thematic review

Thematic review series: Patient-Oriented Research

Design and analysis of lipoprotein tracer kinetics studies in humans

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Abstract **Lipoprotein tracer kinetics studies have for many years provided new and important knowledge of the metabolism of lipoproteins. Our understanding of kinetics defects in lipoprotein metabolism has resulted from the use of tracer kinetics studies and mathematical modeling. This review discusses all aspects of the performance of kinetics studies, including the development of hypotheses, experimental design, statistical considerations, tracer administration and sampling schedule, and the development of compartmental models for the interpretation of tracer data. In addition to providing insight into new metabolic pathways, such models provide quantitative information on the effect of interventions on lipoprotein metabolism. Compartment models are useful tools to describe experimental data but can also be used to aid in experimental design and hypothesis generation. The SAAM II program provides an easy-to-use interface with which to develop and test compartmental models against experimental models. The development of a model requires that certain checks be performed to ensure that the model describes the experimental data and that the model parameters can be estimated with precision. In addition to methodologic aspects, several compartment models of apoprotein and lipid metabolism are reviewed.**—Barrett, P. H. R., D. C. Chan, and G. F. Watts. **Design and analysis of lipoprotein tracer kinetics studies in humans.** ***J. Lipid Res.*** **2006.** 47: **1607–1619.**

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Years of clinical investigation have provided valuable insight into the complexity of human lipoprotein metabolism. The measurement of plasma lipoprotein concentrations provides useful information, but from a functional viewpoint these concentrations reflect the balance between input and output in the lipoprotein system in plasma. The only way to quantify input and output in this system is by undertaking kinetics studies, typically using tracer methodology. These studies, however, are time-consuming and difficult to perform. Nevertheless, they are important to characterize the pathways that result in dyslipidemic states and to describe the in vivo mechanisms of action of treatments designed to regulate dyslipidemia and cardiovascular disease risk in clinical practice. There are many steps to consider when planning such metabolic studies. This review touches on all aspects of the design and analysis of lipoprotein kinetics studies, including philosophical angles, tracer methodology, mathematical modeling, and statistical considerations related to experimental design and analysis.

With the advent of commercial stable isotopes and endogenous labeling protocols, some aspects of performing kinetics studies are now easier than when radioactive tracers and laborious exogenous labeling of lipoproteins were required. However, the number of laboratories and research groups undertaking these types of metabolic studies has changed little, highlighting the complexity of the methods involved.

In addition to the design and modeling aspects of lipoprotein kinetics studies, what we have learned from apolipoprotein B (apoB) and HDL apoA-I and apoA-II tracer kinetics studies is covered in the present series of reviews by Parhofer and Barrett (1) and Rashid, Patterson, and Lewis (2). Other recent reviews of the present field of investigation and its applications include those by Marsh et al. (3, 4) and Barrett, and Watts (5). For basic definitions of terms used in kinetics modeling, the reader is referred to these and other reviews (6, 7).

DESIGN OF TRACER STUDIES

General considerations

Before launching into the details of the analysis and modeling of tracer data, it is important to stress some funda-

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Abbreviations: apoB, apolipoprotein B; PCI, primed, constant infusion; TRL, triglyceride-rich lipoprotein.

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mental, general requirements and considerations for designing successful tracer kinetics studies.

A clear research question, or hypothesis, needs to be defined as a single or series of observational statements that the investigation can realistically answer. This is important to identify the knowledge gap and guide the subsequent study design. Several clinical designs may be used. For observational studies, case-control, matched case-control, and cross-sectional designs can generate preliminary evidence that can be more rigorously tested in controlled intervention trials. All interventional studies should be randomized, placebo-controlled with a parallel group or crossover design. These designs provide the strongest, unbiased evidence for the interventions being tested. Uncontrolled designs undermine the validity of the findings and in our view are of limited value. Of equal importance is a design that uses a sample size that is statistically powered (>80%) to adequately test the null hypothesis with α error of 5% (8). The power of a study is determined by the number of subjects in the study, the variance of the end point, and the expected effect of the intervention or difference in end point between the control and indexed groups. Ethical demands and time and financial investment mandate that a priori power calculations should be routinely undertaken to select and study a realistic and optimal sample size. Good statistical design is an essential prerequisite to establish the scientific credibility of data.

For clinical protocols, all kinetics studies should be designed and conducted to the highest scientific and ethical standards according to present-day requirements of good clinical practice (9). As with all responsible clinical research, the rights, safety, and convenience of volunteers and the risk-to-benefit ratio of the study need careful consideration. Tracer administration should be carried out in an accredited clinical area with adequate supporting resources from qualified and experienced nursing and medical staff. Because of the experimental assumptions required for kinetics data analysis, all studies should be carried out in steady-state conditions. This means that subjects should be studied in the postabsorptive state and not under acute or subacute conditions, such as during recent lifestyle changes or minor illness, when lipoprotein concentrations may not be in steady state. Kinetics studies performed under constant feeding are also permissible and informative and have been widely used by some groups (10–12). Clearly, diet influences lipoprotein metabolism (13–15), but what is important is that kinetics studies be performed while the system is in steady state. Given the slow turnover of some lipoproteins, this may mean that dietary intervention for extended periods (weeks to months) is required for the system to come into metabolic equilibrium.

Preparation of tracers for injection should only be performed in a fully accredited pharmacy department within a hospital setting. As with laboratory methods, all standard operating procedures and details of quality assurance should be documented.

The study of lipoprotein kinetics is a specific branch of systems science. A system is an entity that exists and functions as a unified whole via the interaction of its component parts. The lipoprotein system functions exactly in this way. An understanding of systems thinking (16) will assist investigators in interpreting the major outcomes of kinetics studies, namely the production rate and fractional catabolic rate (FCR). In intervention studies, the concept of balancing feedback can help explain why, for example, the effect of a primary change in catabolism is balanced by other changes in the whole system, including production rate.

Statistical methods, such as general linear modeling, mixed models, and multiple regression methods, are now the preferred tools for end point analysis (17). The general principle of these analyses is broadly based on modeling methods, referred to below, in which a model is fitted to a set of data and the sum of squares of the residuals (or error term) is estimated to assess the degree of fit of the model. When more than one group is being investigated at a given time, or a single group is investigated repeatedly over time, *P* values should be corrected for multiple comparisons. Cross-sectional comparisons without a priori hypotheses also require similar adjustments to *P* values. This approach guards against inappropriate statistical inferences drawn from poorly designed studies with small sample sizes.

A philosophical perspective on tracer kinetics may be useful when using modeling techniques. One could ask: what type of science is mathematical modeling? In as much as it uses a mathematical tool to understand the natural world (e.g., lipoprotein metabolism), mathematical modeling is a special form of "instrumentalist" science (18). This implies that, in contrast with a "realist" approach, it does not deal directly with observable entities, providing data that only approximate reality. However, modeling does rely heavily on investigators generating high-quality, primary, empirical data, and in this sense modeling has a realist component. However, the validity of modeling as a form of scientific investigation relies to a large extent on instrumentalism and, in particular, on the utility of the models hypothesized or tested in a given setting.

Specific considerations

An important objective for tracer protocols is to design the optimal study to maximize the kinetics information content provided by the data. There are a number of issues related to analysis and modeling that need to be considered in more detail below.

LABELING METHODS

Tracers are used in lipoprotein kinetics studies to help characterize the metabolic pathways of lipoprotein metabolism. To be of use, tracers must have certain properties: they must be detectable with adequate sensitivity; they must not perturb the system under investigation; and they must be metabolically indistinguishable from the tracee. The earliest lipoprotein kinetics studies used radioiodinated tracers exogenously attached to lipoproteins to trace VLDL metabolism (19, 20). Since 1985, when Cryer

et al. (21) first reported the use of endogenous labeling with [^{15}N]glycine, the use of exogenous labeling for kinetics studies has declined. However, as discussed below, this has introduced complications with respect to the modeling of apoprotein tracer data.

Exogenous labeling

Exogenous labeling requires the isolation of lipoprotein and ex vivo labeling by radioiodination to create a tracer (22). Radiolabeled lipoproteins are generally administered as a bolus injection, and specific activity is used as a measure of tracer mass; however, it must be assumed that the isotope labels the substance in proportion to the tracee mass (23). Disadvantages of radiolabeling include concerns that preparative isolation and radiolabeling may chemically modify apoB and/or the lipoprotein particle and potentially alter its kinetics (23, 24) and biohazard and ethical concerns with human use. Furthermore, the isolation and subsequent labeling of lipoproteins already circulating in plasma may not provide a tracer that reflects the kinetics of all subpopulations existing within a given lipoprotein fraction (25). Despite this, no studies have demonstrated significant differences in kinetics parameters between exogenously and endogenously labeled lipoproteins. Relative to sources of biological variation within study populations, the differences between labeling protocols are probably negligible.

Endogenous labeling

Amino acids, typically leucine, labeled with stable isotopes have been used as endogenous tracers for the study of lipoprotein metabolism since the mid 1980s (21, 26). Such labeled amino acids are referred to as isotopomers. Different stable isotope-labeled compounds (e.g., [^{15}N]glycine, [^{13}C]phenylalanine, [^{13}C]leucine, and [$^{2}H_3$]leucine) have been used in tracer studies, and the results suggest that all of these act in a similar manner as a tracer (27). Nevertheless, leucine has many advantages as a protein tracer (28) because it is an essential amino acid, is readily available, and is not converted into other amino acids.

With endogenous labeling, proteins and lipids can be labeled, in vivo, from a labeled precursor pool. Although this methodology permits a direct assessment of synthesis pathways, it is complicated by the kinetics of the precursor pool. Endogenous labeling also permits the simultaneous measurement of the kinetics of multiple apoproteins (10, 29) in the same subject. A disadvantage of endogenous labeling with stable (or radioactive) isotopes relates to complex precursor (amino acid) kinetics and the difficulty of quantifying the contribution of this to the kinetics of slowly turning-over lipoprotein fractions. Evidence of tracer recycling is seen in **Fig. 1**. The tracer curves in this figure were simulated using the same VLDL model, although additional precursor compartments were required to describe the synthesis of leucine into VLDL apoB. Because of the complex kinetics of the amino acid precursor, the tracer curve for endogenously labeled VLDL ([$^{2}H_3$]VLDL) is slower than that for exogenously labeled

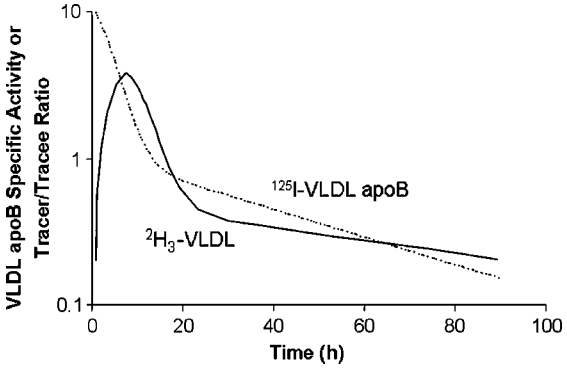

Fig. 1. Model simulated VLDL apolipoprotein B (apoB) tracer curves after injection of exogenously labeled VLDL (^{125}I-VLDL) and endogenously labeled VLDL ([$^{2}H_3$]VLDL). The model and parameter values for the VLDL section of the model were identical for the two tracers. The slower decay for the [$^{2}H_3$]VLDL tracer curve beyond 30 h shows the influence of amino acid recycling.

^{125}I-VLDL. This feature is a reflection of a rate-limiting kinetics process associated with amino acid that is evident in the VLDL product. Despite these limitations, increased safety and greater acceptance by volunteers have made the use of stable isotopes in human lipoprotein kinetics studies almost universal in recent years.

The first endogenous labeling studies administered the amino acid isotopomers as a primed, constant infusion (PCI) for a period of 12–15 h (29). A disadvantage of PCI is that the kinetics of slowly turning-over pools are more difficult to estimate (30, 31). Moreover, the short duration of the kinetics studies made it difficult to estimate the kinetics of apoproteins of interest with relatively slower turnover rates (32). More recently, labeled amino acids have tended to be administered as a bolus and the duration of the experimental protocols extended to 96 h (33, 34). An advantage of the bolus administration of tracer, over that of PCI, is that the dynamics in the product pool (e.g., VLDL apoB) are greater. Such data have higher information content and thus additional knowledge can be gained.

SAMPLING PROTOCOLS

The blood sampling protocol for a study is dependent on the experimental hypothesis and the lipoprotein system under investigation. Blood volumes obtained should be minimized to increase acceptability by volunteers and ethics review bodies as well as to minimize perturbations to the plasma space. Defining the frequency and duration of blood sampling is critical. Choosing a sampling schedule for a kinetics study is made easier by virtue of the fact that most apoprotein and lipid systems being studied today have, to some degree, been investigated (35). Resorting to the theory of optimal design would also benefit in selecting sample times to maximize the information content of the tracer data. Changing the structure of the model, or the hypothesis of a rapidly turning-over compartment of lipo-

proteins, might mean that the sampling schedule should be altered to gain additional information. Further information about the role of optimal design in experiments can be found elsewhere (36).

LABORATORY METHODS

The laboratory methodology associated with lipoprotein kinetics studies is complex and has been reviewed in more detail elsewhere (37, 38). In brief, apoproteins and lipids are isolated from serial plasma samples using ultracentrifugation and a variety of methods so that the tracer attached (exogenous labeling) or incorporated (endogenous labeling) can be quantitated. In VLDL, intermediate density lipoprotein (IDL), and LDL, isopropanol precipitation (39) or tetramethylurea (40) can be used to isolate apoB. For other apoproteins, including apoC, apoE, apoA-I, and apoA-II, polyacrylamide gel electrophoresis or isoelectric focusing is required for isolation. In studies in which radiotracers are used, the specific activity time course is determined by measuring the radioactivity associated with each apoprotein and expressing it as a function of apoprotein mass. Where endogenous labeling is used with stable isotopes, the isolated apoproteins are hydrolyzed, derivatized, and run on a gas chromatograph-mass spectrometer (or isotope ratio mass spectrometer) to measure the ratio of the labeled to unlabeled derivative. As with clinical protocols, full documentation of standard operating procedures with quality assurance is mandatory.

ANALYSIS AND MODELING OF TRACER STUDIES

The analysis of lipoprotein tracer studies can be approached using algebraic functions and noncompartmental models. These forms of data analysis are little more than methods for parameter estimation. In contrast to the process of data analysis, tracer studies can be modeled using compartmental models. The former approach provides limited quantitative information about the system under investigation. Compartmental modeling provides more insight into the system under investigation by permitting the integration of knowledge from other studies and by enabling the modeler to test different structural models against experimental data. To some extent, the method of analysis used is dependent on the information sought and the applicability of the assumptions of the model to the system under investigation. The second of these factors is important when describing the heterogeneity of lipoprotein metabolism.

There are a number of issues related to modeling that need to be considered. These include experimental design; optimizing the test of the hypothesis; the number of component compartments: investigating the complexity of the system; the principle of parsimony: using established criteria to assess model order; and model validity: testing the robustness of the model.

Noncompartmental models and algebraic functions

Kinetic parameters can be estimated using the noncompartmental approach. The formulae for such models rely on the system under study being in steady state and that the pools accessible for tracer input and sampling are kinetically homogeneous. For most lipoprotein kinetics studies, this assumption is not valid (41, 42). Thus, formulae estimating the fractional synthesis rate are unreliable. Further discussion of noncompartmental models in relation to lipoprotein metabolism is provided by Foster et al. (32).

Algebraic functions were also used to describe lipoprotein tracer data (10, 27) in some of the early studies. Fitting a straight line function to tracer data that are inherently nonlinear is not appropriate, and the fitting process was often subjective. A further disadvantage of the linear regression methods was the fact that it failed to account for the delay associated with the synthesis and secretion of apoproteins into plasma. Furthermore, this method required that the enrichment of the immediate precursor be known. These data generally are not available; thus, it was assumed that the plateau enrichment of another apoprotein, such as VLDL apoB, was equivalent to the precursor pool. This method of data analysis is no longer used.

Compartment models

Compartment models provide the gold standard for modeling lipoprotein kinetics data. One major advantage of compartmental models over other models is their ability to take into account the kinetic heterogeneity of lipoprotein metabolism.

A compartment can be defined as an amount of material that is well-mixed, kinetically homogeneous, and distinct from other material in the system. A compartmental model is a collection of compartments that are interconnected in a specific manner. These interconnections permit the transport of material from one compartment to another. In addition, material can enter compartments from outside of the model and from de novo synthesis and can irreversibly leave a compartment to the outside system.

A lipoprotein compartment model is a dual representation of the endogenous material (e.g., apoB) called the tracee and the tracer. The compartment model is a scheme of a system of differential equations describing the mass balance of tracee and tracer under steady-state conditions. Modeling the tracer data enables the coefficients of the differential equations to be determined. From this, and the assumption that most lipoprotein kinetics studies are performed in the steady state, we can calculate transport rates of the endogenous material (tracee) and hence derive production and FCRs. Lipoprotein kinetics studies can also be modeled under non-steady-state conditions (43), but this requires certain complex assumptions being made about production and/or catabolic rates.

Compartment models—beyond the individual

Compartment models are typically fit to the data obtained from a given individual to provide a measure of the

kinetics parameters for that particular individual. These measures, however, are the sum of fixed and random effects that cannot be unbundled when fitting a model to individual data sets. Fixed effects represent the "true" value of the kinetics parameters, and random effects account for intersubject variability (44). As a consequence, the true value of the parameter is unknown and the precision of parameter values is not optimal. With the use of population kinetics analysis, the data from multiple individuals can be fit simultaneously to provide an estimate of the population kinetics parameters and their distribution that accounts for the variation observed within the population. Population analysis provides greater power to determine kinetics parameters because it uses the data from each subject to help fit the model to the data of the next individual. It is an iterative process that generally provides parameter estimates that have greater precision. Furthermore, population kinetics analysis can use parametric or nonparametric methods to identify unimodal or multimodal parameter distributions (in which a study population may be heterogeneous), respectively. Covariates can also be included in the analysis to help explain some of the variability in model parameters. Further information about population kinetics analysis can be found in reference 44.

Identifiability

An important component of model development and testing is identifiability (45). Identifiability refers to the process for evaluating the viability and precision of parameter estimation for a given model. Identifiability analysis should occur a priori, before experiments are performed, and a posteriori, after the fit of a model to experimental data. To some extent, the importance of this process is dependent upon the purpose of the modeling exercise. If the model is being used as an investigative tool rather than to quantify metabolic pathways, then identifiability analysis may not be of paramount importance.

A priori identifiability. Given a particular model with unknown parameters and error-free data, is there sufficient information that will permit the model parameters to be identified? This is a theoretical question and does not necessarily imply that the parameters of the model will be determined with any degree of precision when fitting the model to experimental data (46). If some of the parameters are not identifiable in this ideal context, they clearly would not be identified in a "real" situation, in which there is noise in experimental data. For example, identifiability analysis of a one-compartment model with a single loss pathway ($k_{0,1}$) in which plasma data were available would demonstrate that the model parameters are identifiable. If, however, the compartment had two loss pathways ($k_{0,1}$ and $k'_{0,1}$), then only the sum of the two pathways would be identifiable (36). The values of each of the loss pathways would not be identifiable, because there are an infinite number of possible solutions for the loss pathways constrained to equal the identifiable sum.

A priori identifiability analysis is a powerful tool to aid in model development, because if one knows a priori that the model parameters cannot be identified, the model structure could be modified or additional sampling sites could be specified. Alternatively, additional information could be integrated into the model, or limits could be placed upon the model parameters, to restrict the possible number of model solutions. As models grow in size and complexity, the theory behind identifiability analysis becomes very complicated. A recent review of identifiability analysis, referring to pharmacokinetics models, was published by Yates (47).

A posteriori identifiability. A posteriori identifiability refers to the practical problem of assessing how well a given model describes the experimental data. As shown in **Fig. 2**, the process of model fitting requires input in the form of data, a description of the error model associated with the data, the structure of the model, and initial estimates of the model parameters.

Although good experimental design should lead to the generation of useful data, it is important to appreciate the errors inherent in the data generated. Error models should be formulated from replicates that represent the spectrum of data values generated. These models provide a measure of the confidence of the data and aid in fitting the model to experimental data. A feature of the SAAM II (for Simulation, Analysis, and Modeling) program permits the development of the weighting function that is based upon replicates, and this assigns a numerical weight to each datum during the fitting process. Incorrect weighting of data will lead to poor model fit and incorrect parameter estimates.

In addition to the error model for the data, the initial estimates of the model parameters can affect the fit of the model to the data. Poor initial parameter values (i.e., parameters that are several orders of magnitude away from the true value) may affect the fitting process.

To produce a best fit, software transforms the inputs shown in Fig. 2 into an objective function (weighted nonlinear least-squares) and attempts to minimize the function by adjusting the value of the model parameters. When the criteria of convergence for the objective function have been met, the model has been fitted to the

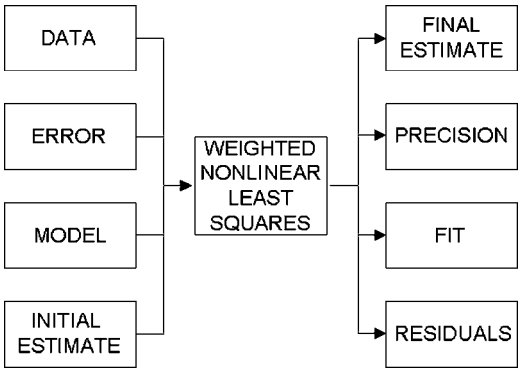

Fig. 2. The modeling problem. The inputs into the weighted nonlinear least-squares model-fitting process and the outputs that result.

experimental data. It is at this point that a posteriori analysis takes place.

The fit of the model to experimental data can be evaluated by eye, ensuring that the model solution is close to the individual data points. Systematic deviations indicate a poor fit of the model to the data. A better way to assess fit is to assess the distribution of the residuals (the difference between model values and data). Ideally, the residuals plot should show random variation with mean zero residual. A runs test can also be performed to test for systematic patterns in the residuals. See Jacques (36) for additional tests of model fit.

The precision of the final parameter estimates should also be examined. The standard deviation and confidence limits of each parameter should be available after a model fit and evaluated. Large standard deviations and confidence limits that cross zero are indicative of poor parameter precision. Parameter precision should be reported in publications that result.

As part of the model development process, tests of model order should be performed. This addresses the issue of the principle of parsimony: pick the model with the least number of parameters that fit the data best. The Akaike information criteria test (48) can be used to differentiate been models based upon the value of the objective function and the number of adjustable parameters. The model with the lowest Akaike information criteria score is the best model. In addition, the *F*-test can be used to assess the change in the objective function with respect to changes in the number of adjustable parameters in the model. Selecting the best model takes care and experience. There are a variety of criteria that must be considered.

Finally, it is important to consider the validity of the model (49). This is not a question of whether the model is true but of assessing its strength or robustness. This is a difficult and somewhat subjective process that relies upon intuition and knowledge of the system to assess whether or not the model is adequate for its purpose. The initial steps in model validation include those described above (tests to assess fit, residuals, precision, and model order) as well as knowledge and plausibility. As models become more complex, this task is more difficult. From a scientific viewpoint, the validity of a model can also be gauged by its simplicity, cohesiveness with experimental data, explanatory potential, and utility. Beyond mathematical tests, cohesiveness and utility are particularly important dimensions for assessing the validity of a model.

MODELING SOFTWARE

The SAAM program, initially developed by Berman and Weiss (50), has been the cornerstone for the modeling of lipoprotein kinetics studies. More recently, the SAAM II program was developed, and today it is frequently used to aid in the design and analysis of lipoprotein tracer data using compartmental models (51, 52). 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 (41). A structural model is a scheme 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 to IDL and subsequently LDL. Once the model is constructed, SAAM II automatically generates the system of first-order, constant coefficient 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 the compartments that are associated with the experimental data. Third, with the model structure and experimental protocol defined and experimental data included, the adjustable parameters of the model are estimated using an iterative process to minimize the difference between the model prediction and observed 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.

MODEL SIMULATION

In addition to fitting models to data, the development of new compartmental models and those already published provide an opportunity to test new experimental protocols and thus simulate experimental data before performing the "real" experiment. Although few simulation studies have been published, Barrett and Parhofer (53) used a two-pool model of LDL apoB metabolism to simulate the effect of LDL apheresis on LDL apoB enrichment. An earlier study by Thompson et al. (54) reported that LDL apheresis had no effect on the catabolism of exogenously labeled LDL. Simulations of the effect of LDL apheresis on the LDL apoB tracer curve were performed using a two-compartment model and two experimental protocols, exogenously and endogenously labeled LDL apoB. The model simulations demonstrated that only large changes in LDL apoB FCR could be detected; therefore, small increases in FCR, although physiologically important, may not be detectable experimentally. Model simulation thus provides an opportunity to test the effect of changes to the experimental protocol and intervention on the expected tracer data.

The first models used to describe VLDL apoB kinetics used a monoexponential function, equivalent to a single-compartment model, to determine FCR (55–57). Al-

though these models provided a good description of the experimental data, with the exception of the early time points, using such a model to simulate an exogenously labeled VLDL kinetics study would reveal that the apoB tracer curve was monoexponential. This has not been the experience of researchers who have performed such studies. The power of models to simulate experiments is largely ignored but should be considered as part of experimental design.

The remainder of this review focuses on compartmental models that have been used to model the protein and lipid moieties of lipoproteins. The models presented represent examples of those that have been published in recent years.

Recent applications of modeling to the study of apolipoprotein and lipid kinetics

VLDL, IDL, and LDL apoB. ApoB, the major structural apolipoprotein of VLDL, IDL, and LDL, is a strong predictor of coronary events. As the only apoprotein to remain with the particle throughout its lifetime and interconversions in the circulation, apoB can be used to trace the kinetics of the apoB-containing lipoproteins and thus provide a description of their metabolism in humans. From apoB tracer data, a compartment model can be developed to estimate kinetics parameters of interest. As an example, **Fig. 3** shows the multicompartment model used to analyze VLDL, IDL, and LDL apoB enrichment data (58, 59).

The model comprises a number of distinct but interconnected components. In this model, leucine tracer is injected into plasma (as a bolus), represented by compartment 2. Compartments 1, 3, and 4 are required to account for the kinetics of the plasma leucine kinetics 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 release of leucine by protein pools that turn over 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 the subsequent lipidation of the apoB protein and secretion into plasma. This model provides for the direct secretion 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 delipidation cascade that represents the sequential delipidation of VLDL particles within the VLDL pool. Delipidation cascades with fewer compartments work well in normolipidemic subjects (60), but the extra model structure is required to describe VLDL apoB kinetics in dyslipidemic subjects. Compartment 10 represents a pool of VLDL particles that are derived from the delipidation cascade that turn over slowly. The fraction of each compartment in the cascade converted to VLDL compartment 10 is the same. VLDL particles in compartment 9 can be converted to IDL or removed directly from plasma. In contrast, in exogenous and endogenous tracer studies in which VLDL has been subfractionated (33, 61), a proportion of VLDL particles are cleared from plasma before conversion to the IDL fraction. 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 to 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. It is assumed that all LDL is cleared via compartment 13.

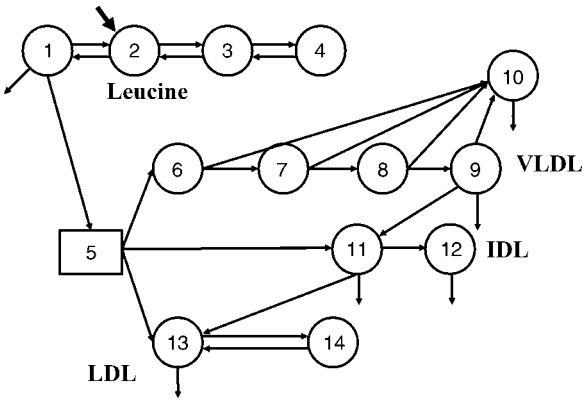

Fig. 3. A modified form of the apoB model developed by Phair et al. (58). Compartments 1–4 describe the kinetics of plasma leucine. Compartment 5 accounts for the delay associated with the synthesis and secretion of apoB from the liver. Compartments 6–9 represent the VLDL delipidation cascade, and compartment 10 is a pool of slowly turning-over VLDL particles. Compartments 11 and 12 are plasma intermediate density lipoprotein (IDL), and compartments 13 and 14 are the plasma LDL and extravascular LDL compartments, respectively.

To further our understanding of the heterogeneity of the VLDL fraction, Adiels and colleagues (62) developed a multicompartment model that allows the kinetics of triglyceride and apoB-100 in $VLDL_1$ and $VLDL_2$ to be assessed simultaneously (**Fig. 4**). As described above, the apoB section of the model incorporates a leucine subsystem together with a compartment that accounts for the synthesis and secretion of apoB from the liver into plasma. The model also includes a glycerol subsystem together with compartments that account for the synthesis of triglyceride in the liver. $VLDL_1$ and $VLDL_2$ particles are secreted into the plasma. Triglycerides are hydrolyzed, as indicated by the dashed lines, and VLDL particles are removed from plasma or converted to other compartments or lipoprotein fractions, as indicated by the solid transfer arrows. Similar to the model developed by Zech et al. (63), this model uses apoB tracer data to describe the transport of VLDL particles and triglyceride tracer data provide information on triglyceride transport and rates of triglyceride hydrolysis. Adiels et al. (62) found that triglyceride and apoB production in $VLDL_1$ and $VLDL_2$ are significantly correlated, suggesting a coupling of the two processes governing the metabolism of these lipoprotein subpopulations. In contrast to the simpler model (Fig. 3),

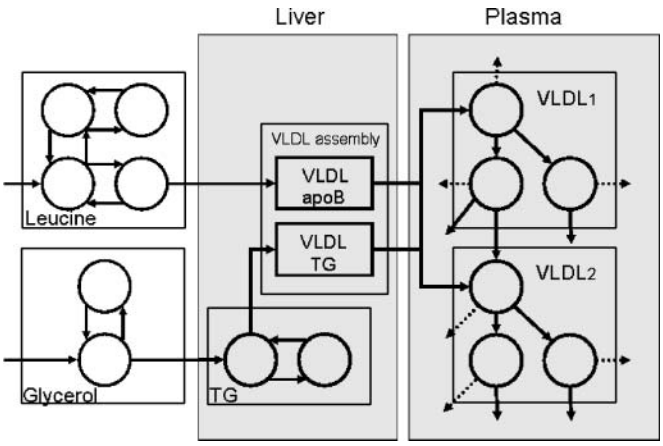

Fig. 4. Compartment model of VLDL apoB and triglyceride developed by Adiels et al. (62). The model includes separate modules for leucine and glycerol. Plasma leucine kinetics are modeled using a four-compartment system that drives the synthesis and secretion of apoB into $VLDL_1$ and $VLDL_2$. Plasma glycerol kinetics are modeled using a two-compartment system connected to fast and slow pathways for triglyceride (TG) synthesis. Plasma apoB and triglyceride kinetics are modeled using a four-compartment hydrolysis chain, in which the kinetics of apoB and triglyceride coupled. For each apoB compartment, there is an equivalent compartment for triglyceride. Triglycerides hydrolyzed from VLDL particles are represented by the dashed arrows, and particles lost from the plasma space are represented by the solid arrows. See Adiels et al. (62) for additional model details.

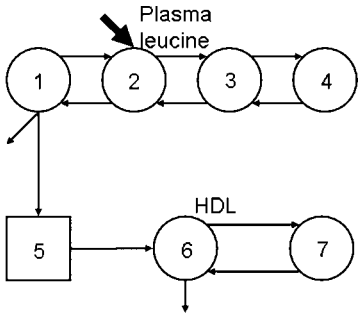

Fig. 5. Compartment model describing HDL apoA-I tracer kinetics (64). Leucine tracer is injected into plasma, represented by compartment 2. Compartments 1, 3, and 4 represent nonplasma leucine compartments. Compartment 5 represents an intrahepatic pool that accounts for the time associated with the synthesis, assembly, and secretion of the apoA-I HDL fraction. HDL apoA-I is represented as a single plasma compartment, compartment 6. ApoA-I is cleared from this compartment and exchanges with an extravascular HDL pool, compartment 7.

in which the differential regulation of the $VLDL_1$ and $VLDL_2$ fractions cannot be measured, this model provides a greater understanding of VLDL metabolism and demonstrates the importance of subfractionating the VLDL pool.

As discussed above, the issue of tracer recycling is a potential confounder in estimating the kinetics of slowly turning-over lipoprotein fractions, including LDL apoB and HDL apoA-I and apoA-II. Three- and four-compartment models are typically used to describe the plasma kinetics of the injected labeled amino acid tracer. Although this submodel may include a recycling component, additional tracer amino acid recycling may be present in the hepatic pool that would affect the resulting LDL apoB tracer curve and hence the estimated FCR. Experimental designs that include exogenous and endogenous labeling protocols should be able to determine the contribution of recycling to tracer data resulting from endogenous labeling. As a consequence, the impact of recycling often means that the kinetics of extravascular LDL and HDL compartments cannot be estimated without imposing model constraints (33).

HDL apoA-I and apoA-II. HDL plays an important role in transporting cholesterol from peripheral tissues directly back to the liver or indirectly via IDL and LDL (and chylomicron) particles, in a process popularly referred to as reverse cholesterol transport. In human plasma, apoA-I and apoA-II are the major apolipoproteins of HDL. **Figure 5** shows a multicompartment model commonly used to describe HDL apoA-I (or apoA-II) tracer data (64). Similar to the apoB model (Fig. 3), compartments 1–4 describe plasma leucine kinetics. This subsystem is connected to a delay compartment (compartment 5) that accounts for the time required for the 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 plasma and in an extravascular 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 has 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.

Fisher et al. (65) proposed several models that described fast and slow apoA-I secretory pathways. These pathways may reflect the secretion of apoA-I from the liver and intestine, but this remains to be confirmed. Despite this, a compartment model that includes two synthesis pathways often provides a better fit to HDL apoA-I tracer data than the standard HDL compartment model.

HDL particles are heterogeneous and can be separated according to their apoprotein content into LpA-I particles, containing apoA-I alone, and LpA-I:A-II particles, containing both apoA-I and apoA-II (66). Taskinen et al. (67), using radiotracers and exogenous labeling, 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 nondiabetic subjects. Using HDL apoA-I and apoA-II tracer data together with the concentration of LpA-I:A-II particles, we have developed and validated a new compartment model that describes the kinetics of LpA-I and LpA-I:A-II particles (**Fig. 6**) (68). Leucine tracer is injected into plasma (compartment 2) and distributes to extravascular compartments 1, 3, and 4. Compartment 1 is connected to an intracellular (hepatic and enterocytic) delay compartment (compartment 5) that accounts for the synthesis, assembly, and secretion of

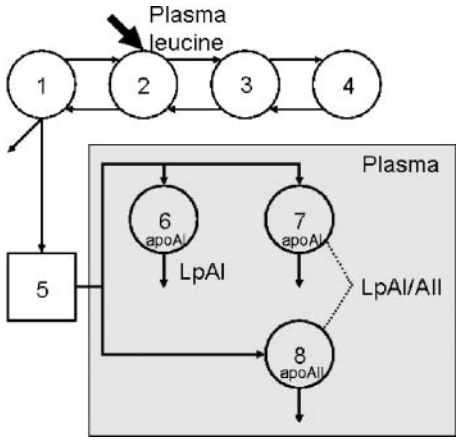

Fig. 6. Compartment model describing apoA-I in LpA-I and LpA-I:A-II particles and apoA-II tracer kinetics (68). Leucine tracer is injected into plasma, represented by compartment 2, and distributes to extravascular compartments 1, 3, and 4. Compartments 1–4 are required to describe leucine tracer kinetics observed in plasma. Compartment 1 is connected to an intracellular (hepatic, enterocytic) delay compartment (compartment 5) that accounts for the synthesis, assembly, and secretion of apoA-I and apoA-II. Compartments 6 and 7 describe the plasma kinetics of HDL apoA-I associated with LpA-I and LpA-I:A-II particles, respectively. The plasma kinetics of HDL apoA-II, which represents the apoA-II component of LpA-I:A-II, are described by compartment 8. See Ji et al. (68) for additional model information. (Reproduced with permission from Ji, J. 2006. High density lipoprotein transport in the metabolic syndrome. *J. Clin. Endocrinol. Metab.* **91:** 973–979. Copyright 2006, The Endocrine Society.)

apoA-I and apoA-II (the time associated with the synthesis and secretion of apoA-II could be different from that for apoA-I). Compartments 6 and 7 describe the plasma kinetics of HDL apoA-I associated with LpA-I and LpA-I:A-II particles, respectively. The kinetics of HDL apoA-II, which represent the apoA-II component of LpA-I:A-II, is described by compartment 8. The model assumed that the kinetics of apoA-II and apoA-I in the LpA-I:A-II particle are the same. The LpA-I/LpA-I:A-II section of the model was validated using plasma HDL apoA-I and apoA-II tracer data in conjunction with the findings of two published studies (69, 70). An extravascular compartment has often been used to help describe the kinetics of HDL apoA-I and apoA-II, although the exact nature of the exchange compartment is unknown. We tested whether adding an extravascular compartment would improve the fit of the model to the tracer data. The tracer data, however, did not support the presence of such a compartment, and this was reflected in the poor precision of the model parameters and a larger value for the Akaike information criteria test.

Rader et al. (70) observed the exchange of apoA-I between LpA-I and LpA-I:A-II particles, consistent with the remodeling of HDL particles. Although the model we developed incorporated such an exchange pathway, the tracer data did not support the existence of such a process. Additional studies that subfractionate the HDL pool according to apoprotein composition are warranted to describe this aspect of HDL metabolism and further develop the HDL compartment model.

Preβ-HDL apoA-I. Subpopulations of HDL particles, small preβ$_0$- and preβ$_1$-HDLs, have been identified as highly effective initial acceptors of cellular cholesterol. Preβ-HDLs appear to play an essential initial role in the reverse cholesterol transport pathway, ensuring the removal of excessive cholesterol from peripheral cells, regressing foam cell formation, and associated atherosclerotic lesions. Chetiveaux et al. (71) recently described a new model for apoA-I metabolism in preβ$_1$-HDL and α-HDL subpopulations (**Fig. 7**). Their tracer studies were of only 14 h duration and used a PCI protocol to administer [$^2H_3$]leucine tracer. In this model, apoA-I is secreted into plasma, where a fraction of the preβ-HDL pool exchanges with α-migrating HDL particles. Although not evident in the tracer data, an exchange pathway between these compartments supports the notion that large HDL particles are recycled to form small HDL particles, which may act as the primary acceptors of cholesterol in the reverse cholesterol transport pathway. Although they presented no evidence of a rapidly turning-over pool of HDL particles in the tracer data, the authors suggest that the preβ$_1$-HDL pool turns over 100 times per day. This model has been used to describe the kinetics of preβ-HDL and α-HDL subpopulations in normal as well as type 2 diabetic subjects (71, 72).

ApoC-III. ApoC-III plays an important role in regulating the metabolism of triglyceride-rich lipoproteins (TRLs) (73). The role of apoC-III in HDL metabolism, however, remains unknown. Compared with apoB and HDL apoA-I, few studies have examined the kinetics of apoC-III. Early apoC-III radiotracer studies supported the complete exchange of apoC-III between the VLDL and HDL fractions (74, 75). Bukberg et al. (76), using similar methodology, also observed rapid, but not complete, exchange of apoC-III between VLDL and HDL. However, recent studies using endogenous labeling with stable isotopes show divergent VLDL and HDL apoC-III tracer curves, suggesting only partial exchange of apoC-III between these fractions (77). The disparity between exogenous and endogenous labeling studies may be attributable to methodological limitations of the isolation of apoC-III from HDL. Recently, our group used Intralipid to extract apoC-III from VLDL and HDL fractions (78). This methodology removed apoC-III from VLDL and HDL free of contamination by apoproteins present on these particles in higher

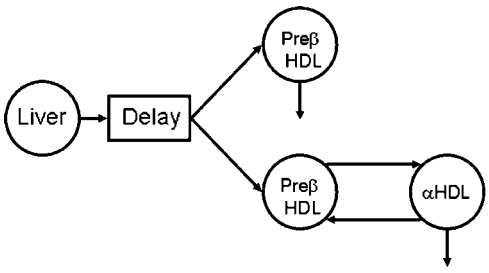

Fig. 7. Multicompartment model for kinetics analysis of apoA-I preβ$_1$-HDL and apoA-I of α-HDL obtained with fast-protein liquid chromatography. See Chetiveaux et al. (71) for additional model information.

concentration. Consistent with the early radiotracer studies, the tracer enrichment curves for VLDL and HDL apoC-III were superimposable, suggesting rapid and complete exchange of apoC-III between the two fractions. A compartment model with a four-compartment leucine subsystem, a delay compartment (for the synthesis and secretion of apoC-III), and a single plasma compartment was used to describe VLDL and HDL apoC-III kinetics. Using this model, we found that the FCRs of apoC-III in VLDL and HDL were not different, supporting the concept of a single, kinetically homogeneous pool of apoC-III in plasma (78).

ApoE. The important role of apoE in lipoprotein metabolism has been well described (79). Radiotracer studies using exogenous labeling methodologies demonstrated kinetic differences between apoE isoforms (80). Furthermore, they provided evidence of rapid exchange or recycling of apoE between the VLDL and HDL fractions. In vitro studies supported these observations but also identified a nonexchangeable pool of VLDL apoE (81). After lipolysis of VLDL triglycerides, this pool of apoE was freely exchangeable. Using stable isotope and endogenous labeling, Millar et al. (82) tested and developed a compartmental model to describe apoE kinetics in humans. The model consists of a single leucine compartment followed by a delay compartment accounting for the synthesis and secretion of apoE into plasma. From this delay, apoE enters plasma associated with the TRL or HDL fraction. A number of models were proposed suggesting a precursor-product relationship between HDL and TRL apoE but no exchange process between TRL and HDL. Reconciling this compartment model with previous observations of rapid exchange remains to be undertaken. The model also included an extracellular compartment (e.g., hepatic lymph) accounting for the removal and reintroduction of TRL apoE from plasma.

Recently, several studies have examined the kinetic properties of apoE in relation to the metabolism of VLDL and HDL lipoprotein fractions (83, 84). Batal et al. (83) developed a simple three-compartment model to describe apoE kinetics in humans. The first compartment represented the plasma amino acid precursor pool. The second compartment was a delay compartment, which accounted for the synthesis, assembly, and secretion of apolipoproteins. The third compartment was the plasma protein compartment. In contrast to the radiotracer studies (80), there was little evidence of apoE exchange between the VLDL and HDL fractions. The longer residence time of VLDL apoE compared with VLDL apoB indicates that apoE moves from particle to particle (exchanges) within the VLDL fraction and perhaps between HDLs before removal from plasma. It remains unclear whether or how much recycling of apoE occurs between the VLDL and HDL fractions. This is analogous to the findings for apoC-III described above.

VLDL triglycerides. Hypertriglyceridemia is an independent risk factor of cardiovascular disease. Furthermore, it is important to appreciate the role of lipids in the progression of cardiovascular disease; thus, studies of the kinetics are important. Triglyceride synthesized in the liver is secreted into plasma on VLDL particles. Therefore, understanding VLDL triglyceride metabolism has important physiological and clinical implications. Patterson et al. (85) designed a four-compartment model to provide a more comprehensive analysis of VLDL triglyceride tracer kinetics based on the model developed by Zech et al. (63). Glycerol tracer is injected into plasma. The model includes a delay compartment that accounts for the time associated with the synthesis, assembly, and secretion of VLDL triglyceride, and another compartment accounts for nonsystemic tracer recycling after a bolus injection of labeled glycerol. As described above, Adiels et al. (62) developed a complex compartment model to assess the kinetics of triglyceride and apoB-100 in VLDL subpopulations simultaneously. Other methods for measuring in vivo lipid metabolism have been reviewed recently (86).

Cholesterol. Methods to assess cholesterol metabolism are complex by virtue of the fact that cholesterol exchanges between lipoprotein fractions. Despite this, methods to assess reverse cholesterol transport are important to evaluate the effectiveness of lipid-lowering therapies. The compartment model developed by Schwartz et al. (87) highlights the complexity of cholesterol metabolism and the need to use multiple tracers to estimate the parameters of importance. Using a simpler approach, Ouguerram et al. (88) developed a [^{13}C]acetate method to assess cholesterol metabolism. However, with the rapid exchange of cholesterol between apoB-containing lipoprotein fractions and HDL, it is difficult to interpret such studies. The use of interventions that affect cholesterol metabolism may demonstrate the utility of this method, however.

CONCLUSION

Lipoprotein kinetics studies have contributed significantly to our understanding of lipoprotein metabolism. Although complex, such studies provide data that enable metabolic pathways to be measured and defined. The science of lipoprotein kinetics relies on the acquisition of reliable empirical data and to a larger extent on the instrumentalist approach provided by mathematical modeling. The basic methodological principles used for the design and analysis of kinetics studies have not changed significantly. In contrast, the tools, specifically the types of tracers, laboratory methods, and equipment and software, used for the modeling and analysis of tracer data are now better than ever. As with all research, the importance of good experimental design cannot be underestimated. Good experimental design includes an awareness of good clinical practice and the regulations required for responsible clinical research. Furthermore, given the investment required for kinetics studies, in terms of both time and finances, it is imperative that studies be statistically powered to address a clear experimental hypothesis.

Kinetics analysis and modeling are tools that can be used to infer information about a biological system. With new knowledge comes increasing complexity and hence the use of models. Compartment models of lipoprotein metabolism enable the system to be described using systems of equations that can be fit to experimental data to provide quantitative information. In addition, different structural models can be tested against experimental data for the purpose of hypothesis generation or simply to find the model that best describes the data.

Compartment models represent the gold standard for the analysis of lipoprotein kinetics studies. These models have provided new knowledge with respect to the mechanisms of production and catabolism that result in dyslipidemic states. In intervention studies, the use of tracer kinetics studies and modeling methods has provided new knowledge of the mechanisms by which lipoprotein concentrations, albeit primarily apoprotein concentrations, are altered. The challenge for the future will be to design kinetics studies that will provide new knowledge of lipid metabolism, specifically the important pathway of reverse cholesterol transport, for which our current knowledge is largely limited to studies of HDL apoA-I metabolism.

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