Procedure Type: Blood Collection

Procedure Title: Insulin Tolerance Test (ITT)

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 for 0-6 hours. Mice that are being fasted will have yellow fasting cage cards placed on their cages 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.

A working insulin solution will be prepared from 10 µL 100 IU/mL pharmaceutical grade human insulin diluted into 6.049 mL in pharmaceutical grade sterile saline and 1.15 mL sterile 0.5% BSA/1 mM HCl shortly before use ***(if you use a different insulin solution, please replace this formula with yours).*** Mice will be injected IP as described below. A range of insulin concentrations is required for insulin resistance/sensitivity that will develop as a result of altered feeding programs (like high fat diet) or genetic manipulation.

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, 0.3 to 1.0 IU/kg of pharmaceutical grade insulin in an injection volume of 3.6 µl/g body weight ***(if you use a different concentration, please replace this with your number)*** will be slowly injected. 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 ITT. The time between injections will be a minimum of 3 days.

After the insulin solution has been administered (T = 0) blood glucose will be determined again as described above at predetermined 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 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 food and water.

How does this procedure fit into or address your overall research goals?
Experiments need to be performed on conscious mice as anesthetics, including isoflurane, have significant effects on blood glucose levels, insulin secretion, heart rate, blood flow, and body temperature and thus would lead to unphysiological results in GTT and ITT assays.
(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 peritonitis may occur. Mice may develop hypoglycemia, which will be evident from monitoring their blood glucose. If so, they will be given a dose of glucose IP of 0.5mg/mL pharmaceutical grade glucose per g BW (volume: 3.3-10 µl per g body weight). Blood glucose values of 36 mg/dl or less will be considered hypoglycemic.

Describe post procedure monitoring that will be performed.
Mice will be monitored for discomfort or complications from the ITT 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., abdominal swelling, discharge at the injection site.

What criteria will be used to determine if animals exhibiting clinical or behavioral changes should be euthanized?
Animals will be euthanized if hypoglycemia is unresponsive to glucose administration, if seizures develop as a result of hypoglycemia, or if animals develop clinical signs often associated with peritonitis such as hunched posture, swollen abdomen, unkempt fur, and lethargy. 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:

(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
Insulin (specify type)	0.5 IU/kg	Intraperitoneal (IP)	Insulin is administered once intraperitoneally.
Pharmaceutical grade sterile saline	3.6 µl/g	Intraperitoneal (IP)	Saline is administered once as part of working insulin solution.
Sterile 0.5% BSA/1 mM HCl	3.6 µl/g	Intraperitoneal (IP)	Sterile 0.5% BSA/1 mM HCl is administered once as part of working insulin solution.
Glucose	3.3 µl per g body weight	Intraperitoneal (IP)	Glucose is administered once intraperitoneally in the event of hypoglycemia.

References:

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3. Heijboer, A. C. *et al.* Sixteen hours of fasting differentially affects hepatic and muscle insulin sensitivity in mice. *J Lipid Res* **46**, 582-588, doi:10.1194/jlr.M400440-JLR200 (2005).
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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8. Heikkinen, S., Argmann, C. A., Champy, M. F. & Auwerx, J. Evaluation of glucose homeostasis. *Current protocols in molecular biology / edited by Frederick M. Ausubel ... [et al.]* **Chapter 29**, Unit 29B.23, doi:10.1002/0471142727.mb29b03s77 (2007).

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