Preliminary Program

11th Annual Course Isotope Tracers in Metabolic Research: Principles and Practice of Kinetic Analysis

October 29 – November 2, 2018
Homewood Suites by Hilton® Nashville Vanderbilt
2400 West End Avenue
Nashville, Tennessee 37203 (also where the course takes place)
615-340-8000

FACULTY

Julio Ayala, Vanderbilt U.

Henri Brunengraber, Case Western Reserve U.

Gary Cline, Yale U.

Stephanie Chung, National Inst. Health

Melanie Cree Green, U. of Colorado

Joanne Kelleher, Mass. Inst. Tech.

Maren Laughlin, National Inst. Health

Owen P. McGuinness, Vanderbilt U. Course Co-Director

Matthew Merritt, U. of Florida

Elizabeth J. Parks, U. of Missouri Course Co-Director

Stephen Previs, Merck

Michelle Puchowicz, U. of Tennessee Health Science Center

Jamey Young, Vanderbilt U.

David Wasserman, Vanderbilt U.

Robert R. Wolfe, U. Arkansas

Course Administration:

Eann Malabanan Vanderbilt U. Program Coordinator
Contact: isotope.tracer@vanderbilt.edu
Phone: 615-343-1065

General Information

Hotel Information and Food and Registration

Check in time for the hotel is 3:00, if you need to be there earlier notify the hotel. The hotel will provide a full hot breakfast and in the evening a light dinner with complimentary social hour each day in the lobby. The course will provide lunch each day as well as snacks and drinks during the break periods. On Wednesday night all course participants are welcome to come to the Wild Horse Saloon (www.wildhorsesaloon.com) for dinner and dancing (6:30-8:30). Buses will be provided to take participants to the event and return you to the hotel. There are plenty of restaurants within walking distance of the hotel. Downtown Nashville is approximately 2 miles from the hotel. The course will end Friday at 11:00 am to allow time for participants to make early afternoon flights.

To make **hotel reservations** call 1 (615) 340-8000 and mention the group code "T18" or group name "Vanderbilt Tracer Course 2018." You may also book directly using the following link
http://homewoodsuites.hilton.com/en/hw/groups/personalized/B/BNAVBHW-T18-20181028/index.jhtml?WT.mc_id=POG

Please make your reservations by September 28th to receive discounted rates for the duration of the course.

Cost for registration:

Academic Faculty ($800 USD)
Industry Scientist ($1,300 USD)
Postdoctoral/Student ($400 USD)

COURSE FORMAT

The course and homework problems will be run on paperless format. Participants are expected to come with a laptop computer equipped with Excel and a wireless Internet connection. 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 by email on Friday, October 26, 2018.** Feel free to print the downloaded material. The faculty will systematically upload any new or additional material (including problems' solutions) on the course webpage. Note that, in order to foster intellectual exchanges without fear of plagiarism, this course will have a closed meeting format, just like a Gordon Conference.

PRESENTATIONS by PARTICIPANTS

There will be trainee presentations on the evening of Thursday, November 1, 2018. 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. Owen McGuinness (owen.mcguinness@vanderbilt.edu) by October 29 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 Thursday evening session, you will have an opportunity to present it later to a selected 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 October 29 (1st day of the class), you will be able to set up appointments with course faculty. The scheduling process will be discussed in more detail in an upcoming email.

Course Schedule

Monday Morning **Start time: 07:30am**

A. 7:30 Registration

B. 8:30 WELCOME (Dr. Maren Laughlin, senior advisor / NIDDK, NIH)

C. 8:40-9:50 PRINCIPLES OF METABOLIC FLUX AND USE OF RADIOACTIVE ISOTOPES (O. McGuinness)

LEARNING OBJECTIVES > **(1)** What is metabolic flux and how can tracer dilution principles be used to quantify flux? **(2)** Responsible Conduct of Research in the use of radioactive isotopes. **(3)** How does one optimize the measurement of radioactivity of compounds labeled with ^{14}C, ^{3}H or ^{32}P? **(4)** How does one measure a metabolic rate using ^{13}C or ^{3}H tracers? **(5)** What are the difficulties and limitations of the use of radioactive isotopes to measure metabolic rates?

SECTIONS > **(A) Basic Principles of Metabolic flux** (define tracer methodology and principle of isotope dilution). **(B) 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)). **(C) 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). **(D) Limitations of the use of isotopes for metabolic studies** (Difference between transfer of label and net flux; Isotopic exchanges; Isotopic equilibration without or with ATP consumption).

D. 9:50-10:10 BREAK

10:10-10:45 PROBLEM BREAKOUT> Attendees will be paired with Tutors who will work on four numerical problems of increasing complexity. The problems are presented to the attendees to teach them how to plan real-life experiments with radioactive isotopes without guessing the amount of radioactivity to be used. An example of the most complex problem is as follows. *You want to measure glucose oxidation in cells, using the production of $^{14}CO_2$ from [U-^{14}C]glucose. You incubate 5 ml of suspension containing 50 mg cells/ml. The glucose concentration is 15 mM. You incubate for 15 min, and collect $^{14}CO_2$ .You want to count about 300 cpm in CO_2, given that counting efficiency for ^{14}C is about 50%. From the literature, you know that the range of glucose oxidation rate is about 0.2 µmol/g x min. Question: how many µCi of [U-^{14}C]glucose must be added to each flask?*

After the problem is introduced, the strategy for solving it is outlined: *The solution of this problem requires 2 steps: (i) calculate the specific activity of glucose that will yield the expected 300 cpm in CO_2, and (ii) calculate how to achieve this specific activity.*

The attendees are given 30 min to find the solutions. The tutors help direct them. Then, the instructor and the attendees discuss how they went about working on the problems. In the next step, the instructor solves the problems in an interactive way. Then, the commented solutions of the problems are uploaded on the course's web site.

E. 10:45-11:30 Problem Discussion

Monday Afternoon Start time: **01:30pm**

A. 1:30-3:00 BASIC CONCEPTS IN MASS SPECTROMETRY (R. WOLFE)

LEARNING OBJECTIVES > (**1**) Gain an understanding of the main mass spectrometry techniques used to investigate metabolic processes with stable isotopes. (**2**) Become familiar with current expressions of isotopic enrichment, including Tracer**:**Tracee Ratio and atom (or mol) percent excess. (**3**) Learn how to measure isotopic enrichment by mass spectrometry (basic approaches). (**4**) Learn how to calculate isotopic enrichment using Gas Chromatography-Mass Spectrometry and LC-MS/MS.

SECTIONS > **(A) 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). **(B) 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; **d)** Use of a standard to calculate enrichment; measurement of ^{13}C-enrichment after combustion; effect of sample size on observed ratio. **(C) Calculation of Enrichment with GC-MS** (definition of total ion chromatogram, mass spectrum, and selected ion monitoring (SIM); identifying appropriate fragment(s) 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 (using multiple ions to calculate isotopic enrichment, using less abundant masses to measure low levels of enrichment, calculation of concentration by internal standard technique).

B. 3:00-3:15 BREAK

3:15-3:50 HOMEWORK BREAKOUT > PROBLEM BREAKOUT > As in the morning session attendees who a paired with the tutors. They will be given problems that ask them to calculate enrichment in the example enrichment of a background and a sample are run in triplicate for $^{13}CO_2$. Separate problem are done for 1-^{13}C Leucine and $^{2}H_3$ leucine. The attendees are given 30 min to find the solutions. The tutors help direct them. Then, the instructor and the attendees discuss how they went about working on the problems. In the next step, the instructor solves the problems in an interactive way. Then, the commented solutions of the problems are uploaded on the course's web site.

3:50-4:20 Problem Discussion

a. **Instrument sensitivity**
b. **Statistical consideration of how many replicates to run.**

Monday Evening

"Free time to explore Nashville"

Tuesday Morning Start time: 09:00am

A. 9:00-10:30 MEASUREMENT OF METABOLIC FLUXES WITH ISOTOPIC TRACERS (R. WOLFE)

LEARNING OBJECTIVES > **(1)** Responsible Conduct of Research in human and animal investigations. (**2**) 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. **(3)** Understand the benefit of priming the substrate pool, how to calculate a tracer priming dose, and the limitations of the primed-constant infusion technique. **(4)** Understand the basic approach for calculating substrate oxidation using a metabolic tracer. **(5)** Understand the calculation of fractional synthetic rate.

SECTIONS > **(A) 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). **(B) 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) Non steady-state kinetics.** (Single and multiple pool models).

B. 10:30-10:45 Break

C. 10:45-11:45 GLUCOSE KINETICS / INCLUDING THE EUGLYCEMIC CLAMP (O. MCGUINNESS)

LEARNING OBJECTIVES > **(1)** Responsible Conduct of Research in such types of investigations in rodents and humans. **(2)** Define the physiological correlates of glucose flux. **(3)** Learn best practices for experimental design optimization and data interpretation to evaluate insulin action.

SECTIONS > **(A) 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 differences among species). **(B) What Are The Sources of Glucose Appearance?** (Understand what 'production' is, from a tissue point of view; define the relative contribution of the liver and kidney to glucose production). **(C) 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). **(D) Assessing Insulin Action** (Choosing a tracer; 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 hyperinsulinemic euglycemic 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; Be able to calculate hepatic and peripheral insulin action

Tuesday Afternoon Start time: **01:30pm**

A. 1:30-2:30 Assessing glucose flux and insulin action using an isotopic tracers 6,6 $^{2}H_2$ glucose (M. CREE GREEN)

LEARNING OBJECTIVES > **(1)** Be able to measure **6,6 $^{2}H_2$ glucose** enrichment in a plasma sample. **(2)** Understand the experimental protocol(s) and design followed to assess glucose flux with application to the clamp as applied to humans (3) How to calculate glucose flux from enrichment data. (A data set will be sent in advance to the class

that will have raw chromatograms that have been quantified. The data set will have a background enrichment sample, basal and clamp enrichments, tracer pump rates, glucose infusion rates)
SECTIONS > (A) **Going from plasma to glucose enrichment to flux working with the data** (B) **The experimental protocol priming the pool and blood sampling protocol** (C) **Consideration of disease and environment in the design**. (D) **From data to interpretation steady state vs. non steady state kinetics**
2:30-2:45 Break
B. 2:45-4:00 LIPID METABOLISM: BASIC KINETICS (E. PARKS)
LEARNING OBJECTIVES > (1) To understand the principles and limitations of various types of measurements of lipid metabolism using stable isotopes. (2) Recognize glycerol and fatty acid availability are very sensitive to insulin and other hormones. (3) Learn to quantify fatty acid oxidation and lipid flux in multiple tissues.
SECTIONS > (A) **Lipolysis and Fatty Acid Release**: Their flux rates can be assessed using glycerol fatty acid tracer as well as substrate cycling between triglycerides and fatty acids. (B) **Fatty Acid Oxidation** (Pathways of fatty acid oxidation; Citric acid cycle exchange reactions; in vivo assessment of CPT activity). (C) **Multiple substrate pools contribute to lipoprotein and intracellular triglyceride synthesis**; limitations of various methods for measuring intracellular lipid synthesis.

Tuesday Evening Start time: 07:00pm

A. INTRODUCTION TO THE NIH GRANTS PROCESS (M. LAUGHLIN)
B. INSULIN AND GLUCOSE CLAMP (BREAKOUT SESSIONS)
- **a.** APPLICATION TO ANIMAL MODELS (O. MCGUINNESS, J. AYALA)
  - i. Unique design considerations for the rodent
  - ii. Tissue glucose uptake using labelled deoxyglucose
  - iii. Data interpretation
  - iv. Tracing oral substrate loads
- **b.** APPLICATION TO HUMAN MODELS (M. CREE-GREEN, S. CHUNG AND R. WOLFE)
  - i. Setup up study practical issues
  - ii. Choose the right tracer(s)
  - iii. Study set up and design
  - iv. Tracing oral substrate loads

Wednesday Morning Start time: **08:30am**

A. 8:30-9:00 MEASURING SYNTHESIS OF ADENINE NUCLEOTIDES, COENZYME A, NUCLEIC ACIDS (H. BRUNENGRABER)
LEARNING OBJECTIVES > (1) Identify problems associated with the use of isotopic tracers for very long experiments (weeks or months). (2) Long-term isotopic experiments occur in an open biological system where unlabeled foodstuffs enter the system continuously. (3) During long-term isotopic experiments, salvaged pathways recycle labeled intermediates into de novo synthesis pathways.
A. 9:00-9:45 Practical applications of Physiological Models using Stable Isotopes I (J. Kelleher)
LEARNING OBJECTIVES> **(1)** To understand methods for describing isotopes in physiological studies. **(2)** To learn a practical method for solving for isotopic mixtures. **(3)** To understand the role of experimental error in developing and testing models. **(4)** To understand the different methods for solving for rates of synthesis and their limitations.
SECTIONS > (A) Describing stable isotope tracers. (B) Solving for tracer contribution to mixtures with simple linear regression. (C) Introduction to Pre-steady state labeling. (D) Solving for the rate of synthesis using nonlinear regression
B. 9:45-10:00 Break
C. 10:00-11:30 METHODS IN PROTEIN METABOLISM I (WOLFE)
LEARNING OBJECTIVES > **(1)** Understand how to use whole body protein turnover techniques. **(2)** Learn how to calculate the rate of synthesis of individual proteins. **(3)** Learn how to measure tissue protein and amino acid kinetics using tracers and transorgan balance techniques.
SECTIONS > **Whole body protein turnover**: **A)** Nitrogen Balance and Catabolic and Anabolic states. **B.)** Stochastic model of whole body protein turnover. **C)** Single amino acid tracer kinetics to calculate whole body protein turnover. **Measurement of Protein FSR**: **a)** Constant tracer infusion. **b)** Flooding dose tracer injection. **c)** Sub-flooding dose tracer injection. **Methods to Estimate Precursor Enrichment for Measurement of FSR**: **a)** Fractional breakdown rate. **b)** Constant tracer infusion. **c)** Bolus injection. **Arterio-Venous Model: a)** Measurement of A-V balance

Wednesday Afternoon Start time: **01:00pm**

A. 1:00-1:30 METHODS IN PROTEIN METABOLISM II (WOLFE) (CONTINUED FROM MORNING SESSION)

B. 1:30-3:00 MEASUREMENTS OF ENERGY EXPENDITURE (S. PREVIS)

LEARNING OBJECTIVES > **(1)** Outline different methods for quantifying energy expenditure (or CO_2 production) . **(2)** Identify the pros/cons for each. **(3)** Outline the general principle of using "doubly labeled water", listing important criteria for the experimentalist. **(4)** Explain the rationale for different data normalization/interpretation.

SECTIONS > **(A) Overview of energy expenditure -** Where does "energy" go? **How Do I Quantify Tissue-Specific Rates of CO_2 Production? a)** Arterio-venous balance is required. **b)** Single *vs.* multiple compartments. **c)** Concerns about mixing/complete perfusion. **How do I quantify substrate-specific rates of CO_2 production? a)** Measure the production of ^{13}C-labeled CO_2. **b)** Concerns about the recovery of a labeled substrate. **How do i quantify total body CO_2 production? a)** Direct calorimetry. **b)** Indirect calorimetry (Direct measurements of gas exchange; indirect measurements of gas exchange (*i.e.:* "doubly-labeled" water)). **How Do I Process the Data and Normalize the Results?** Suggested statistical approach is analysis of covariance. The tool and accompanying learning modules are freely available on the MMPC website.

C. 3:00-3:15 Break

D. 3:15-4:45 MEASURE SYNTHESIS OF PROTEINS, FATS, STEROLS, GLUCOSE & NUCLEIC ACIDS WITH ^2H_2O (S. PREVIS)

LEARNING OBJECTIVES > **(1)** 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. **(2)** Why ^2H_2O is a unique tracer for measuring the synthesis of various macromolecules. **(3)** Explain why one requires knowledge of the labeling of specific hydrogen(s) in a product molecule to accurately determine its rate of synthesis. **(4)** Contrast the pros/cons of using GC-MS *vs.* NMR to measure the labeling of molecules.

SECTIONS > **What can be done with ^2H_2O that cannot be done with other tracers? a)** Simultaneous tracing of multiple processes. **Choice between acute and chronic labeling studies? a)** Source(s) of blood glucose (acute)**. b)** Total triglyceride dynamics (acute and chronic)**. c)** Protein synthesis _ acute and chronic: (Single *vs*. multiple proteins; ii. ^2H_2O *vs.* $H_2{}^{18}O$). **Complementary Approach to Glucose-Insulin Clamping**: a) Measurements of flux during metabolic steady state *vs.* "tolerance" testing.

Wednesday Evening Start time: **06:00pm**

SOCIAL HOUR / DINNER AT WILD HORSE SALOON

BUS WILL TAKE YOU FROM HOTEL TO VENUE

Thursday Morning **Start time: 08:30am**

USE OF POSITIONAL ISOTOPOMER ANALYSIS TO ASSESS PATHWAY FLUXES (G. CLINE, M. MERRITT)

LEARNING OBJECTIVES > **(1)** Understand the basic principles of NMR. **(2)** Understand how the information content of NMR data differs from MS data. **(3)** Understand how metabolic flux information is extracted from NMR data. **(4)** Review common applications of NMR to metabolic flux measurements.

SECTIONS >

- **A. 8:30-9:15 NMR in Tracer Metabolism (M.** Merritt): Basic NMR Principles (Measurement of fractional enrichment, spin-spin coupling, multiplet analysis; measuring ^{13}C and ^2H isotopomer distribution).
- **B. 9:15-10:00 Applications to Biochemical Physiology: Steady State Measurements of Metabolic Fluxes** (G. Cline): Metabolic pathways in isolated cells (TCA cycle, anaplerosis, and substrate cycling); Calculating hepatic fluxes by multinuclear NMR (glycogen synthesis pathways, gluconeogenesis and glycogenolysis, TCA cycle pathways).
- **C. *10:00-10:15 Break***
- **D. *10:15-11:10 in vivo* Applications: Kinetic Analysis of Metabolic Fluxes (G.** Cline**)** - 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
- **E. 11:10-12:00 Using tcaCALC to calculate metabolic flux** (M. Merritt): Tracer analysis to estimate relative pathway fluxes in a hands on data lab using a software called tcaCALC. Isotopomer data will be used to fit a model and impute substrate selection.

Thursday Afternoon Start time: **01:30pm**

USE OF MASS ISOTOPOMER DISTRIBUTION ANALYSIS (KELLEHER, PUCHOWICZ)

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LEARNING OBJECTIVES > **(1)** 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.

- **A.** **1:30-2:00 Analytical Applications** (Puchowicz) **a)** Measurement of low analyte enrichment by oligomerization of analyte. **b)** 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. **c)** Measurement of low ^{2}H-enrichment of analytes by isotope fractionation.
- **B.** **2:00-3:15 Practical applications of Physiological Models using Stable Isotopes II** (Kelleher): Learning objectives: (1) To understand key differences in using stable and radioisotopes. (2) To understand the difference between linear and non-linear models. (3) To understand the complexities of isotope incorporation studies. (4)To develop strategies for identifying and dealing with underdetermined models. Sections: Stable and radioisotopes, which to choose Linear versus nonlinear models, superposition Nonlinear Model for lipid synthesis from ^{13}C precursors. What to do if the model does not fit the data? Overdetermined and underdetermined models.
- **C.** 3:15-3;30 Break
- **D.** **3:30-5:00 OPTIONAL COMPUTER WORKSHOP**
  Metabolic Flux Analysis Workshop (J. Young)> Metabolic Flux Analysis using the MFA Suite of tools with GC-MS data. Investigations of pathway regulation + pathway discovery (metabolomics associated with mass isotopomer distribution).

Thursday **Evening** Start time: **07:00pm**

TRAINEES PRESENTATIONS (10)

Friday **Morning** Start time**: 08:30am**

8:30-10:00 SMALL GROUPS FOR TARGETED QUESTIONS

- **(1) WORKING WITH RAW MASS SPEC AND NMR DATA (KELLEHER; MERRITT; CLINE)**
- **(2) LIPID FLUX (PARKS; BRUNENGRABER; WASSERMAN)**
- **(3) CARBOHYDRATE FLUX (MCGUINNESS, YOUNG, AYALA)**
- **(4) PROTEIN FLUX (WOLFE; CREE-GREEN)**

10:00-10:15 BREAK

10:15-11:15 PATHWAY DISCOVERY THROUGH METABOLOMICS ASSOCIATED WITH STABLE ISOTOPE TECHNOLOGIES (H. BRUNENGRABER).

LEARNING OBJECTIVES > Limitations of non-targeted metabolomics, used as a single research tool, to investigate the regulation of metabolic pathways. Changes in relative concentrations do not reflect changes in flux rates. The association of metabolomics and stable isotope technology allows to follow C, H, N of substrates through the metabolome. This leads to the identification of new pathways and new regulatory mechanisms. Metabolomics should be integrated with classical tools used to investigate metabolism: flux rates, enzyme activity/regulation, balance studies

11:15-11:30 BREAK TO PICK UP LUNCH BOXES

11:45-12:45 INHERENTLY DIFFICULT PROBLEMS (H. BRUNENGRABER).

LEARNING OBJECTIVES > **(1)** Appreciate limitations on the use of isotopes for metabolic studies, using examples of problems, which have challenged investigators for many years. **(2)** Measurement of Cori cycling with labeled lactate. **(3)** Measurement of fatty acid oxidation *in vivo*. **(4)** Measurement of glucose production across a high blood flow organ (kidney, intestine). **(5)** Glyceroneogenesis. **(6)** Ketogenesis vs. pseudoketogenesis. **(7)** Measurement of coenzyme A and nucleic acid turnover with ^{2}H-enriched water. **(8)** Impacts of secondary tracers on the process investigated (e.g., (i) formation of [^{13}C]ketone bodies from infused [^{13}C]fatty acids), and (ii) formation of [^{13}C]glucose from infused [^{13}C]propionate). **(9)** Impact of loads of labeled substrates on metabolic processes being traced.

1:00 End of meeting for your trip home.