Hyperinsulinemic-euglycemic clamp or simply the insulin clamp, is the so-called “gold standard” for investigating and quantifying insulin resistance. It measures the amount of glucose necessary to compensate for an increased insulin level without causing hypoglycemia. During the procedure, insulin is infused intravenously at 0.8–20 mU/kg/min while glucose is infused to maintain euglycemia at the rate that is determined empirically by checking the blood glucose levels every 5 to 10 minutes.

Generally, low-dose insulin infusions are more useful for assessing the response of the liver, whereas high-dose insulin infusions are for assessing peripheral (i.e., muscle and fat) insulin action.

In combination with the use of glucose tracers (i.e., [3-3H]Glucose and [1-14C]-2-deoxyglucose) during the clamp, whole body glucose metabolism (i.e., glucose turnover, glycolysis and glycogen synthesis), endogenous glucose production (i.e., hepatic glucose production), as well as tissue glucose uptake can be evaluated.

The clamp procedure includes three steps, 1) catheterization of jugular vein and carotid artery, 2) glucose clamp per se, and 3) sample process. In order to carry out the study in a way you anticipate, a close collaboration between the core and your laboratory is a prerequisite.

PI’S NAME: __________________ DEPARTMENT: __________________ UM ACCOUNT #: __________________
CONTACT NAME: __________________ PHONE/FAX: __________________ EMAIL: __________________

ANIMAL DESCRIPTION:

<table>
<thead>
<tr>
<th>Group</th>
<th>Strain</th>
<th>Sex/Age</th>
<th>Diet</th>
<th>BW</th>
<th>Fast/Fed Glucose</th>
<th>Fast/Fed Insulin</th>
<th>GTT (glucose/insulin) 0’</th>
<th>GTT (glucose/insulin) 30’</th>
<th>GTT (glucose/insulin) 60’</th>
<th>GTT (glucose/insulin) 120’</th>
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ANIMAL IDENTIFICATION:

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<tr>
<th>Group Name</th>
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<th>Group2:</th>
<th>Group3:</th>
<th>Group4:</th>
<th>Donors*:</th>
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-- Created by Nathan Qi, Associate Director, UM-APC, nathanqi@umich.edu, Phone: (734) 764-7043, Fax: (734) 232-8175
* In case of clamping mice, an infusion of blood cells is usually required to compensate the loss of blood from frequent blood sampling during the procedure. Donor mice can be anyone with similar genetic background regardless of gender and age. However, bigger animals are preferred simply for collecting more blood.

DIETS: we provide LabDiet 5001 as a regular diet.

Is a different diet applied? □ YES □ NO

If yes, please provide the following information about the diet and ensure that enough quantity will be provided when your animals are transferred.

(1) Diet Name: ____________________

(2) Color or Shape Identification: ________________________________

(3) Storage Requirement: □ 4 ºC □ Room Temperature

(4) Special Feeding Instruction, such as feeding time and duration etc. (if none, please state NONE):
____________________________________________________________________________________
____________________________________________________________________________________

CLAMP SETUPS:

(1) Tracers:
□ [3-3H]Glucose only □ [3-3H]Glucose + [14C]2DG □ w/o tracers

(2) Insulin Infusion Rate (mU/kg/min):
□ 0.8 □ 2.5* □ 4.0 □ 16.0 □ 20.0

Note, clamping level of insulin is usually tended to be lower in the more insulin sensitive group even though the insulin infusion rate is kept the same. If the same plasma insulin is required during the clamps, the insulin infusion rate can be adjusted accordingly between groups.

(3) Target Blood Glucose (mg/dL):
□ Hypoglycemia (50~60) □ Euglycemia* (120~130) □ Hyperglycemia (180~)

* In our standard protocol, a continuous infusion of insulin at 2.5 mU/kg/min (or 15 pmol/kg/min) is set to raise plasma insulin within the physiological range while blood glucose is clamped at 120~130 mg/dL (or 6.67~7.22 mmol/L).

(4) Fast Duration:
□ 5hr □ 16hr (overnight) □ Others, indicate: _______________________

Note, 1) In our standard protocol, we use a 5hr fast duration to keep the experimental state as it is when clamping mice or young rats with small body mass. Prolonged food deprivation (e.g. overnight
fast in mice) often causes marked decreases in blood glucose and body mass, a catabolic state that enhances insulin action during a clamp. 2) 8~16 hr fast is commonly chosen in clamping adult rats. A semi-fast may also be considered in which 1/3 of daily amount of food intake is provided at 5PM, before the test at 9AM next morning.

(5) Tissue Collection:
Various tissues can be collected at the end of the steady-state clamp condition. Please checkmark in the table below for specific tissues that you need 1) for assessment of glucose uptake (14C-2DG) and/or, 2) to save for later experimental purposes.

<table>
<thead>
<tr>
<th>Skeletal Muscle</th>
<th>Fat</th>
<th>Liver</th>
<th>Heart</th>
<th>Others</th>
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<tbody>
<tr>
<td>Gastroc</td>
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<td>BAT</td>
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Glucose Uptake

Stored @-80ºC

ISSUES TO BE AWARE:

(1) The room temperature is set at 68~72 °F, with 6:00am to 6:00pm light/dark cycles. Currently we are not able to perform any adjustments on the settings.

(2) Our overall success rate for the clamp is around 80% in mice, which include losses in surgery and those during clamp operations. It is always helpful when extra animals are available specifically in situations where animals are less than 20 grams or extremely obese.

(3) Animals will be placed on a heating pad during recovery from surgery and housed individually thereafter. Are any of your animals genetically modified so that they may experience excessive heat loss when single housed? □ YES □ NO If yes, please contact us for discussion. Note: In case mice are group housed, they will be separated and housed individually in our housing unit for at least 7 days prior to the measurement. Separation from group often causes changes in basal metabolism in small animals, which can be stabilized in most cases in 7~14 days.

ANIMAL TRASFER AND HOUSING REQUIREMENTS:

(1) **Form 8225-C** should be completed in advance for all animal transfers between the core and your lab. Please fax the completed form to us at (734) 763-6492.

(2) Upon arrival, all animals should have ear tag identifications and be housed in clean cages with a wire-top food holder.
Please place your animals up to **4 mice/cage** or **2 rats/cage** with strain names and ID# of each animal clearly labeled on the cage card.

Animals without clear identification or being housed with more than one strain in a cage will not be accepted by the core.

PI'S SIGNATURE: ______________________________ DATE: ____________________________