In vivo lipoprotein kinetic study

Experimental protocol

Food intake, with a leucine poor diet (1700 Kcal.day\(^{-1}\), 55% carbohydrates, 39% fats and 7% proteins), was fractionated in small equal portions which were provided every 2 hours, starting 6 hours prior to the tracer infusion up to the end of the study, in order to avoid important variations in apolipoprotein plasma concentration, as previously performed by our group\(^1\) and others\(^2\). To determine the kinetic of apoB100, the subjects received an intravenous injection of a 0.7 mg.kg\(^{-1}\) bolus of L-[\(^{13}\)C]leucine (99% \(^{13}\)C, Eurisotop, Saint Aubin, France) immediately followed by a 16-hour constant infusion at 0.7 mg.kg\(^{-1}\).h\(^{-1}\). Blood samples were collected at hours 0, 0.25, 0.75, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 14, 15 and 16. Serum was separated by centrifugation and stored at -80°C.

Isolation of apo-B

VLDL (density (d) < 1.006 g.ml\(^{-1}\)), IDL (1.006 < d< 1.019) and LDL (1.019 < d < 1.063) were isolated from plasma by sequential flotation ultracentrifugation, using a 50.4 rotor in a L7 apparatus (Beckman Instruments, Palo Alto, USA). IDL and LDL fractions were then dialyzed against a 10 mmol.l\(^{-1}\) ammonium bicarbonate buffer pH 8.2 containing 0.01% EDTA and 0.013% sodium azide. VLDL, IDL and LDL fractions were delipidated with diethylether-ethanol and apoB100 from each lipoprotein fraction was isolated by preparative sodium dodecyl sulfate-polyacrylamide gel electrophoresis (3%). After staining with Coomassie blue R-250, apoB100 bands were excised from the polyacrylamide gels and hydrolyzed in 6 M HCl at 110°C for 16 h under nitrogen vacuum. Samples were then centrifuged to remove polyacrylamide. Supernatants were then lyophilized in a Speed Vac (Savant Instrument, Farmingdale, New York, USA). Lyophilized samples were dissolved in 50% acetic acid and applied to an AG-50W-X8 200-400 mesh cation exchange resin (Bio-
Rad, Richmond, USA) and aminoacids were recovered by elution with 4N NH₄OH.

**Modeling**

$^{13}$C leucine enrichment was initially expressed in delta ‰ and converted in tracer/tracee ratio prior to modelling. The data were analyzed with the Simulation Analysis And Modeling (SAAM) II program (SAAM Institute, Inc., Seattle, WA) using a multicompartmental model. The model chosen to describe the data is shown in Figure 1 and has already been used by others for apoB kinetic studies performed with stable isotope constant infusion. Development of compartmental models in stable isotope experiment has been previously described in details. A forcing function was used to drive the appearance of leucine tracer into the different lipoprotein fractions. Because labeling of intracellular and plasma leucine is not always similar, we used VLDL-apoB100 plateau enrichment as the forcing function to estimate the intrahepatic leucine precursor pool. The delay compartment accounted for the time required for the synthesis and secretion of apoB100 into the plasma. Plasma VLDL-apoB100 kinetic data are represented by compartments 1 and 2, and plasma IDL-apoB100 kinetic data are represented by compartments 11 and 12. Compartment 21 represents plasma LDL apoB100. Compartments 2 and 12 turned over more slowly than did compartments 1 and 11, respectively. These compartments were required for optimal model fit of the data.

As the experiment was performed in the steady state, fractional synthetic rate equaled fractional catabolic rate (FCR).

- Direct VLDL-apoB100 FCR and indirect VLDL-apoB100 FCR from VLDL to IDL or LDL, expressed in pool.day$^{-1}$ were calculated as follows:
  - Direct VLDL-apoB100 FCR = $M_2 k(0,2)/(M_1 + M_2)$
  - Indirect VLDL-apoB100 FCR→IDL/LDL = $M_1[k(11,1) + k(21,1)]/(M_1 + M_2)$
where $k(i,j)$ is the fractional transfer coefficient from compartment $j$ to $i$, and $M_j$ represents the apoB mass (expressed as concentration per liter of plasma) of compartment $j$.

Total VLDL-apoB100 FCR is the sum of direct VLDL-apoB100 FCR and indirect VLDL-apoB100 FCR→IDL/LDL.

- Direct IDL-apoB100 FCR and indirect IDL-apoB100 FCR from IDL to LDL were calculated as follows:
  - Direct IDL-apoB100 FCR = $M_{12} k(0,12)/(M_{11} + M_{12})$
  - Indirect IDL-apoB100 FCR→LDL = $M_{11}k(21,11)/( M_{11} + M_{12})$

Total IDL-apoB100 FCR is the sum of direct IDL-apoB100 FCR and indirect IDL-apoB100 FCR→LDL.

- LDL-apoB100 FCR = $k(0,21)$

Production rates (PR) of the apoB100 in each lipoprotein fraction were normalized to body weight and calculated as follows:

$$PR = \text{apoB100 FCR (for each lipoprotein fraction)} \times \text{apoB100 pool size /body weight},$$

where apoB100 pool size is calculated by multiplying the apoB100 concentration, in the lipoprotein fraction (VLDL, IDL or LDL) by the estimated plasma volume (4.5% of body weight). In obese subjects (BMI ≥ 30), a correction of plasma volume was performed as previously reported by many authors $^7,^8$. The plasma volume was modified by multiplying by a correction factor to take into account the decrease in relative plasma volume associated with an increase in body weight $^8$.

The Akaike Information Criterion (AIC) was used to compare different models and the model with the lowest AIC value was chosen. Moreover, the physiological plausibility of the model has been verified $^9$. The goodness of fit of the model was assessed by the analysis of the residuals with the runs test $^5$. 


