Isotope Tracers in Metabolic Research. Principles and Practice of Kinetic Analysis, 2nd Edition

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Since 1992, *Radioactive and Stable Isotope Tracers in Biomedicine: Principles and Practice of Kinetic Analysis* by Robert R. Wolfe has been a standard reference for the application of isotopically labeled tracers to the study of in vivo metabolic kinetics. The new second edition contains a number of significant improvements over the first edition, most notably the inclusion of new topics that have undergone considerable growth and development during the past 13 years. In addition, all figures have been updated to provide a more professional appearance, numerous errors have been corrected, and many chapters include new sidebars that provide specific examples and numerical calculations for the covered principles. Augmenting Dr. Wolfe's expertise in metabolism and physiology is a new coauthor, David L. Chinkes, who has made numerous contributions to the mathematical analysis of metabolic kinetics. This book draws on the authors' expertise and considerable literature contributions, and provides a thorough introduction to the subject.

Chapters 1 and 2 introduce tracers and terminology, provide a contrast between radioactive and stable tracers, and cover the measurement of radioactive tracers, specific activity, and radiation dosages. Together, the contents of these two chapters comprised a single chapter in the first edition, but the present division of topics is more practical.

Chapters 3 and 4 cover principles of metabolic kinetic analysis. Chapter 3 ("Calculation of Substrate Kinetics: Single-Pool Model") covers basic principles of metabolic kinetics in systems that are treated as a single pool at metabolic steady state, and the determination of the rate of appearance (Ra) of a tracee in plasma using constant infusion, bolus, and primed constant infusion protocols for the administration of tracers. A discussion of the validity of single-pool models appears, followed by a discussion of complications arising from nonsteady state conditions. New material appears on the principle of isotope dilution and on linear and spline curve fitting. Chapter 4 ("Calculation of Substrate Kinetics: Multiple-Pool Models") includes expanded coverage of noncompartmental models, compartmental models, and identifiability. The chapter includes a lengthy discussion on model validation, and a new section on model simulations (as opposed to fitting data to models).

The topical order has been rearranged in the second edition. Two chapters that are concerned with mass spectrometry instrumentation and the measurement of stable isotope enrichment appear after the two chapters concerned with principles of kinetic analysis. Since the latter uses certain concepts of stable isotope enrichment (e.g., tracer/tracee ratio, atom % excess), it would have been more practical to have the two chapters on the measurement of stable isotope enrichment to immediately follow Chapter 2 that is concerned with the measurement of radioactive tracer enrichment.

Chapter 5 consolidates and abbreviates information that was formerly divided among three separate chapters that dealt with IRMS, GC/MS, and chromatography. This short chapter provides a brief overview of the principles of operation for mass spectrometers commonly used to measure stable isotope enrichment for metabolic tracer kinetic studies. New material on LC/MS is included. Readers are directed to recent texts for a more thorough background on instrumentation.

An understanding of Chapter 6 ("Determination of Isotope Enrichment") is critical to the use of stable isotopically labeled tracers for metabolic kinetics. Various measurements of enrichment, including tracer to tracee ratio (TTR), atom % excess (APE), mole % excess (MPE), and delta (δ) and how they relate to specific instrumentation (IRMS, GC/MS), are explained. TTR is the preferred choice for substrate kinetics, and it is shown how other measures are converted to TTR. Abundant numerical examples are given to illustrate the calculations. Somewhat surprisingly, the discussion of δ from IRMS measurements does not mention international standards (e.g., PDB, SMOW, SLAP) that are used as references for the measurement of absolute ^{13}C, ^{2}H, and ^{18}O abundances. The discussion of GC/MS includes illustrations of fragmentation spectra, positional labeling specificity, and selected ion monitoring. There is a useful description of how to calculate molecular isotopomer distribution abundances from atomic isotopic abundances, including two appendices to the chapter that detail the calculations and describe a computer program available from the authors. Details are given to calculate the TTR from ion abundance ratios measured by GC/MS. This approach assumes that the relative yield of a molecular fragment is the same in the tracer and tracee molecules (i.e., there is no isotope effect in the fragmentation of the molecule) such that measured ion abundance ratios are assumed to equal true molar tracer/tracee ratios. Little consider-

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ation is given to the use of isotopic enrichment standard curves to correct measured ion abundance ratios for isotope effects. The chapter includes new material describing how very low enrichments (TTR down to 0.001%) can be measured by GC/MS using highly substituted tracers.

Chapters 7 to 15 are devoted to individual specific applications, and update material from the first edition with error corrections, improved figures, and significant new material included for some topics. These chapters provide useful backgrounds for each specific application, and keen insight into how tracers have been used to examine these areas of metabolism. Extensive literature bibliographies are included with these chapters, and many include new sidebars to explicitly demonstrate numerical calculations.

Chapters 7 ("Measurement of Substrate Oxidation") and 8 ("Measurement of Total Energy Expenditure Using the Doubly Labeled Water Method") are virtually unchanged from the former edition. Chapter 9 ("Mass Isotopomer Distribution Analysis") is a new chapter that provides a clear and concise overview of how MIDA is used to determine the isotopic enrichment of an inaccessible precursor pool when multiple monomers of that precursor are used to form a polymer product (e.g., ^{13}C-acetate incorporation into palmitate). Various computational approaches to MIDA that have been reported in the literature are reviewed. This chapter would benefit from a detailed numerical example to take the reader from raw GC/MS measurements to final calculated results as has been incorporated into many other chapters.

Chapter 10 ("Glucose Metabolism") is mostly based on the same chapter in the earlier edition, with the addition of new topics including the use of $^{2}H_2O$ to measure gluconeogenesis and MIDA approaches with ^{13}C-labeled glycerol or lactate. A section on lactate kinetics has been updated. Chapter 11 ("Lipid Kinetics") includes updated material on the Ra of multiple free fatty acids and glycerol. New material is included that covers intramuscular glycogen and triglyceride oxidation, the use of ^{2}H-labeled palmitate to measure fatty acid oxidation, the measurement of de novo fatty acid synthesis by ^{13}C-acetate incorporation and MIDA, and the measurement of VLDL-TG kinetics.

The earlier edition devoted three chapters to protein and amino acid metabolism (urea kinetics, amino acid kinetics, and protein synthesis and breakdown). This material has been augmented with new material and reorganized into four chapters (Chapters 12 to 15). Chapter 12 focuses on the assessment of whole-body protein synthesis and breakdown rates by intravenous or oral administration of tracer-labeled amino acids. Chapter 13 narrows the focus from whole-body protein metabolism to the measurement of specific proteins, especially of bulk muscle protein. There is an extensive description of the fractional synthesis rate (FSR) and problems related to knowing the enrichment of the pool used as a precursor for synthesis. A very useful discussion of practical considerations such as the selection of tracer to use, tracer infusion rates, and sampling protocols is included. This chapter includes new material regarding the FSR of DNA, although this is not reflected in the chapter title. Chapter 14 covers the measurement of regional or tissue protein breakdown rates; much of this chapter concerns a novel approach to measure tissue protein fractional breakdown rate (FBR) recently developed by the authors. Finally, Chapter 15 concerns arterio-venous (A-V) balance techniques to measure amino acid kinetics. This chapter includes extensive new material regarding 3- and 4-pool models the authors have developed to measure tissue amino acid inflow and outflow rates, in addition to protein synthesis and breakdown rates, by measuring intracellular amino acid isotopic enrichment as well as tissue protein enrichment in tissue biopsy samples. The reorganized chapters on protein and amino acid metabolism have omitted much of the material on urea kinetics that was in the previous edition.

Finally, Chapter 16 is a new chapter on nuclear magnetic resonance. This chapter contains substantial background on principles of NMR with specific examples of ^{13}C-NMR related to muscle or hepatic glycogen and lipid content and gluconeogenesis.

The book concludes with a four-page glossary and a four-page list of abbreviations, which are very useful additions to the new edition. The 18-page index is much more extensive than the seven pages in the first edition.

Unfortunately, several key topics from the first edition have been omitted, including Chapter 9 ("Selection of Tracer Infusion and Sampling Sites"), Chapter 10 ("Assessment of Body Composition"), and Chapter 11 ("Performance of Tracer Infusions"). The new edition has also omitted numerous practical guides to laboratory techniques that appeared in the previous edition, e.g., how to prepare tracer palmitate/albumin solutions for infusion studies. Most notably, the new edition has omitted two appendices that covered methods for sample processing, derivatizations for GC/MS, and examples of mass spectra.

The new edition provides less practical information concerning how to perform tracer studies, and thus does not fully replace the former edition. Nevertheless, the second edition does contain a number of significant updates, enhancements, and improvements, and should be required reading for anyone who desires to know more about metabolic tracer kinetics.