Accumulation of large very low density lipoprotein in plasma during intravenous infusion of a chylomicron-like triglyceride emulsion reflects competition for a common lipolytic pathway

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Abstract Very low density lipoproteins (VLDL) are produced in the liver and contain apolipoprotein (apo) B-100 and endogenous lipids. By contrast, ingestion of fat leads to formation of chylomicrons containing apoB-48 secreted from the intestine. In this study, a 60-min intravenous infusion of a chylomicron-like triglyceride emulsion was given to healthy young men to examine whether competition between chylomicrons and VLDL for the same lipolytic pathway explains the increase in VLDL seen after meals. The responses of two major VLDL subfractions were determined by measuring the concentrations of apoB-100 in fractions of triglyceride-rich lipoproteins with Svedberg flotation rates of 60–400 (large VLDL) and 20–60 (small VLDL) that were separated from plasma by density gradient ultracentrifugation. A threefold elevation in plasma triglycerides was observed during the infusion together with a consistent linear increase of large VLDL. The rate at which large VLDL accumulated in plasma differed markedly among individuals and was not enhanced by doubling of the infusion rate. The response of small VLDL was more heterogeneous; however, a decrease was seen in most subjects. The combined pattern for the two VLDL species is what would be expected if large VLDL particles are the precursors of smaller VLDL species and if lipolysis of large VLDL is inhibited through competition from the triglyceride emulsion. The extent to which the triglyceride emulsion inhibited the lipolysis of VLDL and/or influenced the synthesis rate of large VLDL was estimated from simultaneous stable isotope studies. The emulsion caused a 75–90% block of the conversion of large VLDL apoB to small VLDL apoB and there was no sign of enhanced synthesis of large VLDL after infusion of the triglyceride emulsion. The corollary of these findings is that chylomicrons and their remnants impede the normal lipolytic degradation of VLDL and could thereby be indirectly implicated in the generation of atherogenic remnant lipoproteins.—**Björkegren, J., C. J. Packard, A. Hamsten, D. Bedford, M. Caslake, L. Foster, J. Shepherd, P. Stewart, and F. Karpe.** Accumulation of large very low density lipoprotein in plasma during intravenous infusion of a chylomicron-like triglyceride emulsion reflects competition for a common lipolytic pathway. *J. Lipid Res.* 1996. **37:** 76–86.

Supplementary key words apoB-100 • kinetic analysis • Intralipid

Plasma triglyceride is gaining increased recognition as a risk factor for coronary heart disease (CHD), although the mechanisms underlying this association have not been settled (1). Triglyceride-rich lipoproteins are a heterogeneous population of lipoprotein particles. Very low density lipoproteins (VLDL) are produced in the liver and contain apolipoprotein (apo) B-100 and endogenous lipids. By contrast, ingestion of fat leads to formation of chylomicrons containing apoB-48 in the intestine. The apoB molecule remains an integral part of both species of triglyceride-rich lipoproteins during their catabolism, whereas the triglyceride content is hydrolyzed by lipoprotein lipase (LPL) resulting in formation of remnant particles. Under normal conditions low density lipoprotein (LDL) is the end product of the metabolism of VLDL in humans. Chylomicrons and their remnants, on the other hand, are removed from plasma at an earlier stage in the lipolytic cascade. More than a decade ago, Zilversmit (2) advanced the hypothesis that postprandial lipoproteins are important in atherogenesis in humans. It has since been shown that both chylomicron remnants and VLDL increase after fat intake (3, 4). In fact, 80% of the postprandial increase of triglyceride-rich lipoprotein particle number is accounted for by VLDL (4), whereas 80% of the

Abbreviations: apo, apolipoprotein; VLDL, very low density lipoprotein; HDL, high density lipoprotein; LPL, lipoprotein lipase; S_f, Svedberg flotation rate; CHD, coronary heart disease.

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postprandial triglycerides is carried by particles containing apoB-48 (5). The explanation for the elevation of VLDL during alimentary lipemia is not obvious. Both an increased secretion of VLDL particles and a reduced rate of VLDL degradation have to be considered. As removal of triglycerides from chylomicrons and VLDL is thought to be achieved by the same lipolytic pathway (6), the LPL-mediated processing of VLDL might be delayed by the appearance in plasma of newly secreted chylomicrons, with an ensuing accumulation of VLDL particles. However, studies of the synthesis rate of VLDL apoB-100 during fasted and fed conditions have been interpreted to indicate that an almost 50% increase in VLDL synthesis occurs after fat intake (7). Based on our observation that the plasma level of large VLDL increases in response to fat ingestion in both normolipidemic and hypertriglyceridemic individuals, whereas the concentration of small VLDL either is unchanged or decreases (3), we hypothesized that postprandial chylomicronemia impedes the lipolysis of VLDL. To study this further in healthy humans, a new model was used in which a short-term intravenous infusion of a triglyceride emulsion provided the source of chylomicron-like triglyceride-rich particles. The metabolism of large and small VLDL apoB-100, respectively, was then determined. We here report results from applying this model in healthy men and its validation by stable isotope kinetic studies and radioisotope turnover studies.

METHODS

Human subjects

Sixteen healthy men aged 26 ± 5 (mean ± SD) years (range 22–37) were examined in Stockholm. Three of them underwent simultaneous stable isotope kinetic studies and triglyceride emulsion infusions and three other subjects belonging to this group underwent repeated intravenous infusions of the triglyceride emulsion to assess reproducibility of the increase in large VLDL and the effect of different infusion rates. Dietary advice was not given to the subjects but they were asked to avoid alcohol the day before the experiment as well as strenuous physical exercise. In repeated experiments subjects were asked to live under similar and stable conditions between the two experiments. Fasting plasma lipid and lipoprotein levels were normal in all participants with plasma triglycerides of 0.93 ± 0.35 (0.56–1.85) mmol/l, plasma cholesterol 4.27 ± 1.06 (3.11–6.27) mmol/l, and high density lipoprotein (HDL) cholesterol of 1.24 ± 0.32 (0.74–1.98) mmol/l. All subjects were nonobese (body mass index of 22.7 ± 1.5 (20.0–25.8) kg/m^2). An additional two healthy normolipidemic young men underwent a radioisotope turnover study in Glasgow in which autologous ^{125}I-labeled VLDL was administered during infusion of the triglyceride emulsion.

The protocol for the study was approved by the ethics committees at the Karolinska Hospital in Stockholm and the Royal Infirmary in Glasgow. All participants gave their informed consent to participate in the study.

Short-term intravenous infusion of a triglyceride emulsion

Participants were admitted to the metabolic ward between 7:00 and 8:00 AM for an intravenous infusion of a triglyceride emulsion (Intralipid® 100 mg/l, Pharmacia, Stockholm, Sweden). They had been fasting for 12 h. The protocol was a modification of that of Peterson et al. (8). Briefly, fasting venous blood samples were drawn on two occasions with an intervening period of 5 min immediately before a bolus dose of either 0.1 g/kg or 0.2 g/kg of body weight of Intralipid was given. The bolus dose was injected within 60 sec through an indwelling intravenous catheter inserted into the antecubital vein of one arm and was immediately followed by an infusion of Intralipid (0.1 or 0.2 g/kg per h) (infusion pump: IMED 922 H, Medical Market AB, Stockholm, Sweden). Blood samples were drawn during the infusion from an intravenous catheter inserted into the other arm at 10, 20, 40, and 60 min after administration of the bolus dose. All blood samples were drawn into precooled sterile tubes (Vacutainer, Becton Dickinson, Meylan Cedex, France) containing Na_2EDTA (1.4 mg/ml) that were immediately put into ice-water. To determine reproducibility and the effect of different Intralipid doses, three subjects underwent intravenous infusions on four occasions. On two consecutive days, an Intralipid infusion of 0.1 g/kg per h (bolus dose 0.1 g/kg) was given. The following week the same procedure was repeated with an infusion rate of 0.2 g/kg per h (bolus dose 0.2 g/kg). These participants were instructed to maintain their level of physical exercise and to avoid alcohol intake and excessive meals between the studies.

Subfractionation of VLDL and determination of apoB-100

Lipoprotein fractions were prepared from plasma samples by density gradient ultracentrifugation, essentially as described by Redgrave and Carlson (9). Plasma was adjusted to density 1.10 g/ml with solid NaCl. A density gradient consisting of 4 ml of 1.10 g/ml plasma and 3 ml each of 1.065, 1.020, and 1.006 g/ml NaCl solutions was then formed in Ultraclear tubes (Beckman, Palo Alto, CA, volume 13.4 ml) that had been coated with polyvinyl alcohol (BDH Chemicals Ltd,

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Poole, England). Ultracentrifugation was performed in a SW40 Ti swinging-bucket rotor (Beckman) at 40,000 rpm and +15°C (Beckman Optima ultracentrifuge). Consecutive runs calculated to float $S_f > 400$ (32 min), S_f 60–400 (3 h 28 min) and S_f 20–60 (14–16 h) particles were made. The density profile of the gradient has been described in detail previously (9). After each centrifugation step, the top 0.5 ml of the gradient containing the respective lipoprotein subclasses was aspirated, and 0.5 ml of density 1.006 kg/l salt solution was used to refill the tube before the next run. All salt solutions were adjusted to pH 7.4 and contained 0.02% NaN_3 and 0.01% Na_2EDTA. Densities were verified to the fourth decimal place (densitometer; Paar, Graz, Austria).

The apoB-100 concentration in isolated fractions of triglyceride-rich lipoproteins was determined according to Karpe and Hamsten (10). Briefly, isolated fractions of apoB-containing lipoproteins were delipidated in a methanol–diethylether solvent system, and the protein pellet was dissolved in 100 µl of 0.15 M sodium phosphate, 12.5% glycerol, 2% SDS, 5% mercaptoethanol, 0.001% bromphenol blue, pH 6.8, and denatured at +80°C for 10 min. Samples were then run on gradient (3–20%) polyacrylamide slab gels with a running buffer consisting of 25 mM Tris, 192 mM glycine, 0.2% SDS adjusted to pH 8.5 at 120 V for 2 h. ApoB-100 isolated from human LDL was used as protein standard. A standard curve consisting of six dilutions of apoB-100 was applied to each set of gels run together (0.1–2.0 µg). Gels were stained with Coomassie G-250 (Serva, Heidelberg, Germany). With this procedure, the chromogenicities of human apoB-48, apoB-100, and apoB-100-containing lipoproteins of varying particle size have been shown to be identical (4, 10, 11). The bands on the gel were scanned using a laser scanner (Ultroscan XL, Pharmacia-LKB, Sollentuna, Sweden) connected to a personal computer that was equipped with a software providing automatic integration of the areas under scanning curves (Gelscan XL, Pharmacia).

Protocol for the stable isotope kinetic study

Three subjects who had been fasting for 11 h were given L-[5,5,5,-$^2H_3$]leucine ([$^2H_3$]leucine, Cambridge Isotope Laboratory, Woburn, MA) starting at 7:00 AM with a small bolus (0.6 mg/kg body weight) followed by a continuous infusion (0.6 mg/kg per h) for 10 h. During this period they continued to fast but were allowed to have noncaloric drinks and to be ambulatory. After 6 h of [$^2H_3$]-leucine infusion, a bolus dose of Intralipid (100 mg/ml) was given (0.15 g/kg body weight) followed by a constant infusion of Intralipid at a rate of 0.15 g/kg per h for 4 h. Blood samples were taken every 30 min throughout the experiment for isolation of VLDL subfractions. More frequent blood samples were taken just after initiation of the leucine infusion to monitor the content of free leucine in plasma.

Protocol for the radioisotope turnover study

Two healthy men underwent this procedure. The methods used to prepare $VLDL_1$ (S_f 60–400 VLDL) is a variant of that described above and described in detail elsewhere (12). Briefly, 250 ml of plasma was obtained by plasmapheresis from subjects who had fasted overnight and $VLDL_1$ was isolated by cumulative gradient ultracentrifugation. This was labeled with Na[^{125}I] by a modification of the iodine monochloride method (13) and sterilized immediately before reinjection by filtration through a 0.45-µm filter (Acrodisc, Gelman Sciences). Subjects were studied on two occasions. On the day of the turnover study (3 days after plasma donation) subjects were admitted at 8:00 AM, having fasted overnight, and an indwelling cannula was placed in a peripheral vein to facilitate repeated blood sampling. Either saline (as a control for both the bolus and infusion) was given or a bolus of Intralipid (100 mg/l, 0.15 g/kg) was injected and a subsequent infusion (0.15 g/kg per h) was administered for 9 h. Two hours after the Intralipid infusion had been initiated, autologous ^{125}I-labeled $VLDL_1$ was administered into a peripheral vein in the other arm. Venous blood samples were collected from the cannula at frequent intervals during 7 h. Subjects remained fasting but were allowed to drink noncaloric drinks. From 2.0-ml aliquots of plasma, $VLDL_1$ and $VLDL_2$ were isolated by a modification of the cumulative gradient ultracentrifugation procedure (14). ApoB was precipitated by the addition of equal volumes of isopropanol (15) and the radioactive content was determined in a gamma counter. This study was conducted entirely in Glasgow.

Preparation of apoB for the stable isotope kinetic study

Isopropanol precipitation was again used to prepare apoB from the $VLDL_1$ and $VLDL_2$ fractions. It was assumed that total apoB was equivalent to apoB-100, even if it is known that fasting samples may contain trace amounts of apoB-48. The apoB protein pellet was hydrolyzed in glass tubes (Schott, Mainz, Germany) in the presence of 0.5–1.0 ml 6 M HCl at 110°C for 20 h and the amino acid hydrolysate was concentrated in a vacuum concentrator centrifuge (SpeedVac SC110AR, Savant Instruments Inc., Farmingdale, NY) and aliquoted into microvials (Chromacol, Herts., UK). After complete removal of HCl, samples were ready for derivatization and mass spectrometric analysis. The free leucine content in 1-ml aliquots of plasma was isolated by precipitating proteins by addition of 1 ml trichloroacetic acid (10%). Amino acids were prepared from the super-

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natant by cation exchange chromatography using 2-ml columns filled with Dowex AG-50W-X8 resin (H^+-form, 50–10 mesh; Bio-Rad, Richmond, CA). The samples were dissolved in a small volume of 1 M HCl and transferred into microvials and dried again to be ready for derivatization. Amino acids were transformed into *tert*-butyl-dimethyl-silyl-(TBDMS-) derivatives by incubation with 50 μl of freshly prepared 1:1 mixture of N-methyl-N-(*tert*-butyl-dimethyl-silyl)-trifluoro-acetamid (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 quadropole GC–MS instrument (Trio 1000, Fisons, Manchester, UK). The method used for the analysis of $^2H_3$-leucine enrichment in protein hydrosylates and plasma amino acids is presented in detail elsewhere (16). The mass spectrometer was operated under electron ionization conditions (EI^+) and ion mass fragments at m/z 277, m/z 276, and m/z 274 monitored by selective ion recording (SIR mode). Peak areas were quantified in arbitrary units by GC–MS data management system (LabBase, Fisons, Manchester, UK). The ratios m/z 277:m/z 276 were multiplied by an average value for the constant ratio of m/z 276:m/z 274 and the resulting m/z 277:m/z 274 values were used to calculate the specific isotopic enrichment (E) (16). Mass spectrometry was performed in Glasgow on samples that had been shipped on dry ice from Stockholm.

The apoB-100 mass content was quantified in the samples as previously described (10).

Kinetic analysis and multicompartment modelling

Isotope enrichments were converted into tracer/tracee ratios at each time point according to the equation of Cobelli et al. (17). The tracer/tracee ratios in $VLDL_1$ and $VLDL_2$ apoB were then multiplied with the appropriate pool size to generate tracer leucine masses (μg $^2H_3$-leucine) assuming, reasonably, that VLDL is restricted to the plasma compartment. These together with the apoB pool sizes in the VLDL fractions were used and the data were set for parameter estimation with the CONSAM/SAAM 30 program (18). The model for $VLDL_1$ and $VLDL_2$ apoB turnover during the leucine/Intralipid infusion experiment consisted of two linked parallel systems, one that explained the behavior of the tracer ($^2H_3$-leucine in $VLDL_1$ and $VLDL_2$ apoB), the other the behavior of tracee (apoB mass in $VLDL_1$ and $VLDL_2$). During the initial period of leucine infusion, the system was in steady state, but after Intralipid administration, non-steady state ensued as $VLDL_1$ and $VLDL_2$ apoB masses changed. The model that fitted the tracer and apoB masses in both periods satisfactorily was a simplified version of the full $VLDL_1$, $VLDL_2$ apoB model described in an earlier publication (12). Both VLDL subfractions had two compartments arranged in a standard "delipidation chain format". Direct hepatic production of apoB-100 occurred at the level of compartment 1 in $VLDL_1$ and compartment 3 in $VLDL_2$ (see Figs. 4 and 5). As there was not enough information in the tracer curves to determine the values of internal delipidation rates as well as $VLDL_1$ and $VLDL_2$ conversion and $VLDL_2$ catabolism, L(2,1) was set equal to L(3,2) and L(4,3) was set equal to L(0,4). The abrupt change in $VLDL_1$ or $VLDL_2$ apoB tracer leucine content was modelled using the time interrupt system in SAAM. This permitted an instantaneous change to be made in the value of rate constants at specific times, i.e., at 6 h into the turnover when the Intralipid infusion was begun.

Plasma leucine achieved a constant tracer/tracee ratio throughout the 10 h of infusion. Its kinetic behavior was described by a previously established four-compartment subsystem in which average normal values were used as rate constants. These values were similar in most subjects and did not significantly influence VLDL apoB kinetic parameters. A delay of about 30 min between the loss of tracer from plasma and its appearance in $VLDL_1$ or $VLDL_2$ was included in the model.

Radioactivity changes in $VLDL_1$ and $VLDL_2$ apoB after injection of ^{125}I-labeled VLDL were modelled in a fashion similar to the leucine data. An additional compartment was required in $VLDL_1$ to account for the tail observed during the control saline infusion. Again, internal delipidation rates were dependent on the next step in the chain, i.e., L(2,1) was set equal to L(3,2) and L(4,3) equal to L(0,4). The VLDL apoB FCR is the weighted sum of L(0,5), L(0,2), and L(3,2). The $VLDL_1$ to $VLDL_2$ conversion rate is L(3,2). No mass data were used in generating kinetic rate constants for the radioactivity experiments.

Subjects were studied in non-steady state experiments, i.e., during the first 2–7 h of Intralipid infusion, because this is representative of the physiological situation that occurs following a meal. Further examination of the acute perturbation of tracer and tracee in going from steady to non-steady state is a particularly powerful method to determine kinetic parameters.

Major plasma lipoproteins

Major fasting plasma lipoproteins (VLDL, LDL, HDL) were determined by a combination of preparative ultracentrifugation and precipitation of apoB-containing lipoproteins followed by lipid analysis (19). Total cholesterol (20) and triglycerides (21) were determined in triplicate in plasma and in the lipoprotein fractions. Lipids were first extracted with chloroform–methanol (22). Cholesterol and triglycerides were then determined on an Ultrolab (LKB Pharmacia).

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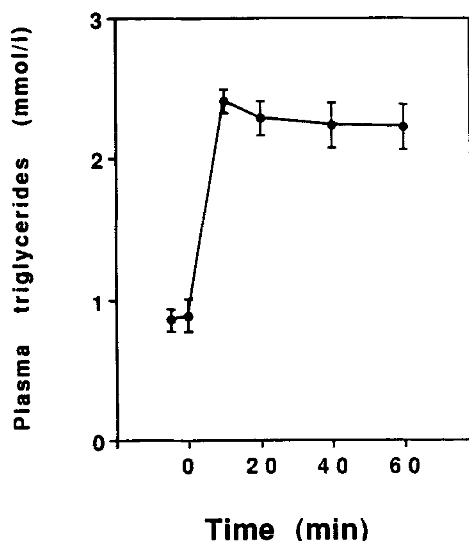

Fig. 1. Line plot of the plasma triglyceride concentrations in 13 men during a 1-h infusion of 10% Intralipid (0.1 g/kg per hr). A bolus dose of 0.1 g/kg of body weight was given at 0 min. Bars indicate SEM.

Statistics

Conventional methods were used for calculation of means, standard deviations (SD), and standard error of means (SEM) (23).

RESULTS

Responses of plasma triglycerides and apoB-100 in VLDL subfractions to intravenous infusion of a triglyceride emulsion

The mean fasting plasma triglyceride concentration increased threefold from 0.9 ± 0.4 mmol/l (mean ± SD) to 2.4 ± 0.3 mmol/l after 10 min infusion of Intralipid at a dose of 0.1 g/kg per h and then leveled off at approximately 2.2 mmol/l (**Fig. 1**). The mean plasma concentration of apoB-100 in the S_f 60–400 lipoprotein fraction, reflecting the plasma level of large VLDL, doubled during the 60 min of infusion (**Fig. 2A**). The increase seemed to be linear as illustrated by the individual patterns of increase. Almost all subjects exhibited a twofold increase, irrespective of the fasting level of S_f 60–400 apoB-100. Nor was the rate of rise of S_f 60–400 apoB-100 correlated to the fasting plasma HDL cholesterol level ($r = 0.03$, $P = 0.93$, n = 13, Pearson correlation coefficient). Subjects in the lower range of the distribution for the fasting plasma concentration (0–10 mg/l) increased to 10–20 mg/l, whereas subjects in the upper range on average increased from 15 mg/l to 30 mg/l (Fig. 2A). Taken together, this means that large VLDL accumulates in plasma when a chylomicron-like triglyceride emulsion is present at a concentration corresponding to what is obtained after intake of a mixed meal (3, 4, 10). Furthermore, the rate at which accumulation of large VLDL occurs in plasma differs markedly even among healthy normolipidemic young adults. In contrast, the concentration of apoB-100 in the S_f 20–60 lipoprotein fraction, reflecting the plasma level of small VLDL, decreased in most subjects (Fig. 2B). The individual pattern of the apoB-100 response in the S_f 20–60 fraction was far more heterogeneous than that observed for the S_f 60–400 fraction. One subject tended to increase, whereas the two subjects with the highest fasting plasma concentration of small VLDL apoB-100 exhibited marked decreases.

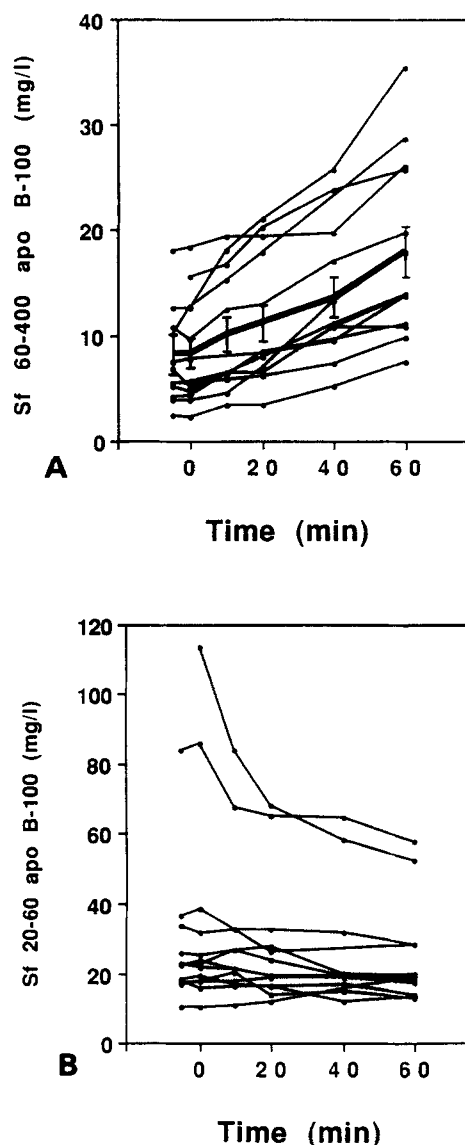

Fig. 2. A and B. Line plots of the individual S_f 60–400 (A) and S_f 20–60 (B) apoB-100 concentrations in 13 men during a 1-h infusion of 10% Intralipid (0.1 g/kg per hr). A bolus dose of 0.1 g/kg body weight was given at 0 min. The mean S_f 60–400 apoB-100 concentration is shown in Fig. 2 A. Bars indicate SEM.

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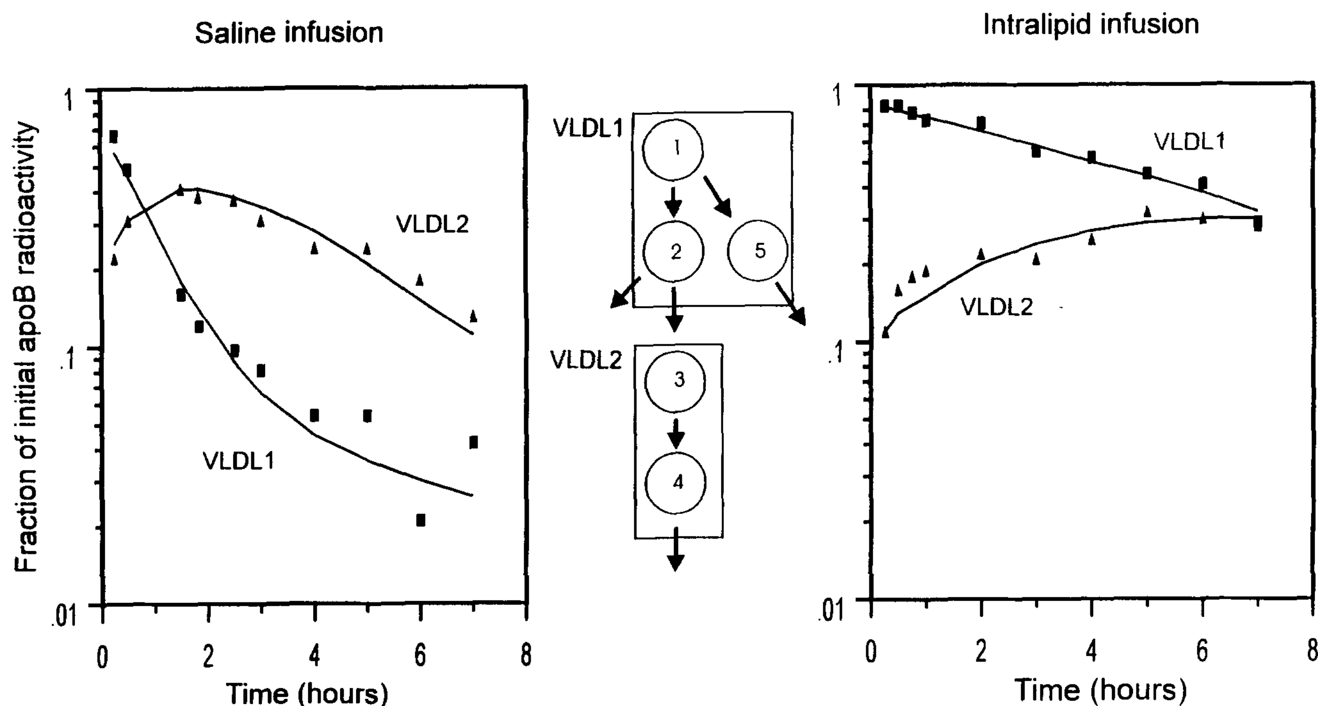

Fig. 3. Plasma decay curves of ^{125}I-labeled apoB in large S_f 60–400 ($VLDL_1$) and small S_f 20–60 VLDL ($VLDL_2$) from subject AD after injection of ^{125}I-labeled $VLDL_1$ in the fasting state during control saline infusion (left panel) and during a second occasion an infusion of a chylomicron-like triglyceride emulsion (Intralipid 100 mg/ml) (right panel). A bolus dose of Intralipid (0.15 g/kg) was injected and followed by an Intralipid infusion (0.15 g/kg per h) for 9 h. Two hours after the start of the infusion, autologous ^{125}I-labeled $VLDL_1$ was administered. The multicompartment model used to determine $VLDL_1$ and $VLDL_2$ apoB kinetic parameters is depicted in the middle. The initial radioactivity in $VLDL_1$ apoB was distributed among the compartments 1, 2, and 5 as described in ref. 12. In the basal state, rapid conversion of $VLDL_1$ to $VLDL_2$ occurred through the delipidation chain compartments 1 → 2 → 3. The rate constant L(2,1) was set equal to L(3,2). The slowly decaying component of the $VLDL_1$ apoB curve was represented by compartment 5 and the loss L(0,5). In $VLDL_2$ loss was from compartment 4 [L(0,4)] and the internal rate L(4,3) was set equal to L(0,4).

Reproducibility and effect of different infusion rates

When the Intralipid infusion rate was doubled (from 0.1 to 0.2 g/kg per h) the plasma triglyceride concentration increased from approximately 3 to 5 mM (data not shown). The apoB-100 content in the S_f 60–400 fraction again showed a consistent linear increase, but the slope was no greater than that observed on the lower Intralipid dose. On the other hand, the baseline plasma S_f 60–400 apoB concentration showed the expected day-to-day variation. The response of S_f 20–60 apoB-100 was neither affected by different doses nor by intra-individual variability.

Metabolism of $VLDL_1$ (S_f 60–400) apoB during Intralipid infusion

In the control saline infusion experiments, ^{125}I-labeled $VLDL_1$ apoB in the subjects studied (**Fig. 3**) disappeared rapidly and the majority of radioactivity appeared in the $VLDL_2$ density interval. The $VLDL_1$ FCR (**Table 1**) and the $VLDL_1$ and $VLDL_2$ conversion rate were in the range seen previously in normal subjects (12). During Intralipid infusion, it was clear that $VLDL_1$ apoB clearance was markedly impaired, the FCR in the subjects fell to 15 and 25% of the value observed in the control experiment. The appearance of radioactivity in $VLDL_2$ apoB was very slow (Fig. 3) with a peak level in the product lipoprotein at approximately 7 h compared to 2 h in the saline experiment. The $VLDL_1$ and $VLDL_2$ conversion rate [L(3,2)] during Intralipid was reduced dramatically (Table 1).

Acute effects of Intralipid infusion on $VLDL_1$ and $VLDL_2$ apoB kinetics

In these experiments we took advantage of an established technique, that of primed constant infusion of a stable isotope-labeled amino acid precursor to follow

TABLE 1. Influence of a chylomicron-like triglyceride emulsion (Intralipid) on the fractional catabolic rate of large S_f 60–400 ($VLDL_1$) and the conversion to small S_f 20–60 VLDL ($VLDL_2$)

Patient	$VLDL_1$ FCR		$VLDL_1$ to $VLDL_2$ Conversion[a]	
	Before IL	During IL	Before IL	During IL
	pool/h		*pool/h*	
AD	0.75	0.11	1.26	0.18
TW	0.58	0.20	1.32	0.35

For explanation, see Fig. 4.
[a]L(3,2).

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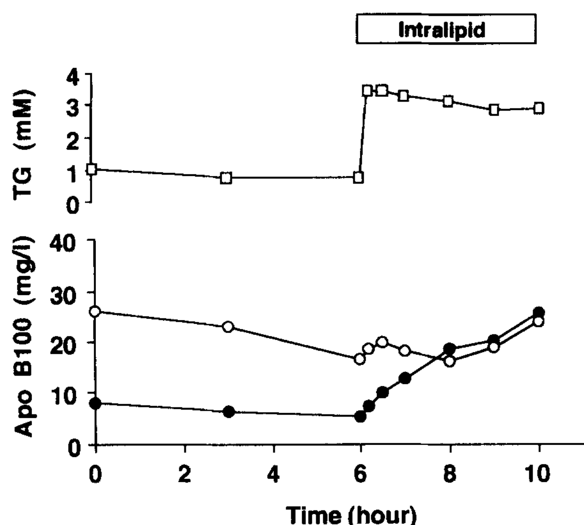

Fig. 4. Line plot of the mean (n = 3) value of plasma triglyceride level and the apoB-100 concentration in the $VLDL_1$ and $VLDL_2$ fractions. [$^2H_3$]leucine is infused during the entire experiment. At 6 h a bolus dose of Intralipid is given followed by an infusion. Filled circles are $VLDL_1$ (S_f 60–400 lipoprotein fraction) and open circles are $VLDL_2$ (S_f 20–60 fraction).

the perturbation in VLDL apoB kinetics as Intralipid was introduced mid-way through a turnover experiment. As tracer and tracee levels were both in steady state prior to giving Intralipid, we were able to determine the effect of infusion of the emulsion on both simultaneously.

The mean response of $VLDL_1$ and $VLDL_2$ apoB-100 and plasma triglycerides of the three subjects is shown in **Fig. 4.** The response of a typical subject is shown in **Fig. 5** for tracer content in $VLDL_1$ and $VLDL_2$ apoB and for tracee (lipoprotein apoB mass). In the early period of the turnover (up to 6 h), $VLDL_1$ apoB leucine content exhibited a plateau consistent with the fact that this lipoprotein has a rapid fractional synthesis rate, i.e., a short residence time (equal to the reciprocal of the FSR) (**Table 2**) and the plasma precursor enrichment being constant. $VLDL_2$ apoB tracer content was approaching a plateau value at 6 h. Intralipid administration caused an abrupt change. $VLDL_1$ apoB $^2H_3$-leucine content rose steeply while that of $VLDL_2$ apoB fell, although not as dramatically. Using the time interrupt facility in SAAM L(3,2) and L(0,2) were set to new low values as shown in Table 2. For the patient in Fig. 5 (MA), no other changes

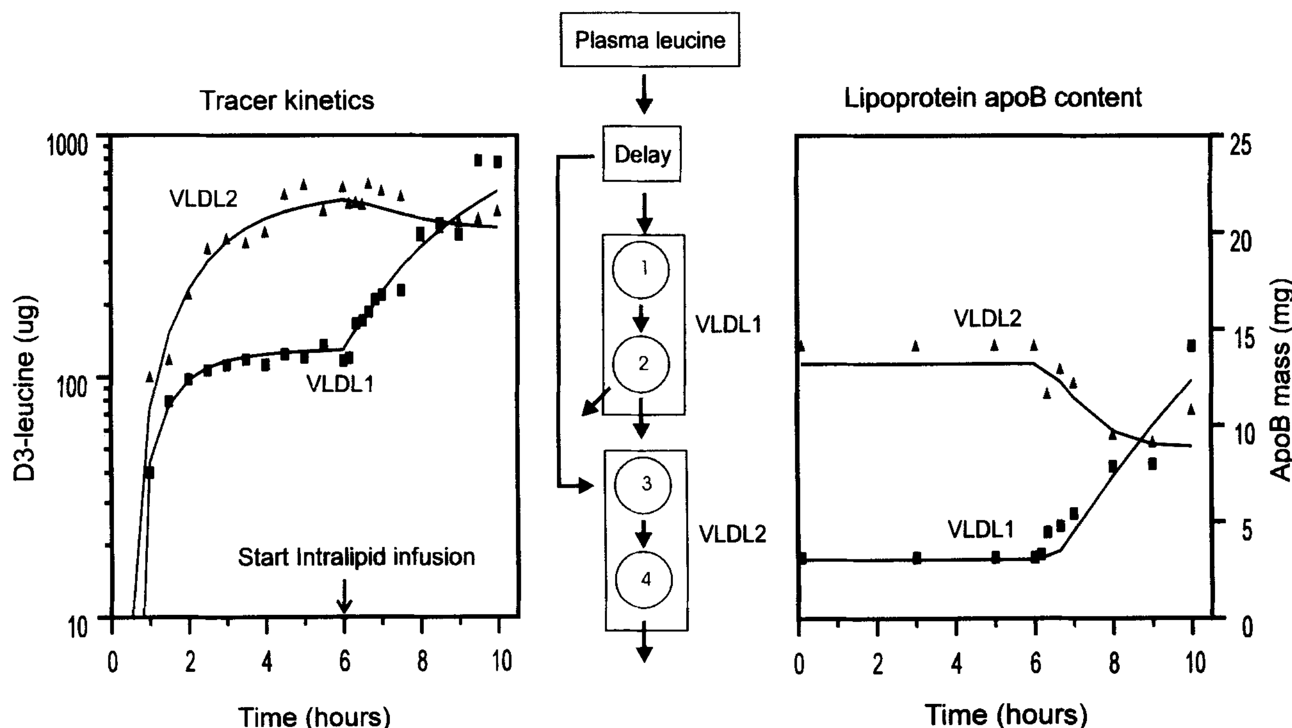

Fig. 5. Plasma [$^2H_3$]leucine-enrichment curves for apoB in large S_f 60-400 ($VLDL_1$), left panel, and small S_f 20-60 VLDL ($VLDL_2$), right panel, from subject MA. [$^2H_3$]-leucine was given as a small bolus (0.6 mg/kg) followed by a continuous infusion (0.6 mg/kg per h) for 10 h. After 6 h of [$^2H_3$]-leucine infusion, a bolus dose of Intralipid (0.15 g/kg) was given followed by a constant infusion of Intralipid (0.15 g/kg per h) for 4 h. The multicompartment model is shown in the middle. After a delay of 30 min, [$^2H_3$]-leucine entered $VLDL_1$ and $VLDL_2$ apoB. Input was from the delay compartment to compartment 1 in $VLDL_1$ and compartment 3 in $VLDL_2$. L(4,1) was set equal to L(3,2) and L(4,3) was set equal to L(0,4). As in the radioactivity model, some tracer was lost from $VLDL_1$ without going to $VLDL_2$, i.e., L(0,2). At 6 h, upon administration of Intralipid, a time interrupt was used to reduce L(3,2) by 98%. L(0,4) was left unchanged. These alterations produced a satisfactory fit to both the tracer data and the observed masses during the non-steady state.

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TABLE 2. Metabolism of VLDL apoB determined by kinetic studies using stable isotopes in three subjects undergoing infusion of a chylomicron-like triglyceride emulsion (Intralipid)

	VLDL$_1$ ApoB					VLDL$_2$ ApoB				
	Production	Initial	Residence	VLDL$_1$ to VLDL$_2$ Conversion[a]		Production	Initial	Residence	VLDL$_2$ Elimination Rate[b]	
Patient	Rate	Mass	Time	Before IL	During IL	Rate	Mass	Time	Before IL	During IL
	mg/h	*mg*	*h*	*pool/h*		*mg/h*	*mg*	*h*	*pool/h*	
MA	23.8	26.2	1.09	1.57	0.03	34.3	117.4	2.32	0.90	0.90
EH	21.6	23.4	1.08	2.29	0.30	57.1	45.3	1.11	3.81	3.81
PS	8.0	6.9	0.86	3.20	0.37	16.9	20.6	0.96	2.09	1.00

For explanation of how parameters were derived, see Figs. 4 and 5. The residence time of VLDL$_1$ or VLDL$_2$ apoB was the reciprocal of the fractional synthesis rate (equal to fractional catabolic rate) of lipoprotein determined during the initial 6-h steady state period. The values for L(3,2) and L(0,4) during Intralipid infusion were those that pertained after a "time interrupt" at 6 h into the [$^2H_3$]leucine infusion.
[a]L(3,2).
[b]L(0,4).

were necessary to fit both the tracer and tracee in the new, non-steady state. In the two other patients (EM and PS) a further alteration was required in that there was an observed virtually instantaneous increment in the VLDL$_1$ apoB tracer content upon giving Intralipid that could not be accounted for by simply reducing L(0,2) and L(3,2) to very low values (see dashed line at 6 h in **Fig. 6**). We postulated that Intralipid displaced VLDL$_1$ bound to capillary sites and this reduced the apparent volume of distribution of the tracer. When this facet was incorporated into the model (i.e., a 20% reduction in VLDL$_1$ volume of distribution in EH and a 30% reduction in PS), a satisfactory fit was obtained to observed tracer data. These figures were derived from the best fit of the tracer curves. The rates of VLDL1 apoB production in the 6-h steady state period are in agreement with values seen in a group of normal subjects studied previously (12). Administration of Intralipid was postulated to cause a block in VLDL$_1$ to VLDL$_2$ conversion as seen in the radioactive experiments above. When L(3,2) was

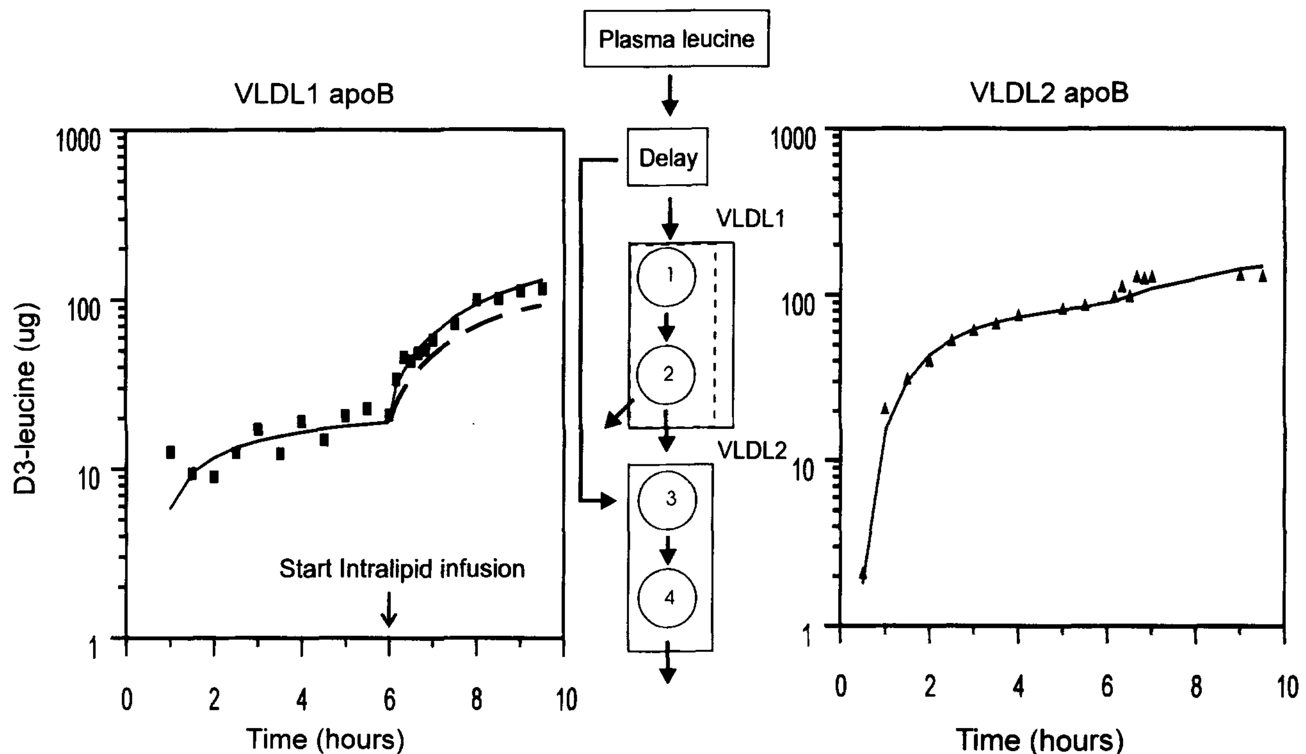

Fig. 6. Plasma [$^2H_3$]-leucine-enrichment curves for apoB in large S_f 60–400 (VLDL$_1$) from subject PS (left panel). [$^2H_3$]-leucine was given as a small bolus (0.6 mg/kg) followed by a continuous infusion (0.6 mg/kg per h) for 10 h. After 6 h of [$^2H_3$]-leucine infusion, a bolus dose of Intralipid (0.15 g/kg) followed by a constant infusion of Intralipid (0.15 g/kg per h) for 4 h. In order to fit the observed apoB mass, data for this subject after administration of Intralipid, L(3,2) was reduced by 88%, L(0,4) was reduced by around 50%, and the distribution volume of the VLDL$_1$ pool was reduced by 30% (middle). The [$^2H_3$]-leucine-enrichment curves for apoB in small S_f 20–60 VLDL (VLDL$_2$) are shown to the right. The dashed line shows the fit without correction for a change in the distribution volume.

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reduced to the low values seen in Table 2 at the 6-h time interrupt, both tracer and tracee curves produced satisfactory fits. An alternative hypothesis in which the L(3,2) was left unchanged and $VLDL_1$ apoB synthesis increased fivefold was tested. This failed to generate a fit to the observations (data not shown). Furthermore, it was not possible to fit observed data with the combination of increased $VLDL_1$ synthesis and a reduced L(3,2).

In subjects MA and EH (Table 2), $VLDL_2$ apoB tracer content and apoB mass declined during Intralipid infusion. However, in PS, $VLDL_2$ tracer content increased slightly (Fig. 6) and this was accounted for by setting L(0,4) 50% lower at the 6-h time interrupt (compared to the steady state value, Table 2). Data were not compatible with a partial block in L(3,2).

Influence of Intralipid on VLDL apoB-100 measurements

To determine whether the presence of Intralipid in plasma influenced the VLDL subfractionation or the determination of VLDL apoB-100, two aliquots of 13 ml of fasting plasma were incubated at 37°C for 60 min with either 234 μl 10% Intralipid or 228 μl distilled water and 6 μl glycerol (control sample). The volume of Intralipid added was calculated to correspond to a triglyceride elevation of approximately 2 mmol/l. Each incubate was then dispensed into three separate samples from which S_f 60–400 and S_f 20–60 VLDL were subsequently isolated for determination of the respective apoB-100 concentrations. The contents of S_f 60–400 and S_f 20–60 VLDL apoB-100 in the plasma incubated with Intralipid were 4.2 ± 1.1 and 10.1 ± 0.4 mg/l, respectively. The corresponding levels in the control samples were 4.5 ± 1.0 and 10.1 ± 0.5 mg/l, respectively. It could thus be concluded that the presence of Intralipid in plasma affected neither the VLDL subfractionation nor the measurements of large and small VLDL apoB-100.

DISCUSSION

VLDL particles are formed in the liver by coupling lipids to the structural protein, apoB-100. The rate of synthesis of VLDL depends on the amount of available lipid substrate rather than on the production of apoB (24). In fact, Sniderman and Cianflone (25) have suggested that substrate delivery to the liver is the major determinant of hepatic VLDL apoB-100 secretion. In plasma, large (S_f > 60) VLDL particles are then either eliminated as such or catabolized to smaller (S_f 20–60) remnants through lipolysis of triglycerides by LPL bound to the endothelium.

A recent study in which direct quantification of apoB-48 and apoB-100 was performed in fractions of triglyceride-rich lipoproteins has clearly demonstrated that the plasma concentration of large VLDL (S_f 60–400 apoB-100) increases after an oral fat load both in healthy subjects and in normo- and hypertriglyceridemic patients with CHD (3). Whether this is due to enhanced liver secretion, decreased removal from plasma, or to competition between large VLDL and chylomicrons for the same lipolytic pathway, or a combination thereof, could not be determined in that study. In the present study we used a short-term intravenous infusion of a triglyceride emulsion to specifically address the issue of competition for lipolysis between the chylomicron-like emulsion and VLDL. An acute threefold increase in plasma triglycerides was accompanied by a consistent linear increase of S_f 60–400 apoB-100 during the 60-min infusion of the chylomicron-like triglyceride emulsion. In contrast, the S_f 20–60 apoB-100 content decreased in most subjects. Clearly, the most likely explanation for the rise in S_f 60–400 VLDL is delayed lipolysis of the particle due to competition for the sites of LDL action. Recently, Karpe and Hultin have shown (26) that endogenous triglyceride-rich lipoproteins accumulate in rat plasma due to a failure to compete for a common lipolytic pathway with the chylomicron-like triglyceride emulsion used in the present experiments. This contention is strongly supported by the evidence of the kinetic studies reported here. First, the increase in $VLDL_1$ apoB was remarkably linear for a prolonged period (up to 4 h). The nature of the rise would have been predictably different, i.e., an early rapid rise followed by a new plateau after 2–3 h in $VLDL_1$ apoB tracer leucine content in the subjects shown in Figs. 5 and 6, if increased synthesis of $VLDL_1$ had been the underlying mechanism. Second, the increase in $VLDL_1$ apoB in the studies without the isotopes was apparent as early as 10 min after giving Intralipid and the increment was unaffected by doubling the dose of the emulsion. Third, direct measurement of $VLDL_1$ apoB clearance rates with a radioactive tracer showed a dramatic reduction in $VLDL_1$ to $VLDL_2$ conversion during Intralipid infusion. The stable isotope experiments were most informative. Not only did they allow for a quantitative assessment of $VLDL_1$ apoB production and removal but they also provided evidence for a reduction in the volume of distribution of $VLDL_1$ apoB during Intralipid administration. A possible explanation for this phenomenon is that large VLDL particles, which are known to be a good substrate for LPL, are transiently bound to the LPL attached to the endothelium. When a competing substrate for LPL appears in plasma, the marginalized VLDL may then be released, which, in turn, results in an apparent reduction in the distribution volume of the lipoprotein.

In the present study, Intralipid has been used as a chylomicron-like substance. Few studies have compared

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the metabolism of "true" chylomicrons with the metabolism of Intralipid. Intralipid triglycerides do, however, seem to be cleared almost as rapidly as chylomicron triglycerides (27, 28). From a compositional point of view, there are differences between Intralipid and chylomicrons. Intralipid is not a completely homogeneous emulsion as there is a surplus of phospholipids forming large bilayer-like structures, which together with the triglyceride emulsion particles bind apolipoprotein Cs and E (29). An alternative explanation of the present finding could therefore be that the presence of Intralipid depletes endogenous lipoproteins, i.e., VLDL of functional apolipoproteins. Hypothetically, a severe loss of apoC-II could reduce LPL-mediated lipolysis of VLDL and the accumulation of VLDL seen after Intralipid infusion could therefore be attributed to such an effect. Similarly, a sudden loss of VLDL-bound apoE could reduce LDL receptor-mediated uptake of VLDL.

Heterogeneity in the rate at which VLDL enters the plasma compartment may, indeed, have significant clinical implications. Overproduction of VLDL particles is, for instance, thought to be the underlying pathogenic mechanism in a majority of cases with familial combined hyperlipidemia (25), a condition that is present in a substantial proportion of patients with premature CHD (30). Unfortunately, simple methods to quantify VLDL production in clinical studies have been lacking so far. Because the block in the conversion of large to small VLDL was almost complete during Intralipid infusion, the rising plasma concentration after administration of Intralipid could be used for calculating the production rate of large VLDL. Using the present data, a linear regression between the total large VLDL apoB mass and time gave the following rates of accumulation in the three subjects who also participated in the kinetic study of VLDL using stable isotopes: 20.0, 25.6, and 7.2 mg/h. The corresponding production rates for large VLDL calculated from the stable isotope studies were 23.8, 21.6, and 8.0 mg/h.

In summary, use of a short-term intravenous infusion of a triglyceride emulsion in healthy men has clearly indicated that the considerable VLDL elevation observed in plasma during alimentary lipemia is secondary to competition between chylomicrons and VLDL for the same lipolytic pathway. The clinical corollary of this finding is that chylomicrons and their remnants might be implicated in atherogenesis by impeding the normal LPL-mediated catabolism of VLDL. Furthermore, repeated determinations of the accumulating VLDL apoB-100 in plasma during a short-term intravenous infusion of a triglyceride emulsion holds prospects for a simple method to measure the basal hepatic secretion of large VLDL.

This study was supported by grants from the Swedish Medical Research Council (8691), the Swedish Heart-Lung Foundation, the Marianne and Marcus Wallenberg Foundation, the King Gustaf V 80th Birthday Fund, the Professor Nanna Svartz' Fund, the Nordic Insulin Fund, the Swedish Margarine Industry Fund, and British Heart Foundation (PG/90124). Dr. Hamsten is Career Investigator of the Swedish Heart-Lung Foundation.

Manuscript received 21 June 1995 and in revised form 9 October 1995.

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