Eur J Pediatr (1997) 156 [Suppl 1]: S75–S77

T. Demant · C. J. Packard

Studies of apolipoprotein B-100 metabolism using radiotracers and stable isotopes

Abstract Apolipoprotein B metabolism can be investigated in-vivo either by exogenous radiolabeling of preformed lipoproteins or by endogenous labelling of de-novo synthesized apo B using a stable isotope substituted amino acid tracer. The potential of both methods and results obtained by in-vivo studies in genetically determined dys- or hyperlipidaemic subjects will be discussed.

Key words Apolipoprotein B · Lipoprotein metabolism · Stable isotope kinetics · Turnover study

Abbreviations *Apo B* apolipoprotein B · *Apo E* apolipoprotein E · *IDL* intermediate density lipoprotein · *LDL* low density lipoprotein · *VLDL* very low density lipoprotein

Apolipoprotein B is the main protein component of the atherogenic lipoproteins in humans. Cell culture studies and molecular biology techniques as applied extensively in recent years are crucial for the identification of the principal structures and mechanisms which govern apo B metabolism. However, the complex interactions of the full array of different metabolic regulators cannot be investigated by a reductionist approach. In contrast, in-vivo studies involving the organism as a whole are necessary for the quantitative analysis of metabolic processes which result from the interplay of a multitude of different metabolic principles.

Very-low-density lipoproteins (VLDL), which comprise large triglyceride-rich $VLDL_1$ and smaller, relatively cholesterylester-rich $VLDL_2$ particles are secreted from the liver into the plasma compartment. Here they are either gradually delipidated to form intermediate-density lipoprotein (IDL) and eventually low-density lipoprotein (LDL), or they are cleared from the circulation by specific binding to cell surface receptors such as the remnant receptor or the LDL receptor. For the in-vivo investigation of apo B metabolism basically two study protocols are available. Lipoproteins can be isolated from plasma, tracelabelled with a radioactive substance such as radioiodine and re-injected into the donor subject in order to follow their disappearance from the plasma compartment. Alternatively, a stable isotope labelled amino acid, e.g. d_3-leucine or d_5-phenylalanine, is injected, either as a bolus or as a primed constant infusion, and subsequently serves as an endogenous label of de-novo synthesised apo B protein. In either case, multiple plasma samples are collected during the 12 h following tracer injection and daily thereafter in the fasting state for another 10–14 days. From these samples four apo B-containing lipoproteins, i.e. $VLDL_1$, $VLDL_2$, IDL and LDL, are prepared by cumulative gradient ultracentrifugation. Apo B is isolated by selective precipitation and, in the case of radioactive tracer studies, specific activity is measured directly, or, where stable isotope labelling is used, apo B is hydrolysed and tracer/tracee ratios are determined by gaschromatography mass spectrometry [1]. Rates of production, delipidation and catabolism of apo B-containing lipoproteins can be calculated from observed tracer kinetics by use of a multicompartmental model of lipoprotein metabolism, as depicted in Figs. 1 A, B, and an appropriate computer programme dedicated to kinetic analysis and metabolic modelling [2].

Differentially labelled radio-iodinated $VLDL_1$ and $VLDL_2$ tracers have been used in turnover studies in a number of patients suffering from different genetically determined disorders of apo B metabolism. In a study of three subjects with inherited lipoprotein lipase deficiency [3] it could be shown that the delipidation of $VLDL_1$ was reduced to about 10% of normal. In line with recent in-vitro findings [4] it also became apparent that direct catabolism of $VLDL_1$ was impaired in these subjects point-

PD Dr. T. Demant (✉)
Institut für Klinische Chemie, Klinikum Großhadern,
Marchioninistr. 15, 81377 München, Germany

Prof. C. J. Packard
Department of Pathology and Biochemistry,
Royal Infirmary NHS Trust, Glasgow, G31 2ER, UK

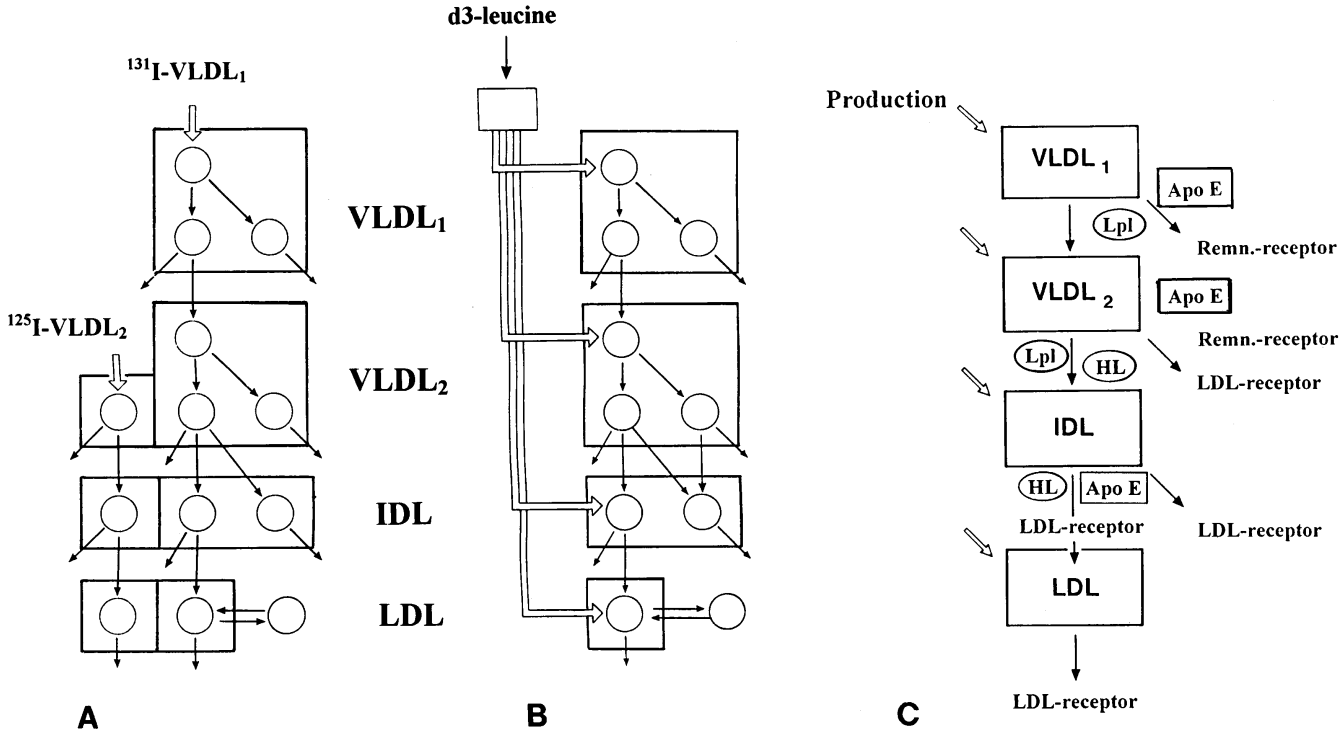

Fig. 1 Model of apo B metabolism. **A** model used for analysis of radioactive VLDL turnover studies, **B** model used for the analysis of stable isotope data and **C** model of apo B metabolism indicating the sites of action of apolipoproteins, lipolytic enzymes and lipoprotein receptors

ing at a role of lipoprotein lipase in the binding of $VLDL_1$ prior to receptor-mediated uptake and degradation. IDL and LDL, on the other hand, were rapidly catabolised, probably due to LDL-receptor independent mechanisms activated in the presence of severe hypertriglyceridaemia. VLDL tracer kinetics in a subject with an inherited defect of hepatic lipase were distinctly different from the above [5]. Whereas $VLDL_1$ metabolism was basically normal, $VLDL_2$ transer into IDL was reduced (–65%) and IDL to LDL transfer was almost entirely blocked. The two studies together confirmed a complementary activity of both lipolytic enzymes which previously had been demonstrated only in cell-free in-vitro systems. In a third study we investigated a group of five patients homozygous for familial hypercholesterolaemia, a condition characterised by the absence of functional LDL receptors [6]. In these subjects LDL-cholesterol levels were 330–579 mg/dl, 3–5 times above normal. Again $VLDL_1$ delipidation and catabolism were unimpaired but further delipidation of $VLDL_2$ and IDL as well as direct catabolism of IDL and LDL were significantly reduced, implying a dual function of the LDL receptor which not only mediates cellular uptake of IDL and LDL but also enhances delipidation at the bottom end of the VLDL to LDL delipidation cascade. Finally, we investigated the impact of the apo E polymorphism on the metabolism of apo B-containing lipoproteins [7]. Many epidemiological studies provide evidence that the apo E-2 isoform which is present in a heterozygous or homozygous form in about 10%–15% of the population and which does not bind to the LDL receptor is associated with a 10%–15% decrease of plasma and LDL-cholesterol levels while the apo E-4 isoform present in about 20%–25% is correlated with a 5%–10% LDL-cholesterol increase. In order to elucidate the underlying metabolic mechanisms, VLDL turnovers were conducted in 14 normolipidaemic subjects homozygous for either apo E-2, apo E-3 or the common wild-type allele apo E-3. It turned out that in apo E-2 homozygotes, $VLDL_1$ and $VLDL_2$ were cleared more slowly and that again the generation of LDL from IDL precursors was diminished while direct catabolism of IDL was enhanced. In apo E-4 homozygotes, on the other hand, the only significant difference in comparison with apo E3 controls was a reduced fractional catabolic rate for LDL.

Taken together these findings provide a better understanding of how lipolytic enzymes, apolipoproteins and lipoprotein receptors interact in the metabolic processing of apo B-containing lipoproteins (Fig. 1 C). The results can be summarised as follows:

1. $VLDL_1$ metabolism is largely dependent on lipoprotein lipase and functional apo E but independent of the LDL receptor route.
2. $VLDL_2$ is cleared from the plasma compartment partly through an apo E dependent receptor and partly through the LDL receptor. Further delipidation is mediated by both lipoprotein lipase and hepatic lipase.
3. IDL is eliminated from plasma by interaction with the LDL receptor, further delipidation is critically dependent on the action of hepatic lipase.
4. Hepatic lipase and LDL receptors, both present on the surface of hepatic sinusoidal cells, seem to co-operate

with functional apo E in the generation of LDL from IDL precursors.

5. Lower LDL and apo B levels in apo E-2 homozygotes result from a faster catabolism of IDL and an impaired IDL to LDL transfer. Higher apo B concentrations in apo-E4 subjects are caused by a delay in LDL catabolism.

Recently, we have used stable isotope substituted multiple labelled amino acids such as d_3-leucine or d_5-phenylalanine for studies of apo B metabolism in normolipidaemic subjects [8]. Tracer/tracee ratios were analysed using the metabolic model shown in Fig. 1 B. Rates for production, transfer and catabolism for $VLDL_1$, $VLDL_2$, IDL and LDL were in good agreement with the rates determined in the previous studies based on teh radio-iodine VLDL turnover technique. Since the incorporation of a labelled amino acid occurs in all newly synthesised proteins, the stable isotope approach allows for the direct determination of production rates across the whole density range of apo B-containing lipoproteins. This is in contrast to radio-tracer studies where synthetic rates can only be inferred from steady state conditions for the tracee molecules. It also means that in the same tracer study not only apo B but also any other protein which is built and then released into the plasma compartment can be investigated. This has been demonstrated specifically for apo B in conjunction with albumin in a subject suffering from the nephrotic syndrome [9]. The fact that stable isotopes are free of any radiation hazards makes this approach particularly attractive for metabolic studies in paediatric patients.

References

1. Demant T, Packard CJ, Stewart P, Bedynek A, Calder AG, Shepherd J, Seidel D (1994) Sensitive mass spectrometry techniques for the measurement of human apolipoprotein B metabolism in-vivo. Clin Chem 40 : 1825–1827
2. Packard CJ, Gaw A, Demant T, Shepherd J (1995) Development and application of a multicompartmental model to study very low density lipoprotein subfraction metabolism. J Lipid Res 36 : 172–187
3. Demant T, Gaw A, Watts GF, Durrington P, Buckley B, Imrie CW, Wilson C, Packard CJ, Shepherd J (1993) Metabolism of apo B-100 containing lipoproteins in familial hyperchylomicronemia. J Lipid Res 34 : 147–156
4. Beisiegel U, Weber W, Bengtsson-Olivecrona G (1991) Lipoprotein lipase enhances the binding of chylomicrons to low density lipoprotein receptor-related protein. Proc Natl Acad Sci USA 88 : 8342–8346
5. Demant T, Carlson LA, Holmquist L, Karpe F, Nilsson-Ehle P, Shepherd J (1988) Lipoprotein metabolism in hepatic lipase deficiency: studies on the turnover of apolipoprotein B and on the effect of hepatic lipase on high density lipoprotein. J Lipid Res 29 : 1603–1611
6. James RW, Martin B, Pometta D, Fruchart JC, Duriez P, Puchois P, Farriaux JP, Taquet A, Demant T, Clegg RG, Munro A, Oliver MF, Packard CJ, Shepherd J (1989) Apolipoprotein B metabolism in homozygous familial hypercholesterolemia. J Lipid Res 30 : 159–169
7. Demant T, Bedford D, Packard CJ, Shepherd J (1991) Influence of apolipoprotein E polymorphism on apolipoprotein B-100 metabolism in normolipemic subjects. J Clin Invest 88 : 1490–1501
8. Demant T, Packard CJ, Demmelmair H, Stewart P, Bedynek A, Bedford D, Seidel D, Shepherd J (1996) Sensitive methods to study human apolipoprotein B metabolism using stable isotope-labeled amino acids. Am J Physiol 270 : E1022–E1036
9. Demant T, Mathes C, Bedynek A, Bosch T, Seidel D (1995) Simultanous measurement o apolipoprotein B and albumin metabolism using gas chromatography – mass spectrometry and multicompartmental data analysis. Eur J Clin Invest 33 : A26