Lipoprotein transport in the metabolic syndrome: methodological aspects of stable isotope kinetic studies

Dick C. CHAN, P. Hugh R. BARRETT and Gerald F. WATTS
Lipoprotein Research Unit, School of Medicine and Pharmacology, University of Western Australia and The Western Australian Institute for Medical Research, Perth, WA 6847, Australia

ABSTRACT

The metabolic syndrome encapsulates visceral obesity, insulin resistance, diabetes, hypertension and dyslipidaemia. Dyslipidaemia is a cardinal feature of the metabolic syndrome that accelerates the risk of cardiovascular disease. It is usually characterized by high plasma concentrations of triacylglycerol (triglyceride)-rich and apoB (apolipoprotein B)-containing lipoproteins, with depressed concentrations of HDL (high-density lipoprotein). However, lipoprotein metabolism is complex and abnormal plasma concentrations can result from alterations in the rates of production and/or catabolism of these lipoprotein particles. Our in vivo understanding of kinetic defects in lipoprotein metabolism in the metabolic syndrome has been achieved chiefly by ongoing developments in the use of stable isotope tracers and mathematical modelling. This review deals with the methodological aspects of stable isotope kinetic studies. The design of in vivo turnover studies requires considerations related to stable isotope tracer administration, duration of sampling protocol and interpretation of tracer data, all of which are critically dependent on the kinetic properties of the lipoproteins under investigation. Such models provide novel insight that further understanding of metabolic disorders and effects of treatments. Future investigations of the pathophysiology and therapy of the dyslipoproteinemia of the metabolic syndrome will require the development of novel kinetic methodologies. Specifically, new stable isotope techniques are required for investigating in vivo the turnover of the HDL subpopulation of particles, as well as the cellular efflux of cholesterol into the extracellular space and its subsequent transport in plasma and metabolic fate in the liver.

INTRODUCTION

The concurrence of visceral obesity, insulin resistance, dyslipidaemia, hypertension and a pro-inflammatory/thrombotic state within an individual has generated the concept of the metabolic syndrome [1,2]. Epidemiological studies have shown that individuals with the metabolic syndrome have a 3-fold increase in CVD (cardiovascular disease) and a significant increase in cardiovascular mortality [3–6]. A recent estimate of the prevalence of the metabolic syndrome suggests that 25% of adults in the United States have this condition [7,8]. It increases with age in both sexes, and the majority of subjects do not have diabetes [7,9].
The metabolic syndrome has now been described in several ethnic groups, the prevalence being particularly high in Asian Indians and certain minority groups, such as Indigenous Australians [10,11]. In recognition of its clinical importance, the recent NCEP (National Cholesterol Education Program) Adult Treatment Panel III has identified the metabolic syndrome as an important therapeutic target for the management of CVD.

Dyslipidaemia is a cardinal feature of the metabolic syndrome that accelerates the risk of cardiovascular disease [12,13]. Key mechanistic advances in our understanding of this type of dyslipidaemia have recently been made with the use of stable isotope tracers and mathematical modelling. The present review focuses on the principles and use of these methods in investigating lipoprotein metabolism in vivo, and the accompanying review [13a] deals with the application of these methods to the study of the pathophysiology and therapeutic regulation of lipoprotein transport in the metabolic syndrome.

**DEFINING THE METABOLIC SYNDROME: IMPORTANCE OF DYSLIPIDAEMIA**

The concept of the metabolic syndrome as a cluster of cardiovascular risk factors, including central obesity, insulin resistance, hypertension and dyslipidaemia, was introduced in the late 1980s. Thereafter, several descriptions, including insulin-resistance syndrome, Reaven’s syndrome, syndrome X and ‘the deadly quartet’ have been used to refer to this nexus of abnormalities [14,15]. To aid research and clinical practice, several definitions of the metabolic syndrome have been proposed by various expert and authoritative groups (Table 1) [1,2,9]. The prevalence of the metabolic syndrome increases with advancing age and its development is positively related to childhood obesity [16].

Dyslipidaemia, specifically high plasma triacylglycerols (triglycerides) and low HDL (high-density lipoprotein)-cholesterol concentrations, is a common feature of all the definitions given in Table 1. The prevalence of the metabolic syndrome, including dyslipidaemia, increases from normal, through IGT (impaired glucose tolerance) to frank diabetes mellitus [4,13]. However, within populations, most subjects with the metabolic syndrome defined by whatever criterion have normal or IGT and are not diabetic [4]. The metabolic syndrome is on average present in 50% of patients with clinical manifest atherosclerotic vascular disease [17]. Dyslipidaemia is a simplified description for the, so-called, ‘atherogenic lipoprotein triad’ characterized by accumulation of triacylglycerol-rich lipoproteins and small dense LDL (low-density lipoprotein) particles with depressed HDL-cholesterol in plasma [18,19]. Accordingly, dyslipidaemia has been shown to be a major independent risk factor for CVD and stroke in the metabolic syndrome, including Type II diabetes mellitus [6,13,20]. Observational data in different populations employing factor analysis have also underlined that dyslipidaemia tends to co-express in the syndrome with insulin resistance and diabetes [21,22].

**BRIEF OVERVIEW OF LIPOPROTEIN PHYSIOLOGY**

Lipoprotein physiology has been reviewed in detail elsewhere [23,24], and is critical to understanding the dyslipidaemia of the metabolic syndrome and the concepts presented and discussed in the reviews. Briefly, in the exogenous pathway, dietary esterified triacylglycerols and cholesterol are combined with apo (apolipoprotein) B-48 [25], phospholipid and free cholesterol to produce nascent chylomicrons in the enterocyte. Chylomicron particles are then secreted into lymphatics and, via the thoracic duct, flow into the circulation where they acquire apoE and apoC from other lipoproteins [26]. Triacylglycerols are then removed from chylomicrons by the action of the enzyme LPL (lipoprotein lipase). Chylomicron remnants are then removed from the circulation by hepatic apoE/apoB receptors.

### Table 1 Clinical definitions of the metabolic syndrome

<table>
<thead>
<tr>
<th>Variable</th>
<th>WHO*</th>
<th>NCEP†</th>
<th>EGIR‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin resistance</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>IGT/IFG/diabetes</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Obesity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMI or WC/WHR</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>WHR &gt; 0.90</td>
<td>WC &gt; 102 cm</td>
<td>WC &gt; 94 cm</td>
</tr>
<tr>
<td>Women</td>
<td>WHR &gt; 0.85</td>
<td>WC &gt; 88 cm</td>
<td>WC &gt; 80 cm</td>
</tr>
<tr>
<td>Dyslipidaemia</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Triacylglycerol</td>
<td>&gt; 1.7 mmol/l</td>
<td>&gt; 1.7 mmol/l</td>
<td>&gt; 2.0 mmol/l</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>&lt; 0.9 mmol/l</td>
<td>&lt; 0.9 mmol/l</td>
<td>&lt; 1.0 mmol/l</td>
</tr>
<tr>
<td>Blood pressure</td>
<td>&gt; 140/90 mmHg</td>
<td>&gt; 130/85 mmHg</td>
<td>&gt; 140/90 mmHg</td>
</tr>
<tr>
<td>Microalbuminuria (albumin excretion rate)</td>
<td>&gt; 20 µg/min</td>
<td></td>
<td></td>
</tr>
</tbody>
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* The WHO defines the metabolic syndrome as the presence of ≥ 2 of the variables in addition to Type II diabetes, impaired glucose tolerance or insulin-resistance (fasting insulin level >75 percentile for the non-diabetic population).
† The NCEP guidelines define the metabolic syndrome as the presence of ≥ 3 or more of the variables.
‡ The EGIR defines the metabolic syndrome for non-diabetic individuals as the presence of ≥ 2 of the variables in addition to insulin-resistance (defined as a fasting insulin or HOMA (homeostasis model assessment) score >75 percentile for the reference population).
In the endogenous pathway, VLDL (very-LDL) transports hepatically synthesized triacylglycerols and cholesterol in the circulation to extrahepatic tissues [27]. Nascent VLDL contains one molecule of apoB-100 per particle, and acquires cholesterol esters, apoC and apoE, from HDL. VLDLs differ in size according to their triacylglycerol content. The larger VLDL species are lipolysed by LPL, and most of the remnant particles formed by this process are removed directly by the liver. Some larger VLDL species are, however, subjected to further LPL-mediated lipolysis, resulting in the formation of small dense LDL particles. Smaller VLDL species are the major direct precursors of LDL particles [28–30]. The lipolysis of VLDL is facilitated by the apoC-II and inhibited by the apoC-III content of the lipoprotein particle [31]. IDL (intermediate-density lipoprotein), sometimes termed VLDL remnant, is a transient intermediate in the delipidation cascade from VLDL to LDL. IDLs are lipolysed by hepatic lipase, converting into LDL, or taken up by the liver via ligand interaction of its apoE component with the LDL receptor and/or LRP (LDL-related protein) [32]. LDL is the major carrier of cholesterol in the circulation. The cellular uptake and degradation of LDL particles is facilitated by a saturable mechanism involving the interaction of the apoB-100 component of LDL with LDL receptors located on plasma membranes [33].

As shown in Figure 1, apoB-100 is the main structural protein that remains associated with VLDL, IDL and LDL particles through their transport in the plasma. Hence apoB-100 is a suitable marker for tracing the metabolism of these lipoprotein particles. This is a fundamental principle employed in studies of the metabolism of the apoB-containing lipoproteins based on the use of stable isotopically labelled amino acid precursors.

In humans, excess cholesterol from peripheral tissue is transported back to the liver by a process called RCT (reverse cholesterol transport) [34]. Briefly, discoidal apoA-I discs are produced by enterocytes and hepatocytes or dissociate from triacylglycerol-rich lipoproteins following lipolysis by LPL. The lipid-poor apoA-I-containing particles interact with peripheral cells and acquire phospholipids and free cholesterol through ABCA1 (ATP-binding cassette protein A1), and possibly by SR-B1 (scavenger receptor B1) [35]. PLTP (phospholipid transfer protein) also facilitates the first step of RCT by transferring phospholipids from cellular membranes and lipoprotein surfaces to form pre-β-HDLs [36]. Once associated with nascent HDL, free cholesterol is esterified by the enzyme LCAT.
(lecithin:cholesterol acyltransferase). The nascent HDL (pre-β HDLs) particles are converted into spherical small dense HDL_{3}. The maturation of HDL_{3} particles requires further acquisition of free cholesterol and subsequent cholesterol esterification converts HDL_{3} into larger less dense cholesterol-rich HDL_{2} particles. The majority of free cholesterol in HDL can be taken up selectively by the liver through the action of SR-B1 [37]. Cholesteryl ester can also be selectively transferred to apoB-containing lipoprotein in exchange for triacylglycerols through the action of CETP (cholesteryl ester transfer protein).

In contrast with apoB-100 in VLDL, IDL and LDL, apoA-I and apoA-II do not remain permanently associated with the HDL particle. Nevertheless, as illustrated later, with the assistance of stable isotopically labelled amino acids and modelling methods, studies of the kinetic behaviour of these two apoproteins can provide valuable insight into the subtleties of HDL metabolism in vivo.

**METHODOLOGICAL ASPECTS OF TRACER STUDIES OF LIPOPROTEIN METABOLISM**

Measurements of plasma lipid and lipoprotein concentrations are traditionally employed to characterize disorders of lipoprotein metabolism. However, lipoprotein metabolism is complex and abnormal plasma concentrations can result from alterations in the rates of production and/or catabolism of the various lipoprotein particles. Static measures of either lipid or lipoprotein concentrations do not provide information on the underlying pathogenic mechanisms. Tracer studies, whether utilizing radioactive or stable isotopes, provide data from which mechanistic models can be developed and tested against experimental data. Such models provide novel insight providing further understanding of metabolic disorders and effects of treatments [38,39]. With advances in GC–MS (gas chromatography–mass spectrometry) technology and widespread availability of inexpensive stable isotopes, the endogenous labelling of apoB with amino acid precursor molecules has, of late, been increasingly employed in human metabolic studies [40–48]. To understand how this approach has furthered our knowledge of dyslipoproteinaemia in the metabolic syndrome, we now review in more detail the mathematical and biochemical principles involved.

**Principles of tracer methodology**

**Terms and conditions**

The term ‘trace’ is defined as the substance of interest, for example apoB, to be traced kinetically. A ‘tracer’ is a labelled substance, for example stable isotopically labelled leucine, introduced into the ‘system’ to infer information about the kinetics of the tracee. ‘System’ refers to a biological construct in which the substance or material under investigation exists, including whole body, organ, cellular or subcellular. For the purpose of lipoprotein studies, the system should be maintained in a steady state, where the rates of input and output for a given unlabelled tracee substance are equal and time-invariant. To validate its use in kinetic studies, a tracer should have the following properties: (i) be physically, chemically and biologically identical with the tracee, (ii) be quantitatively detectable by the observer, and (iii) should not perturb the system under investigation [49].

**Data analysis**

Tracer data generated in lipoprotein metabolism studies can be analysed using a variety of methodologies. However, with increasingly complex experimental protocols, compartmental analysis is being used more frequently as a technique to dissect the complexities of the system under investigation. A compartment is defined as an amount of material that is well mixed, kinetically homogeneous and distinct from other material in the system. A compartment model is a collection of compartments, connected in a specific structure to permit the movement of material among the compartments [50]. When an endogenously incorporated tracer, such as [2H3]leucine, is injected into the system, it is incorporated into molecules under investigation and labels the components of a system with a stable isotope tracer or tag. The tracer enables the dynamic fate of the latter to be traced through its metabolic pathways. The movement of material in the system can be described by a set of differential equations, thereby allowing quantitative information on the system to be estimated. When considering the development of a system model, for example apoB metabolism, a simple compartmental model may not describe adequately the kinetic heterogeneity present within the system. Hence a more complex compartmental model should be constructed to account for the increased information content of the data. A model that is overly complex, on the other hand, will not be supported by the experimental data and, hence, will have little predictive value [51]. More detailed reviews of the mathematical principles of kinetic modelling have been published elsewhere [50,51].

**Tracers**

From the earlier description of lipoprotein physiology, it is understood that lipoproteins can be labelled in their lipid and/or protein components. Amino acids labelled with stable isotopes have chiefly been used as tracers for the study of lipoprotein metabolism for more than a decade. The term stable isotope refers to a non-radioactive isotope of a given atom that is present in a molecule in a biological system at a lesser abundance than the lightest naturally occurring isotope. The most commonly used stable isotopes for metabolic tracer studies are 2H, 13C, 15N and 18O. The molecules under investigation are chemically identical, but differ in their molecular mass;
Figure 2 Principle of endogenous labelling of apoB-100 with an amino acid labelled with a stable isotope and detection by GC–MS

Newly synthesized apoB-100 is incorporated with labelled (●) and unlabelled (○) amino acids, d3-leucine, [2H3]leucine.

that is they are, so-called, mass isotopomers. The choice of a stable isotopically labelled amino acid for a metabolic study should be based upon knowledge of metabolism, abundance in the protein of interest and the availability and cost of the label [50]. However, the selection of the amino acid tracer used for endogenous labelling does not appear to affect the estimation of lipoprotein kinetic parameters [52]. Figure 2 summarizes the principle of endogenous labelling of apoB-100, as an example, with an amino acid labelled with a stable isotope ([2H3]leucine) and analysis by GC–MS. [2H3]Leucine infused into the circulation is taken up by the liver and incorporated into the apoB-100 molecule (and all other hepatically synthesized proteins).

In addition to apo turnover, stable isotopes and GC–MS may be employed to assess the kinetics of triacylglycerol, cholesterol and fatty acids [53–55]. Although fewer studies of this type have been reported to date, they have provided new insight into other aspects of the regulation of lipid and lipoprotein metabolism. The stable isotopes employed are the following: [3H5]glycerol and [13C]palmitate for VLDL-triacylglycerol metabolism; [1,2-13C]acetate and [13C]palmitate for fatty acid metabolism and hepatic lipogenesis; [13C5]cholesterol and [2H4]cholesterol for intestinal cholesterol absorption [56]; and [13C]acetate for assessing RCT [57].

Tracer administration: clinical protocols

A tracer can be administered intravenously as either a bolus injection or a primed constant infusion. Historically, the primed constant infusion approach has been used because the analysis of tracer data appeared simpler [40]. Theoretically, the bolus administration of tracer is better than the primed constant infusion, because the tracer data display greater dynamics by virtue of the pulse administration of the tracer [58]. The primed constant infusion is suitable for lipoproteins with rapid turnover, such as VLDL. However, it requires a longer time to achieve a plateau of isotopic enrichment in lipoproteins with a low rate of turnover, such as LDL apoB and HDL apoA-I. This limits the reliable estimation of lipoprotein kinetic parameters, especially when assumptions about the plateau of isotopic enrichment have to be made in studies lasting less than 15 h. Moreover, lipoprotein kinetic studies of only 12–15 h produce limited kinetic information and thus limit the type of analysis employed to interpret the tracer data and derive kinetic parameters [59]. These studies therefore have limited ability to observe and model kinetic heterogeneity. On the other hand, long infusion protocols have the disadvantage of tracer recycling, although this can, to some extent, be accounted for in modelling the tracer data. Hence we consider that a single bolus injection of tracer is more suitable to the study of lipoprotein metabolism, including lipoproteins with a slow rate of turnover, such as apoB in LDL and apoA-I/apoA-II in HDL. The bolus dose also makes it easier to measure the interconversion of lipoprotein particles; it may also facilitate the determination of newly synthesized particles, because the intracellular precursor enrichment is greater at the start of the study. Moreover, the clinical protocol of the bolus approach is more convenient for both subjects and researchers. The bolus administration of tracer has also been widely employed to study lipid kinetics, such as triacylglycerol, cholesterol and fatty acid metabolism. Some stable isotopes may also be taken orally, a good example of which is the use of [2H5]cholesterol to study cholesterol absorption [56].

Volunteers who are considered medically fit to undergo studies are admitted to the metabolic ward in the morning after a 14-h fast. Subjects are typically studied in a semirecumbent position and allowed to drink water only. For studies of apoB-48 kinetics, constant feeding to achieve a higher steady state of chylomicron concentration above fasting levels rate may be required [60,61]. A single bolus injection of tracer or a primed constant infusion is administrated via a teflon annula placed in a superficial vein of an antecubital fossa. Venous blood samples are collected from a cannula placed in the
superficial vein of an antecubital fossa in the other arm at baseline; following a rapid bolus injection of isotope, further blood samples may be collected at 5, 10, 20, 30 and 40 min and 1, 1.5, 2, 2.5, 3, 4, 5, 6, 8 and 10 h. Subjects are then given a snack and allowed to go home. Additional fasting blood samples are collected in the morning of the following 4 days during the same week (24, 48, 72, and 96 h) to provide kinetic information on lipoproteins with relatively low turnover rates, such as LDL-apoB and HDL apoA-I and apoA-II.

For the determination of rate of cholesterol absorption, \[^{13}C_5\]cholesterol and \[^{2}H_6\]cholesterol are administered intravenously and orally respectively. The percentage cholesterol absorption is determined from the ratio of \[^{13}C_5]/[^{2}H_6]\) in plasma cholesterol after at least 3 days to allow for tracer equilibration [56]. This methodology could be modified and applied to investigate whole-body cholesterol synthesis and RCT. For the determination of VLDL-triacylglycerol kinetics, a bolus of labelled \[^{2}H_5\]glycerol is administered intravenously [53]. Blood samples are obtained at 0, 5, 15, 30, 45 and 60 min and then every hour for 12 h to determine plasma glycerol and VLDL-triacylglycerol tracer-to-tracee ratios. With the use of stable isotope \[^{13}C\]palmitate, fatty acid kinetics could also be examined in a clinical protocol, as for VLDL-triacylglycerol kinetics [55].

**Kinetic analysis of lipoprotein metabolism**

Parameters of lipoprotein metabolism are determined from the mathematical analysis of apo tracer/tracee mass ratio data. Three different methods have been used for the analysis of lipoprotein kinetics: linear regression, a mono-exponential function and compartmental modelling. The third is the most complex and appropriate approach to analyse isotopic enrichment, and provides information not obtainable with the other two methods of analysis [38,50]. Figure 3 illustrates three different compartmental models that we have employed to provide information on the kinetic behaviour of apoB, apoA-I (or apoA-II) and chylomicron remnant-like remnants respectively, in subjects with metabolic syndrome. More complex compartmental models have been developed which account for additional heterogeneity present within some lipoprotein fractions and this will be discussed in a later section.

**Simulation, analysis, and modelling software (SAAM II)**

The SAAM II program is frequently used to aid in the design and analysis of lipoprotein tracer data using compartmental models [62,63]. Compartmental models can be developed by incorporating what is known or hypothesized about the system under study. The development of a compartmental model requires a number of discrete, but related, processes: development of a structural model, specification of the experiment being performed on the model, fitting and parameter estimation, and finally model comparison [62]. A structural model is a schematic that shows the relationships between compartments in the model. The basis for this structure comes from knowledge of the system and new experimental data. For example, the simplest model of apoB metabolism would include compartments that showed the conversion of VLDL into IDL and subsequently LDL. Once the model is constructed, SAAM II will automatically generate the system of ordinary differential equations from the compartmental model structure. Additional user-defined equations or constraints can be incorporated into the model. The second process involves specifying the experiment that is performed on the model. This includes specifying the sites and protocol of tracer administration and compartments that are sampled or associated with experimental data. Thirdly, with model structure and experimental data, the parameters of the models can be estimated by adjusting the parameter values (e.g. the synthesis delay time required for the secretion of apoB in the liver has an initial assigned value of 0.5 h) in a process...
known as ‘optimization’. This process adjusts the value of the adjustable parameters to obtain a ‘best fit’ between the model calculated values and the experimental data. This process provides the ‘best’ estimates of model parameters together with a measure of their precision. Finally, in the development of a new model, alternative model structures should be developed and fitted to the same experimental data in a process to identify the best model. This process often proves informative in generating new hypotheses that can be subsequently tested experimentally. SAAM II has been employed for the development of many of the contemporary models of lipoprotein metabolism.

Some key definitions
With the ‘best fit’ of the model to the experimental data, metabolic parameters of lipoprotein metabolism can be estimated. For example, the rate of tracee appearance and disappearance from the circulation can be determined. Several terms defined below are employed to describe these parameters.

FCR (fractional catabolic rate) is defined as the fraction of tracee irreversibly removed from a pool per unit time (e.g. pools/day). Secretion rate is defined as the rate at which the tracee enters the pool. This is a derived parameter, being the product of the FCR and tracee pool size. Pool size is the mass of tracee in a specific pool determined as the product of plasma volume and tracee concentration. Conversion rate is defined as the fraction of tracee converted from one pool to another.

**APOB-100: VLDL, IDL and LDL KINETICS**

Figure 3(A) shows the multicompartmental model we have used to analyse VLDL-, IDL- and LDL-apoB enrichment data [64]. Leucine tracer is injected into plasma (as a bolus), represented by compartment 2. Compartments 1, 3 and 4 are required to account for the dynamics of the plasma leucine kinetic data. Compartment 1 is in rapid equilibrium with the plasma compartment and 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 protein pools that turnover slowly. This subsystem is connected to an intrahepatic delay compartment (compartment 5) that accounts for the time required for the synthesis and secretion of apoB into plasma. This represents a complex process that includes the translation of apoB protein from amino acids and subsequent lipiddation of the apoB protein and secretion into plasma. This model provides for the direct secretation of apoB into the VLDL, IDL and LDL fractions. Compartments 6–10 are used to describe the kinetics of apoB-100 in VLDL. Compartments 6–9 represent a delipiddation cascade that represents the sequential delipiddation of VLDL particles within the VLDL pool. Compartment 10 represents a pool of VLDL particles that are derived from the delipiddation cascade that turnover slowly. The fraction of each compartment in the cascade converted into VLDL compartment 10 is the same. VLDL particles in compartment 9 can be converted into IDL or removed directly from plasma. Plasma IDL kinetics are described by two compartments, compartments 11 and 12. Compartment 12 represents a slowly turning over pool of IDL particles. IDL in compartment 11 can be converted into LDL, compartment 13, or be removed directly from plasma. The LDL section of the model consists of two compartments. Compartment 13 describes plasma LDL, and compartment 14 is an extravascular LDL exchange compartment. Since LDL is removed chiefly by the hepatic receptor pathway, it is assumed that all LDL is cleared via compartment 13. VLDL- and LDL-apoB metabolic parameters are derived when the compartment model is fitted simultaneously to the VLDL-, IDL- and LDL-apoB tracer/tracee ratio and apoB mass data.

Figure 4(A) shows the isotopic enrichment of VLDL-, IDL- and LDL apoB (a), HDL apoA-I and apoA-II (b) and chylomicron remnant-like particle emulsion (c) in a representative subject. The observed values and model-predicted values are shown.
Figure 5  A complex compartment model describing apoB-100 metabolism in VLDL1, VLDL2, IDL and LDL fractions

A subject with metabolic syndrome. The tracer curves demonstrate a precursor product relationship between the VLDL-, IDL- and LDL-apoB fractions. Despite this, some apoB enters directly into the IDL- and LDL-apoB fractions. The exact nature of this input, direct secretion of IDL and LDL particles versus the rapid conversion of VLDL into IDL and LDL, has yet to be demonstrated. Different compartment models have been developed to describe apoB tracer kinetics observed in different forms of dyslipidaemia. As discussed before, the main differences between models are due to varying experimental protocols (i.e., bolus injection versus primed, constant infusion, or study period) and the isolation of different lipoprotein fractions (such as VLDL and LDL subfractions). For example, Packard et al. [65] have developed a complex model of apoB metabolism (Figure 5) using apoB radiotracer data following the injection of 131I-labelled VLDL1 (Sf 60–400) and 125I-labelled VLDL2 (Sf 20–60). This model highlights kinetic heterogeneity within VLDL subfractions and permitted the direct input of apoB into the VLDL1, VLDL2, IDL and LDL fractions, and direct removal of apoB from each fraction.

APOA-I and APOA-II: HDL KINETICS

Figure 3(B) shows a multicompartamental model used to describe HDL-apoA-I (or apoA-II) leucine tracer/tracer ratios [66]. Compartments 1–4 describe plasma leucine kinetics, as for the apoB model. This subsystem is connected to a delay compartment (compartment 5) that accounts for the time required for synthesis and secretion of apoA-I (or apoA-II) from liver and intestine into plasma. Compartments 6 and 7 describe the kinetics of apoA-I (or apoA-II) in the plasma HDL fraction and in an extravascular-plasma compartment respectively. Compartment 7 may represent an exchange between the plasma and extravascular compartments, such as intestinal and lymphatic spaces, although the exact nature of this compartment is yet to be described. The loss from compartment 6 describes the removal (degradation) pathway for apoA-I (or apoA-II) via both the liver and kidney. Figure 4(B) shows the isotopic enrichment of HDL apoA-I and apoA-II after a primed infusion of [3H3]leucine (1 mg/kg of body weight bolus and 1 mg·kg−1·h−1 infusion for 6 h) in a subject with the metabolic syndrome. The tracer curves for the two apoproteins are similar, consistent with the notion that apoA-I and apoA-II are present on the same particle [i.e. HDL LpA (lipoprotein A)-I/A-II]. Other models have also been employed to examine the kinetic behaviours of apoA-I and apoA-II in HDL. Taskinen et al. [67], employing radiotracers and exogenous labelling, studied the kinetics of LpA-I and LpA-I/A-II particles in diabetic subjects and attributed the low LpA-I/A-II concentrations observed in diabetic subjects to a reduced secretion rate of these particles relative to non-diabetic subjects. In a recent placebo-controlled study of the effect of fenofibrate on HDL kinetics in subjects with the metabolic syndrome, we measured the kinetics of HDL apoA-I and apoA-II [66]. Using these tracer data and the concentration of LpA-I/A-II particles a new compartmental model was developed (Figure 6). The LpA-I/A-II concentration data were used to split the apoA-I tracer data into two components that describe the kinetics of LpA-I and LpA-I/A-II particles. In developing this model, it was assumed that the kinetic properties of apoA-I and apoA-II on the LpA-I/A-II
Lipoprotein metabolism in the metabolic syndrome: methodological aspects

Figure 7  Compartment models of HDL apoA-I metabolism


particle were identical. Using this new model, we demonstrated recently in a placebo-controlled trial that fenofibrate selectively increases the synthesis of LpA-I/A-II particles in subjects with the metabolic syndrome (P. H. R. Barrett and G. F. Watts, unpublished work).

New models

When employing endogenous labelling methods, HDL kinetic studies are complicated by virtue of the recycling of the amino acid precursor. As a consequence, the terminal slope of the HDL tracer curve provides limited kinetic information, and the kinetic parameters associated with the extravascular HDL compartment cannot often be determined with precision. In contrast, the early rising section of the HDL apoA-I tracer curve, following the bolus administration of labelled amino acid, provides rich kinetic information. Fisher et al. [68] proposed several compartment models, one of which hypothesized the existence of fast and slow turning over plasma pools of apoA-I (Figure 7, Model A). Model A has a plasma leucine pool (compartment 17), which serves as a forcing function followed by a delay (compartment 19), reflecting the synthesis and secretion time for apoA-I. ApoA-I initially appears in a rapidly turning over compartment (compartment 2) that represents approx. 15 % of the HDL apoA-I pool. The apoA-I in this leaves the plasma space and, after delay (compartment 10), reappears in plasma as a more slowly turning over pool of HDL apoA-I (compartment 1). This compartment is in equilibrium with two extravascular compartments (compartments 3 and 4). This model supports the concept that the major pool of HDL apoA-I particles is derived from a smaller, more rapidly turning over, pool of apoA-I. Alternative models, such as that shown in Figure 7 (Model B), incorporate different synthesis pathways, hepatic and intestinal, that can more fully describe the HDL apoA-I tracer data. Experience with this model is at present limited. Recently, Ouguerram et al. [57] also proposed a novel kinetic model to assess cholesterol transport between HDL and apoB-containing lipoproteins. With the addition of HDL apoA-I and apoB tracer data, such an approach could provide important information to assess RCT in vivo.

APOB-48 AND CHYLOMICRON REMNANT KINETICS

ApoB-48 is an ideal marker for studying chylomicrons and chylomicron remnants metabolism [25,69]. Direct determination of apoB-48 in plasma following a fat load allows assessment of the number of circulating chylomicron and chylomicron remnants and has become an important tool to study intestinally derived postprandial lipoproteins [70]. However, it does not provide reliable information on the kinetics of the system. The metabolism of apoB-48 has been examined in the fed state using a primed constant infusion of stable isotopically labelled leucine. Welty et al. [60,61] proposed a three-compartmental model for determination of kinetic parameters for apoB-48. This study approach has been used to elucidate key features of apoB-48 metabolism in normal and pathological states. Recently, we have proposed [71] a functional test to measure chylomicron remnant metabolism. In brief, a cholesteryl-[13C]oleate-labelled remnant-like emulsion is prepared with lipid composition and size similar to chylomicron remnants. After injection into the bloodstream, the chylomicron remnant-like emulsion is cleared from the plasma and metabolized by the liver. The lipid components are degraded and the fatty acids are oxidized by mitochondria and peroxisomes to form CO2, which is expired in the breath. A three-compartmental model (Figure 3C) is developed and fitted to 13CO2 breath data to provide estimates of the fractional catabolic rate of the remnant-like emulsion. Figure 4(C) shows the enrichment of 13CO2 in the breath following injection of the emulsion in an obese subject. There is a rapid increase in enrichment of 13CO2 with a peak at approx. 4 h, reflecting the catabolism of remnant-like particles via lipolysis and hepatic removal. Readers should refer to the literature for further details of this text [60,61,71].

CONCLUSIONS

The metabolic syndrome is an escalating public health problem related to adverse gene–environment interactions that result in progressive increase in body weight and phenotypic expression of a spectrum of metabolic and cardiovascular risk factors. The metabolic
syndrome is frequently associated with visceral obesity, insulin resistance and dyslipidaemia, which, in turn, is causally related to an increased risk of Type II diabetes and CVD. The use of stable isotope, MS and modelling methods has played a fundamental role in advancing our knowledge of this important metabolism disorder. However, this approach is time consuming, expensive and requires a high level of expertise. Its success relies on a multi-disciplinary team involving biochemists, physicians and biostatisticians, so that these studies are best carried out in specialized research centres. These types of investigations not only provide a rationale for how to optimally employ existing therapies for dyslipidaemia in metabolic syndrome, but also assist in drug discovery and drug development. Development of new tracer methodologies for investigating in vivo cholesterol efflux from cells, cholesterol transport in body, the turnover of the HDL subpopulation of particles and other apo (e.g. apoC-III and apoE) are required to unravel further the complexities of abnormal lipoprotein metabolism in the metabolic syndrome. In the accompanying review [13a], we will deal with the application of stable isotopy and modelling method to the study of lipoprotein transport in metabolism syndrome.

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