

Procedure: IACUC-65  
Date: October 17, 2024  
Enabled by: IACUC  
Supersedes: August 22, 2024

**UC Davis  
Institutional Animal Care and Use Committee (IACUC)**

Title: ***Insulin and Glucose Tolerance Testing Policy for Mice and Rats***

**I. Purpose:**

This policy provides guidelines for conducting Insulin Tolerance Testing (ITT) and Glucose Tolerance Testing (GTT) in mice and rats to assess insulin sensitivity and glucose metabolism, respectively. All ITT and GTT procedures must be described in an approved IACUC Animal Care and Use Protocol.

**II. Policy:**

- A.** Rodents should be acclimated for a sufficient period (e.g., 3 days) before ITT or GTT to minimize cortisol levels. Fasting duration prior to the test should be standardized and specified in the protocol, taking into account the age, strain, weight, and sex of the mice or rats. Rodents are typically fasted 5-6 hours (morning fast). Overnight fasting (14-16 hours) is generally not recommended as it elevates ketone levels and induces a stressed metabolic state. Fasting mice or rats should have water available ad libitum. Anesthesia can induce hyperglycemia and is not recommended for these procedures. Testing at a consistent time of day is also strongly recommended.
- B. GTT:**
1. Blood glucose levels should be measured using validated glucometers or laboratory methods to ensure accuracy.
  2. Adequate training must be provided to personnel responsible for blood glucose monitoring to ensure consistency and reliability of measurements.
  3. Administer glucose orally or intraperitoneally (IP) at the specified dose (e.g., 1-2 g/kg body weight) as described in the approved Animal Care and Use Protocol and following the Policy on Acceptable Maximum and Absolute Maximum Volumes for Research Compound Administration.
  4. Blood Glucose Measurements: Monitor blood glucose levels at regular intervals post-glucose administration (e.g., 15, 30, 60, 90, 120 minutes).
- C. ITT:**
1. Blood glucose levels should be measured using a reliable method (e.g., tail vein sampling) before insulin administration.

2. Standard (fast acting) insulin (e.g., Humulin R or Novolin R) should be administered at a standardized dose (e.g., 0.2-2.0 U/kg) and route (e.g., IP or intravenously, IV) as specified in the approved protocol. Intermediate/long-acting insulin (e.g., Humulin N or Novolin N) cannot be used for ITT. Insulin cannot be administered to mice or rats with blood glucose levels below the reference value (<70 mg/dL).
3. Blood glucose levels should be measured at specified time points (e.g., 0 (baseline) 15, 30, 60, 90, 120 minutes) using a validated glucometer or laboratory method following insulin administration to assess glucose clearance.
4. Treatment Threshold: If blood glucose levels fall below the predetermined minimum threshold (e.g., 30-35 mg/dL) or if the mice display signs of severe hypoglycemia (seizures, ataxia, severe lethargy), administer glucose solution orally or IP to prevent hypoglycemia immediately and terminate the ITT for the affected animal.

**D. Post-Testing:**

1. Rodents should be returned to their housing conditions with access to food and water ad libitum after the test.
2. Any adverse effects observed during or after the test should be documented and reported to CVS for veterinary consultation and the IACUC if not listed in the adverse effects section of the Animal Care and Use Protocol.

**III. Resources:**

1. Ayala, Julio E et al. "Standard operating procedures for describing and performing metabolic tests of glucose homeostasis in mice." *Disease models & mechanisms* vol. 3,9-10 (2010): 525-34. doi:10.1242/dmm.006239
2. Hahn, Margaret K et al. "In vivo techniques for assessment of insulin sensitivity and glucose metabolism." *The Journal of endocrinology* vol. 260,3 e230308. 31 Jan. 2024, doi:10.1530/JOE-23-0308
3. UCSF "Glucose Monitoring on Small Samples of Blood in Rats and Mice IACUC Standard Procedure"  
[https://iacuc.ucsf.edu/sites/g/files/tkssra751/f/wysiwyg/STD%20PROCEDURE%20-%20Misc%20Rodent%20Procedures%20-%20Glucose%20Monitoring%20on%20Small%20Samples%20of%20Blood%20in%20Rats%20and%20Mice\\_0.pdf](https://iacuc.ucsf.edu/sites/g/files/tkssra751/f/wysiwyg/STD%20PROCEDURE%20-%20Misc%20Rodent%20Procedures%20-%20Glucose%20Monitoring%20on%20Small%20Samples%20of%20Blood%20in%20Rats%20and%20Mice_0.pdf)
4. UC Berkley "Insulin Tolerance Test (ITT)"  
<https://olac.berkeley.edu/sites/private/preApproved/ITT%20approved%2001-2022.pdf>
5. IACUC-50 "Acceptable Maximum and Absolute Maximum Volumes for Research Compound Administration"  
<https://research.ucdavis.edu/wp-content/uploads/IACUC-50.pdf>