

Procedure Type: Blood Collection

Procedure Title: Glucose Tolerance Test (GTT)

Species: Rat or Mouse

Pain/Distress Category: *C or E

*Fasting of up to 8 hours during the day (lights on) is Category C

Procedure Description Tab:

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Mice will be fasted with free access to water either for 14-18 hours (overnight fast) or for 5-6 hours (morning fast). Please see rationale below. Mice that are being fasted will be marked with yellow fasting cage cards in accordance with ACUC Policy for Fasting Animals, Special/Regulated Diets/Water/Housing.

Following the fast, a baseline glucose measurement is established as described below.

1. Restrain mouse in rodent restraint apparatus or lid from home cage.
2. Warm tail to dilate vessels (heat lamp, warm water, or warm compress) if needed.
3. Using a clean sharp scalpel blade or scissors, nick the lateral tail vein ~3 cm from the tip of the tail.
4. Transfer a drop of blood onto the glucose test strip or touch the test strip of a calibrated glucometer to the incision site.
5. The incision for blood collection should be minimal and not cause blood to flow without gentle massaging at the site.
6. If bleeding continues, apply pressure to puncture site with gauze pad until bleeding stops.

Mice will be injected IP as described below. If body composition is known, doses should be adjusted to lean body mass.

1. Animals will be restrained manually or placed in a plastic decapicone bag to facilitate restraint. Restraint will be < 5 minutes.
2. The needle will be inserted into the lower right or left quadrant of the animal's abdomen, in between the midline and the medial side of the hind leg.
3. Prior to injecting the compound, the syringe will be aspirated to confirm proper placement. If fluid is visualized within the hub, the needle will be removed and a fresh needle will be utilized for the subsequent attempt in a new location.
4. Once placement has been confirmed, 2mg/g of a 20% pharmaceutical grade glucose in a sterile 0.9% saline solution will be slowly injected (***If you use a different glucose solution formula, please replace this formula with yours***). Doses will comply with ACUC Guidelines for "Dosing Techniques and Limits." (***If doses will not comply with ACUC Guidelines, insert variation with justification here.***)
5. If bleeding occurs at the injection site, pressure will be applied until hemostasis is achieved.
(Insert step 6 if animals will receive more than one injection.)

6. Each animal will experience a maximum of 3 IP injection(s) for GTT. The time between injections will be a minimum of 3 days.

Alternatively, glucose solution will be administered orally via gavage catheter or with a feeding needle as described below.

1. The animal will be restrained by scruffing the loose skin across the shoulders, ensuring that the animal cannot move its head. Hold the head in a vertical alignment with the esophagus.
2. The appropriate size feeding needle will be selected and confirmed by measuring the length of the needle to reach the last rib of the animal.
3. The needle tip is inserted between the incisors and first premolars and directed towards the back of the throat. The needle is then inserted down the esophagus. Care is taken to not force the needle, but instead the needle should fall by gravity alone with no resistance felt.
4. Once placement has been confirmed, 2 g glucose/kg body weight of the same 20% w/v aqueous glucose solution as for IP injections will be slowly infused (***If you use a different glucose solution formula, please replace this formula with yours.***) Doses will comply with ACUC Guidelines for “Dosing Techniques and Limits.” (***If doses will not comply with ACUC Guidelines, insert variation with justification here.***)
5. Upon completion the needle will be removed vertically.
(Insert step 6 if animals will receive more than one OG dosing.)
6. Each animal will experience a maximum of 3 OG dosing(s). The time between doses will be a minimum of 3 days.

After the glucose solution has been administered (T = 0) blood glucose will be determined again as described above at pre-determined time points (e.g., T = 15, 30, 60, 90, 120, 150, and 180 min) using a glucometer. To measure blood glucose at the prescribed time points, the initial incision will be massaged to initiate blood flow. If blood flow is prevented due to the formation of a clot, it will be carefully removed with a scalpel. At the end of the experiment, mice will be returned to their cages and provided with unrestricted of food and water.

How does this procedure fit into or address your overall research goals?

In mice, overnight fasting depletes liver glycogen stores and, unlike in humans, enhances insulin stimulated glucose utilization. Thus overnight fasting will only be performed if the scientific focus is on glucose utilization (e.g. effects on muscle uptake of glucose). Otherwise, a more physiologically relevant 5- to 6-hour fast will be used to assess insulin sensitivity.
(Insert additional protocol-specific rationale here.)

Please list any clinical effects or changes from the normal health and behavior of an untreated animal which may occur as a result of this procedure.

While negative clinical effects from IP injections are not expected, hematoma formation, tissue trauma, and infection may occur. Similarly, while clinical effects from OG injections are not

expected, respiratory aspiration, and esophageal trauma may occur. All effects due to the glucose solution are expected to be subclinical.

Describe post procedure monitoring that will be performed.

Mice will be monitored for discomfort or complications from the GTT procedure for 30 minutes initially, and then once the next day. We will look for excessive bleeding, hematoma formation, tissue trauma, or signs of infection (e.g., swelling, discharge and malaise).

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?

If the animal is moribund as defined by the ACUC "Guidelines for Humane Endpoints in Animal Studies", if adverse effects are noted or if abnormal signs persist despite treatment as directed by the veterinarian, the animal will be euthanized. Animals will be euthanized if they develop clinical signs often associated with peritonitis such as hunched posture, unkempt fur, and lethargy. Animals will be euthanized if they experience respiratory distress, swelling in the periesophageal area, malaise or hemorrhage associated with complications from oral gavage. Endpoints will comply with ACUC's "Guidelines for Humane Endpoints in Animal Studies."

Anesthetic Regimen tab:

Not applicable.

Peri procedure Care/Analgesics tab:

Not applicable.

Other Agents Utilized tab: (If you use a different glucose solution formula, please replace this formula with yours)

(Insert each compound/agent to be administered via injection as separate entries. Describe vehicle within each entry or insert separately (for example if being used for the control group).)

Agent Name	Dosage (in mg/kg if possible) and volume	Route	Describe timing, frequency and duration of administration
Glucose solution	2g/kg	Intraperitoneal (IP)	20% glucose solution is administered once intraperitoneally.
Glucose solution	2g/kg	Oral	20% glucose solution is administered

			once via oral gavage.
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References:

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4. Argmann, C. A. & Auwerx, J. Collection of blood and plasma from the mouse. *Current protocols in molecular biology / edited by Frederick M. Ausubel ... [et al.] Chapter 29*, Unit 29A.23, doi:10.1002/0471142727.mb29a03s75 (2006).
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6. Pomplun, D., Mohlig, M., Spranger, J., Pfeiffer, A. F. & Ristow, M. Elevation of blood glucose following anaesthetic treatment in C57BL/6 mice. *Hormone and metabolic research = Hormon- und Stoffwechselforschung = Hormones et metabolisme* **36**, 67-69, doi:10.1055/s-2004-814104 (2004).
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