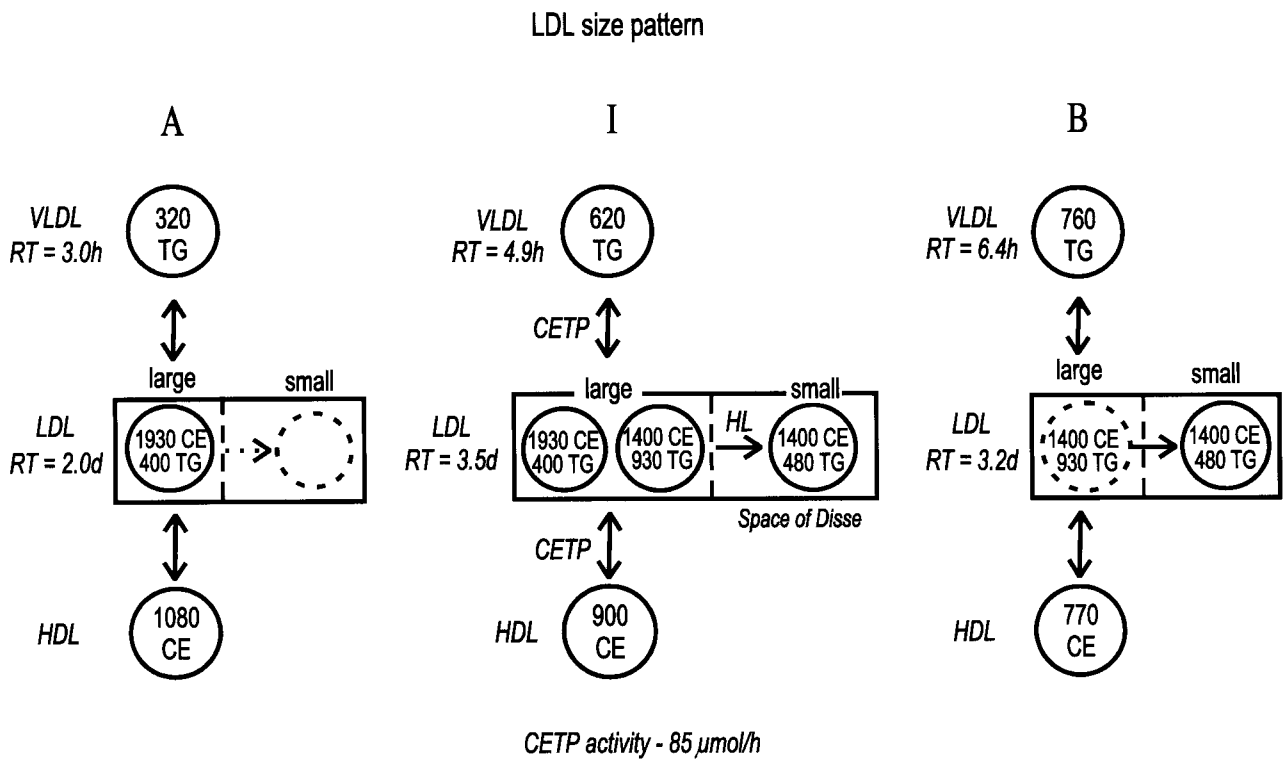

Fig. 7. Metabolic model for formation of small dense LDL. Mean residence time for LDL was calculated for each group (A, I, B). Likewise, the weighted mean residence time for VLDL particles was determined as: ($VLDL_1$ apoB RT × $VLDL_1$ apoB pool + $VLDL_2$ RT × $VLDL_2$ apoB pool)/($VLDL_1$ + $VLDL_2$ apoB pool). Estimates of mean plasma levels of VLDL triglyceride (µmol/l), HDL cholesteryl ester (µmol/l), and LDL cholesteryl ester and triglyceride (in µmol/l) are given inside the circles for each group. LDL cholesteryl ester and triglyceride contents were determined in a previous compositional analysis of isolated intermediate size LDL (d 1.034–1.044 g/ml; LDL-II in reference 3) and small dense LDL (d 1.045–1.060 g/ml; LDL-III in reference 3) in 37 controls whose plasma lipid levels were in the range of the subjects in the present study (M. J. Caslake and C. J. Packard, unpublished observations). Intermediate-sized LDL was composed (g/100 g) of 24.1% protein (of which 97% was apoB), 9.2% free cholesterol, 37.5% cholesteryl ester, 10.5% triglyceride, and 18.6% phospholipid, whereas small dense LDL was 24.4% protein (97% apoB), 7.3% free cholesterol, 27.6% cholesteryl ester, 12.9% triglyceride, and 17.1% phospholipid. Cholesteryl ester content of HDL was taken as 70% of total HDL cholesterol (46). VLDL triglyceride levels were calculated from published compositions of $VLDL_1$ and $VLDL_2$ and the apoB concentrations for these subfractions in the three LDL-size phenotype groups (see reference 9: $VLDL_1$ triglyceride:protein weight ratio = 4.30; $VLDL_2$ triglyceride:protein ratio = 2.29; in the present group of subjects apoB was 36 ± 3% of total protein in $VLDL_1$ and 70 ± 8% of total protein in $VLDL_2$). LDL is subject to continuous inter-particle lipid exchange while in the plasma compartment. It is suggested that the cholesteryl ester content of intermediate-sized LDL (1930 µmol/l) has to be reduced to that normally seen in small LDL (1400 µmol/l) before the conversion in size to a small dense particle can occur. This requires the replacement of 530 µmol/l CE with 530 µmol/l TG. The activity of cholesteryl ester transfer protein (CETP) at about 85 µmol CE/h/liter plasma (47) may also be rate-limiting under some circumstances.

high is enough to generate some small LDL, while in pattern B individuals most of LDL is remodelled due to the long residence time and the higher ratio of VLDL triglyceride to HDL cholesteryl ester. It has been shown in vitro (45) that small dense LDL binds less well to LDL receptors, in accord with our findings that the LDL FCR is reduced by about 60% in subjects with pattern B and with the data of Teng et al. (10). The model in Fig. 7 suggests that the reason for this is that LDL with a prolonged residence time will be preferentially converted to small-sized particles.

In conclusion, it appears that in normal to moderately hyperlipidemic men, the nature of apolipoprotein B-containing particles secreted from the liver, which can vary across a spectrum from $VLDL_1$ to LDL, has an impact upon subsequent lipoprotein catabolism. In contrast (at least within this population) total apoB production is not a determinant of plasma cholesterol and triglyceride levels. The efficiency of VLDL clearance is the primary regulator of VLDL levels in normal men and this, in turn, is a major controlling factor in small dense LDL formation.

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APPENDIX 1. Apolipoprotein B kinetics in very low density lipoprotein subfractions, $VLDL_1$ and $VLDL_2$

	$VLDL_1$ (S_f 60–400)				$VLDL_2$ (S_f 20–60)			
			Fractional Rate of				Fractional Rate of	
Subject	Production	Pool	Direct Catabolism	Transfer to $VLDL_2$	Direct Production	Pool	Direct Catabolism	Transfer to IDL
	mg/d	*mg*	*pool/d*	*pools/d*	*mg/d*	*mg*	*pools/d*	*pools/d*
1	1184	18	27.2	37.3	200	66	2.8	10.8
2	980	37	0.0	26.4	20	48	0.5	20.4
3	452	21	0.1	21.1	15	47	0.5	9.5
4	462	23	9.2	11.1	146	126	0.1	3.0
5	1249	69	10.1	8.1	476	111	1.6	7.7
6	780	62	0.0	12.6	166	111	0.6	8.0
7	751	44	0.0	17.0	123	97	0.0	9.0
8	1230	47	18.3	7.8	30	131	0.0	3.0
9	1000	59	7.2	9.9	280	114	0.2	7.3
10	553	27	9.8	10.4	271	96	0.5	4.2
11	1183	53	13.8	8.7	61	103	0.2	4.8
12	2074	123	11.1	5.7	364	182	0.8	5.0
13	1258	127	4.0	5.9	230	236	0.1	2.2
14	665	136	1.7	3.2	90	156	0.2	3.2
15	943	100	0.1	9.3	247	162	2.7	4.5
16	600	78	0.1	7.6	558	267	0.0	1.8
17	714	86	0.0	8.3	324	280	0.0	3.7
18	1549	150	6.3	4.0	463	462	0.0	2.2
19	1155	133	1.7	6.9	782	332	1.3	3.9
20	1377	193	3.2	3.9	354	321	0.4	3.0

APPENDIX 2. Apolipoprotein B kinetics in intermediate and low density lipoprotein

	IDL (S_f 12–20)				LDL (S_f 0–12)		
			Fractional Rate of				
Subject	Direct Production	Pool	Direct Catabolism	Transfer to LDL	Direct Production	Pool	Fractional Catabolic Rate
	mg/d	*mg*	*pools/d*	*pools/d*	*mg/d*	*mg*	*pools/d*
1	370	177	1.85	4.23	60	860	0.94
2	96	113	1.06	8.41	116	1429	0.75
3	259	342	0.23	1.81	138	1508	0.50
4	559	534	0.02	1.74	308	1962	0.63
5	137	243	1.05	3.03	1	1998	0.37
6	42	337	0.59	2.15	158	1845	0.48
7	143	351	0.01	2.88	142	1861	0.62
8	60	328	0.01	1.40	276	1730	0.42
9	130	433	0.55	1.69	1	2612	0.28
10	215	321	0.32	1.92	373	2219	0.44
11	79	255	0.57	1.70	31	1559	0.30
12	145	488	0.31	1.85	271	2791	0.42
13	86	498	0.89	1.20	2	3407	0.18
14	14	370	0.25	1.14	27	1901	0.24
15	173	209	1.03	2.42	253	1336	0.57
16	1	527	1.29	0.89	83	3003	0.18
17	39	403	0.32	2.34	56	2226	0.45
18	430	663	0.07	2.19	2	3853	0.38
19	1	482	1.00	1.67	160	3588	0.27
20	266	505	0.85	1.60	1	2892	0.28

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