2014 Course Schedule

ISOTOPE TRACERS in METABOLIC RESEARCH:

Principles and Practice of Kinetic Analysis

NOVEMBER 10 - 14, 2014

WYNDHAM Hotel *at Playhouse Square*

1260 Euclid Avenue
Cleveland OHIO 44115

FACULTY

Douglas E. BEFROY

Henri BRUNENGRABER - *Course Co-Director*

Gary CLINE

Joanne KELLEHER

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Owen McGUINNESS

Matthew MERRITT

Elizabeth PARKS

Stephen PREVIS

Michelle PUCHOWICZ

Robert R. WOLFE - *Course Co-Director*

General Information

COURSE **FORMAT**

The course and homework problems will be run on paperless format. Participants are expected to bring a wireless laptop computer, running MS Excel, if possible. All course material (including slides and problem sets) will be available for download a few days before the course starts.

> Registered participants will receive the link and password to download the course material on Friday, NOVEMBER 7, 2014 **by email**. Feel free to print the downloaded material.

Note 1. The faculty will systematically upload any new or additional material (including problems' solutions) on the course webpage.

Note 2. In order to foster intellectual exchanges without fear of plagiarism, this course will have a closed meeting format, just like a Gordon Conference.

COURSE **CONTENT**

We would like as much as possible to tailor faculty presentations to the interests of the attendees. Therefore, please feel free to send us a list of topics you would wish to be expanded upon, before Monday, NOVEMBER 3, 2014. Please outline these topics in as much detail as you feel comfortable.

Feel free to suggest a topic which is **not** currently listed in the syllabus and send your suggestions to Dr. Brunengraber at hxb8@case.edu

PRESENTATIONS by PARTICIPANTS

There will be trainee presentations on the evening of Wednesday, NOVEMBER 12, 2014. Specifically, 10 participants will have the opportunity to outline their research project (planned *or* ongoing) involving isotopic tracers. We thus invite you to prepare a 7-8 minute slide presentation, which will summarize your project, emphasizing the protocols that use isotopes, the quantitative data you expect to obtain, and any questions you have on the validity of protocols and data interpretation. Each presentation will be followed by comments from the faculty and attendees.

Please notify Dr. Brunengraber at hxb8@case.edu by Monday, NOVEMBER 3, 2014 if you wish to make such a presentation (please let us know the title of your presentation). If your presentation is not selected for the Wednesday evening session, you will have an opportunity to present it later to a select faculty member (*see below*).

ONE-ON-ONE MENTORING

Participants are also invited to set up 30-minute One-on-One Mentoring/Discussion Sessions with any course faculty.

Starting Monday, November 10, 2014 (*1st day of the course*), you will be able to set up appointments with course faculty. The scheduling process will be discussed in more detail in an upcoming email.

Monday, NOVEMBER 10, 2014

morning • **7:30 am**

CONTINENTAL BREAKFAST • SIGN-IN • MEET & GREET

7:30 - 8:30 am

Wyndham Hotel

WELCOME

8:30 am

MAREN **LAUGHLIN**, Senior Advisor, NIDDK, NIH

USE OF RADIOACTIVE ISOTOPES

HENRI **BRUNENGRABER**

A. LEARNING OBJECTIVES

- Responsible Conduct of Research in the use of radioactive isotopes
- How does one optimize the measurement of radioactivity of compounds labeled with ^{14}C or ^{3}H.
- How does one measure a metabolic rate using ^{13}C or ^{3}H tracers?
- What are the limitations of the use of radioactive isotopes to measure metabolic rates?

B. SECTIONS

- Measurement of beta radioactivity by scintillation counting; conversion of cpm to dpm (external standards, automatic quench correction, internal standards); how does one deal with counting artifacts (quenching, chemiluminescence).
- Principles of measurement of metabolic rates; notion of specific activity of labeled precursor; problems and solutions with variations of specific activity of precursor (how does one avoid dealing with one equation and two variables).
- Limitations of the use of isotopes for metabolic studies; difference between transfer of label and net flux; isotopic exchanges;

  (Difference between **exchange** flux and **net** flux)

C. HOMEWORK BREAKOUT

(SMALL GROUPS)

BASIC CONCEPTS IN MASS SPECTROMETRY

ROBERT R. **WOLFE**

A. LEARNING OBJECTIVES

- Gain an understanding of the main mass spectrometry techniques used to investigate metabolic processes with stable isotopes.
- Become familiar with current expressions of isotopic enrichment, including Tracer:Tracee Ratio and atom (or mol) percent excess.
- Learn how to measure isotopic enrichment by mass spectrometry (basic approaches).
- Learn how to calculate isotopic enrichment using gas chromatography-mass spectrometry and LC-MS/MS.

B. SECTIONS

1. BASIC DESCRIPTION OF INSTRUMENTATION

- Isotope ratio mass spectrometry (IRMS).
- Gas chromatography mass spectrometry (GC-MS).
- Gas chromatography-combustion-isotope ratio mass spectrometry (GC-C-IRMS).
- Liquid chromatography-mass spectrometry (LC-MS; LC-MS/MS).

2. CALCULATION OF ENRICHMENT WITH IRMS

- Correction of enrichment for background enrichment.
- Tracer:Tracee Ratio (TTR) *vs.* Molar Percent Enrichment (MPE).
- Skew correction factor to correct for the fact that the natural distribution of mass isotopomers is the same in the sample and the background.
- Use of a standard to calculate enrichment.
- Measurement of ^{13}C-enrichment after combustion.
- Effect of sample size on observed ratio.

3. CALCULATION OF ENRICHMENT USING GC-MS

- Definition of total ion chromatogram, mass spectrum, and selected ion monitoring (SIM).
- Identifying appropriate fragment to monitor.
- Calculation of theoretical abundance.

- Calculation of isotopic enrichment using SIM.
- Effect of skewed abundance of tracer, skew correction factor.
- Overlapping spectra correction.
- Calculation of TTR when TTR > 1
    i. Using multiple ions to calculate isotopic enrichment
    ii. Using lees abundant masses to measure low levels of enrichment
    iii. Calculation of concentration by internal standard technique

C. HOMEWORK BREAKOUT

(SMALL GROUPS)

MEASUREMENT OF METABOLIC FLUXES WITH ISOTOPIC TRACERS

ROBERT R. **WOLFE**

A. LEARNING OBJECTIVES

- Responsible Conduct of Research in human and animal investigations.
- Gain a conceptual and practical understanding of calculating the rate of substrate appearance (**Ra**) by tracer dilution using a single pool model with radioactive and stable isotopes.
- Understand the benefit of priming the substrate pool, how to calculate a tracer priming dose, and the limitations of the primed-constant infusion technique.
- Understand the basic approach for calculating substrate oxidation using a metabolic tracer.
- Understand the calculation of fractional synthetic rate.

B. SECTIONS

1. TRACER KINETICS-SINGLE POOL MODELS

- Constant infusion of tracer.
- Influence of changes in uptake on calculation of rate of appearance.
- Calculation of Ra with a bolus injection of tracer.
- Priming the pool.
- Estimation of Ra in the non-steady state.
- Minimizing errors by curve fitting.

2. INCORPORATION STUDIES

- Principles and calculation of substrate oxidation at the whole body level using tracers, including use of Atom Percent Excess *vs.* Tracer:Tracee Ratio.
- Bicarbonate recovery factor.
- Improving the estimation of true precursor enrichment.
- Priming the bicarbonate pool.
- Determination of carbon dioxide production with labeled bicarbonate.
- Problems in determining oxidation with tracers.
- Labeled CO_2 reincorporation.
- Contribution of naturally occurring ^{13}C to apparent CO_2 enrichment.
- Fractional synthetic rate.
- Synthetic rate.

C. HOMEWORK BREAKOUT

(SMALL GROUPS)

GLUCOSE KINETICS (INCLUDING THE EUGLYCEMIC CLAMP)

OWEN **McGUINNESS**

A. LEARNING OBJECTIVES

- Responsible Conduct of Research in such investigations.
- Define the physiological correlates of glucose flux.
- Learn best practices for experimental design optimization and data interpretation to evaluate insulin action.

B. SECTIONS

1. OVERVIEW OF GLUCOSE KINETICS

- Define steady state.
- Define the relationship between glucose concentration and glucose mass in the body.
- Identify sites and relative rates of glucose production and consumption and how these rates differ among species.

2. WHAT ARE THE SOURCES OF GLUCOSE APPEARANCE?

- Understand from a tissue point of view what production is.
- Define the relative contribution of the liver and kidney to glucose production.

3. HOW DO WE GET STARTED?

- Choosing a tracer.
- Understand how the sites of sampling and infusion can influence the measured rates of glucose flux.
- Know how to optimize the study design to maximize steady state conditions.

4. ASSESSING INSULIN ACTION:

- Know how fasting status influences insulin action differently in mice and humans.
- Define what insulin action is in the liver and the periphery.
- Understand what a euglycemic hyperinsulinemic clamp is and how to deal with variable rates of endogenous insulin and glucagon secretion.
- How to recognize and deal with tracer/model assumption errors (non steady-state and negative endogenous production rates).
- Be able to evaluate data used to calculate hepatic and peripheral insulin action.
- Understand the principles used in assessing tissue specific glucose uptake.

5. DATA PRESENTATION:

- Know what information to include in a manuscript so as to adequately interpret the data.

C. HOMEWORK BREAKOUT

(SMALL GROUPS)

LIPID METABOLISM: BASIC KINETICS

ELIZABETH **PARKS**

A. LEARNING OBJECTIVES

- To understand the principles and limitations of various types of measurements of metabolic rates using stable isotopes.

B. SECTIONS

1. LIPOLYSIS AND FATTY ACID RELEASE

- Pros and cons of glycerol as a tracer to measure lipolysis.
- Measurement of fatty acid flux with labeled fatty acid tracers.
- Triglyceride-fatty acid substrate cycling limitations.

2. FATTY ACID OXIDATION

- Possible pathways of fatty acid oxidation.
- Citric acid cycle exchange reactions.
- Acetate correction factor.
- *in vivo* assessment of CPT activity.

3. TECHNIQUES FOR INVESTIGATING LIPOPROTEIN METABOLISM

INTRODUCTION TO THE NIH GRANTS PROCESS

MAREN **LAUGHLIN**, Senior Advisor (NIDDK, NIH)

INSULIN AND GLUCOSE CLAMP

OWEN **McGUINNESS**

and

(SMALL GROUP DISCUSSIONS)

USE OF POSITIONAL ISOTOPOMER ANALYSIS TO ASSESS PATHWAY FLUXES

GARY **CLINE**, MATTHEW **MERRITT**, DOUGLAS **BEFROY**

A. LEARNING OBJECTIVES

- Understand the basic principles of magnetic resonance.
- Understand how the information content of NMR data differs from MS data.
- Understand how metabolic flux information is extracted from NMR data.
- Review common applications of NMR to metabolic flux measurements

B. SECTIONS

PART I. NMR IN TRACER METABOLISM (45 MIN, MERRITT)

- Basic NMR principles:
  a. Measurement of fractional enrichment, spin-spin coupling, multiplet analysis
  b. Measuring ^{13}C and ^{2}H isotopomer distribution

PART II. APPLICATIONS TO BIOCHEMICAL PHYSIOLOGY: STEADY STATE MEASUREMENTS OF METABOLIC FLUXES (60 MIN, CLINE)

a. Metabolic pathways in isolated cells:
   TCA cycle, anaplerosis, and substrate cycling

b. Calculating hepatic fluxes by multinuclear NMR:
   glycogen synthesis pathways, gluconeogenesis and glycogenolysis,
   TCA cycle pathways.

PART III. *IN VIVO* APPLICATIONS: KINETIC ANALYSIS OF METABOLIC FLUXES (60 MIN, BEFROY)

a. Practical aspects of performing *in vivo* experiments (homogeneity, localization, lipid suppression etc.)
b. Conventional ^{13}C labeling strategies (Brain / Muscle)
c. Alternative ^{13}C labeling strategies (Brain / Liver)
d. Complementary *in vivo* techniques

PART IV. EVOLVING TECHNIQUES (30 MIN, MERRITT)

a. Hyperpolarization-intracellular fluxes

MEASURING SYNTHESIS OF ADENINE NUCLEOTIDES, COENZYME A, AND NUCLEIC ACIDS

HENRI **BRUNENGRABER**, JOANNE **KELLEHER**

A. LEARNING OBJECTIVES

- Identify problems associated with the use of isotopic tracers for very long experiments (weeks or months).
- Long-term isotopic experiments occur in an open biological system where unlabeled foodstuffs enter the system continuously.
- During long-term isotopic experiments, salvaged pathways into de novo synthesis pathways recycle labeled intermediates.

B. SECTIONS

- Comparing synthetic rates investigated with $^{2}H_2O$ *vs.* [^{13}C]substrates. (BRUNENGRABER)
- Whole pathway kinetics. Upgrading our understanding of pathway labeling. (KELLEHER)
- Information obtained on the scrambling of label into related pathways. (BRUNENGRABER)

METHODS IN PROTEIN METABOLISM

ROBERT R. **WOLFE**

A. LEARNING OBJECTIVES

- Understand the use of whole body protein turnover techniques.
- Earn how to calculate the rate of synthesis of individual proteins.
- Learn how to measure tissue protein and amino acid kinetics using tracers vascular catheters.

B. SECTIONS

1. WHOLE BODY PROTEIN TURNOVER

- Catabolic and anabolic states
- Energy cost of protein synthesis
- Stochastic model of whole body protein turnover
- Comparison of tracers
- Isotopic determination of urea production
- Single amino acid tracer kinetics to calculate whole body protein turnover

2. MEASUREMENT OF PROTEIN FSR

- Constant tracer infusion
- Flooding dose tracer injection
- Sub-flooding dose tracer injection

3. METHODS TO ESTIMATE PRECURSOR ENRICHMENT FOR MEASUREMENT OF FSR 4

- Fractional breakdown rate
- Constant tracer infusion
- Bolus injection

4. ARTERIO-VENOUS MODEL

- Measurement of A-V balance
- 3-pool and 4-pool models of protein kinetics and amino acid transport
- Measurement of tissue oxidation rate
- Technical aspects of performing A-V balance studies from mouse to human

TRAINEES PRESENTATIONS (10)

MEASUREMENTS OF ENERGY EXPENDITURE

STEPHEN **PREVIS**

A. LEARNING OBJECTIVES

- List different methods for quantifying energy expenditure (or CO_2 production)
- Identify the pros/cons for each.
- Outline the general principle of using doubly-labeled water, listing important criteria for the experimentalist.
- Explain the rationale for different data normalization/interpretation.

B. SECTIONS

1. OVERVIEW OF ENERGY EXPENDITURE

- Where does “energy” go?

2. HOW DO I QUANTIFY TISSUE-SPECIFIC RATES OF CO_2 PRODUCTION?

- Arterio-venous balance is required
- Single *vs.* multiple compartments
- Concerns about mixing/complete perfusion

3. HOW DO I QUANTIFY SUBSTRATE-SPECIFIC RATES OF CO_2 PRODUCTION?

- Measure the production of ^{13}C-labeled CO_2
- Concerns about the recovery of a labeled substrate

4. HOW DO I QUANTIFY TOTAL BODY CO_2 PRODUCTION?

- Direct calorimetry
- Indirect calorimetry
  i. Direct measurements of gas exchange
  ii. Indirect measurements of gas exchange (*i.e.:* doubly labeled water)

5. HOW DO I PROCESS THE DATA AND NORMALIZE THE RESULTS?

USE OF $^{2}H_2O$ FOR MEASURING SYNTHESIS OF PROTEINS, FATS, STEROLS, GLUCOSE AND NUCLEIC ACIDS

STEPHEN **PREVIS**

A. LEARNING OBJECTIVES

- Explain the general use of precursor:product labeling ratios in biochemical research, list general equations for calculating rates of synthesis in short-term vs. long-term studies, i.e. those that run over several hours *vs.* those that run over several days, respectively.
- Suggest reasons that make $^{2}H_2O$ a unique tracer for measuring the synthesis of various macromolecules.
- Explain why one requires knowledge of the labeling of specific hydrogen(s) in a product molecule in order to accurately determine its rate of synthesis.
- Contrast the pros/cons of using GC-MS *vs.* NMR to measure the labeling of molecule.

B. SECTIONS

1. WHAT CAN ONE DO WITH $^{2}H_2O$ THAT CANNOT BE DONE WITH OTHER TRACERS?

- Simultaneous tracing of multiple processes

2. CHOICE BETWEEN ACUTE AND CHRONIC LABELING STUDIES?

- Source(s) of blood glucose (acute)
- Total triglyceride dynamics (acute and chronic)
- Protein synthesis (acute and chronic)
    i. Single *vs*. multiple proteins
    ii. $^{2}H_2O$ *vs.* $H_2{}^{18}O$

3. COMPLEMENTARY APPROACH TO GLUCOSE-INSULIN CLAMPING

- Measurements of flux during metabolic steady-state vs. “tolerance” testing

C. HOMEWORK BREAKOUT

(SMALL GROUPS)

USE OF MASS ISOTOPOMER DISTRIBUTION ANALYSIS

HENRI **BRUNENGRABER**, JOANNE **KELLEHER**, MICHELLE **PUCHOWICZ**

A. LEARNING OBJECTIVES

- To understand the differences between mass isotopomers and positional isotopomers (the latter was be discussed in detail in the morning session)
- To appreciate the multiple uses of mass isotopomer distribution for metabolic investigation, with the understanding that mass isotopomer distributions and positional isotopomer distributions yields complementary insights on metabolic regulation

B. SECTIONS

1. PUCHOWICZ: ANALYTICAL APPLICATIONS

   (i) measurement of low analyte enrichment by oligomerization of analyte; (ii) use of hexamethylenetetramine to amplify the ^{2}H-enrichment on glucose carbons which can be converted to formaldehyde; measurement of low ^{2}H- or ^{18}O-enrichment of water; (iii) measurement of low ^{2}H-enrichment of analytes by isotope fractionation.

2. KELLEHER: ESTIMATING BIOSYNTHESIS FROM LABELING: Building a model from the ground up (syntheses of fatty acids, cholesterol, glucose, nucleic acids).

3. BRUNENGRABER: INVESTIGATIONS OF PATHWAY regulation + pathway discovery (metabolomics associated with mass isotopomer distribution).

C. HOMEWORK BREAKOUT

(SMALL GROUPS)

- Errors in calculations of rates of glucose metabolism associated with the steady state assumption.

Thursday, NOVEMBER 13, 2014

EVENING · **7:00 pm**

SOCIAL HOUR

(CASH BAR)

DINNER

METABOLOMICS ASSOCIATED TO MASS ISOTOPOMER ANALYSIS

• HENRI BRUNENGRABER

A. LEARNING OBJECTIVE

- Appreciate how the discovery potential of metabolomics for pathway discovery can be enhanced by associating metabolomics and isotopomer analysis.

INHERENTLY DIFFICULT PROBLEMS

HENRI **BRUNENGRABER** AND **COURSE FACULTY**

B. LEARNING OBJECTIVE

- Appreciate limitations on the use of isotopes for metabolic studies, using examples of problems, which have challenged investigators for many years.
- Measurement of Cori cycling.
- Measurement of fatty acid oxidation in vivo.
- Measurement of glucose production across a high blood flow organ (kidney intestine).
- Glyceroneogenesis.
- Measurement of coenzyme A and nucleic acid turnover with 2H-enriched water.
- Impact of secondary tracers.