

GLUCOSE CLAMPING THE CONSCIOUS MOUSE: A LABORATORY COURSE

**Offered by the Vanderbilt-NIDDK Mouse Metabolic Phenotyping Center
(MMPC), Nashville, Tennessee**



**For more information, please contact
Amanda Zetans 615-343-1065 or
MMPC@vanderbilt.edu**



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Offered by the Vanderbilt-NIDDK Mouse Metabolic Phenotyping Center (MMPC),
Nashville, Tennessee

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AIM: To familiarize participants with methods, protocols and quantitative tools need to perform glucose clamps in the conscious mouse.

JUSTIFICATION: The National MMPC Program was initiated in 2001 to provide phenotyping services to identify and characterize mouse models of diabetes and related disorders. Since the inception of the program numerous mouse genotypes have been studied and much has been learned. This education program is for investigators that anticipate the regular need to perform clamps, sterile surgery, or metabolic investigations in mice. The hope is that participants will be able to transfer the standardized methodology used by the MMPC to their labs, thereby eliminating the need for repeated animal transfer.

Schedule *

	MONDAY	TUESDAY	WEDNESDAY	THURSDAY	FRIDAY
8 am	Welcome/Introduction	Tracer Methods For Quantifying Metabolism <i>In vivo</i>	Considerations in Clamping the Conscious Mouse	Interpretation of Insulin Clamps	Active Data Management and Interpretation
9 am	Anesthesia and Sterile Surgery Supplies, Tools and Equipment	Post-Op Monitoring Demonstration		“Hands On” Post-Operative Care	
10am ↓	Surgical Procedures And Demonstrations	“Hands On” Surgery	“Hands On” Surgery	“Hands On” Surgery	“Hands On” Insulin Clamp
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1 pm ↓ 4 pm	“Hands On” Surgery	“Hands On” Surgery	Insulin Clamp Demonstration	“Hands On” Insulin Clamp	Elective

* Participants can follow this schedule or select “Fast Track Option”.

Maximum Enrollment 10 participants

Target Audience Scientists with needs to assess metabolism, particularly sensitivity of metabolism to insulin, in the conscious mouse.

Course Directors David Wasserman, PhD
Owen McGuinness, PhD
Julio Ayala, PhD

Faculty Owen McGuinness, PhD
Masa Shiota, DVM, PhD
David Wasserman, PhD
Julio Ayala, PhD
Phil Williams

Staff Tasneem Ansari
Deanna Bracy
Freyja James
Li Kang
Alicia Lorance
Lianli Ma
Carlo Malabanan
Merrygay James

Coordinator and Contact Amanda Zetans 615-343-1065, MMPC@vanderbilt.edu

* **Fast Track Option** Surgery and experimental stations will be available for participants familiar with a lecture topic who wish to use the time to work on surgical or other experimental skills.

Course Description:

Glucose Clamping the Conscious Mouse

Welcome/Introduction

Introduction to the Goals of the National MMPC Program and the Vanderbilt MMPC. Aims of the Short Course and IACUC issues will be discussed.

Anesthesia and Sterile Surgery

Assessing depth of anesthesia and maintaining a sterile field will be emphasized.

Supplies, Tools and Equipment

Supplies and preferred vendors will be presented. Catheter preparation will be demonstrated.

Considerations in Clamping the Conscious Mouse

As with any experimental model, numerous factors must be considered. Effect of strain, sampling site, fast duration, use of isotopes, and dose of insulin are factors that must be considered and will be discussed.

Three basic clamps are used at the Vanderbilt MMPC. (1) The hyperinsulemic euglycemic clamp where insulin is infused at a constant rate and glucose is infused at a variable rate to maintain basal glucose levels based on feedback from frequent glucose measurements. This clamp is used to test insulin action. (2) The hyperinsulinemic hypoglycemic clamp where insulin is infused at a constant rate but blood glucose is allowed to fall and is fixed at a hypoglycemic level. The hypoglycemic clamp is used to test hypoglycemic counterregulation. (3) The hyperglycemic clamp technique requires that blood glucose be fixed at a hyperglycemic level sufficient to evoke an insulin response. The hyperglycemic clamp technique is used to assess beta-cell secretory function.

Tracer Methods for Quantifying Metabolism *In vivo*

Radioactive or stable isotopes can be used to measure substrate flux or the activity through a pathway *in vivo*. Isotopic techniques can be used during insulin clamps to measure sites of insulin action. Basic principles for measuring metabolism using isotope dilution methods, simple product/precursor conversion rates, and tissue isotope accumulation will be discussed with specific application to carbohydrates.

Interpretation of Insulin Clamps

Insulin clamps provide a wealth of data particularly when combined with isotopes. Insulin doses, sites of action, and pathways involved can all be assessed. How to maximize the power of clamp data will be discussed. Examples will be given from the literature to illustrate interpretative issues that can arise.

Surgical Procedures and Demonstrations

Live narrated demonstration of sterile catheterization of carotid artery and jugular vein in the anesthetized mouse. Instructors will use a viewing screen so that participants can see exposure from the perspective of the surgeon.

Post-Operative Monitoring Demonstration

Experiments are typically done 5 to 7 days after surgery. This section will describe and demonstrate post-operative care used to ensure mice are healthy and catheters are patent on the day of the experiment.

Insulin Clamp Demonstration

Live narrated demonstration of an insulin clamp in the conscious mouse. Every facet of the hyperinsulinemic euglycemic clamp will be viewed by participants with an explanation of procedures will be discussed.

“Hands On” Post-Operative Care

Participants will assess surgical recovery and wound healing. Techniques to maintain catheter patency will be undertaken.

“Hands On” Insulin Clamp

Participants will conduct a hyperinsulinemic euglycemic clamp under the supervision of staff.

“Hands On” Surgery

Participants will have the opportunity to perform sterile surgery under the guidance of MMPC staff. Anesthesia, proper exposure, proper use of surgical tools, and sterility will be emphasized.

Active Data Management and Interpretation

Data sets from a variety of clamp experiments will be analyzed in an interactive manner. Emphases will be on the calculations and interpretation of results derived from radioactive isotopes. Assessment of non-steady state conditions will also be discussed. Web-based workshop will be available and updated periodically. We recognize that participants will not necessarily be experts in clamps and tracers during their short time with us. The purpose of the web-based workshop is to allow attendees to continue learning about the complex problems associated with data analysis as they set up the surgical and experimental aspects of the clamp after they return home.

Elective

Time will be set aside on the final day to further engage in “Hands On” sessions or to learn about an MMPC resource or technique that was not covered in the course. Participants will have the option to meet with other MMPC faculty and staff and see first-hand practice of techniques ranging from energy balance to microassay to echocardiography.