# Glucose Tolerance Test in Mice

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**[Abstract]** Glucose tolerance test is a standard procedure that addresses how quickly exogenous glucose can be cleared from blood. Uptake of glucose from the blood by cells is regulated by insulin. Impairment of glucose tolerance (*i.e.,* longer time to clear given amount of glucose) indicates problems with maintenance of glucose homeostasis (*e.g.,* insulin resistance, diabetes, carbohydrate metabolism, etc). According to the WHO, in a standard oral glucose tolerance test (OGTT), the glucose level should be below 7.8 mmol/L (140 mg/dl) at 2 h in humans. Levels between this and 11.1 mmol/L (200 mg/dl) indicate “impaired glucose tolerance”, and any level above 11.1 mmol/L (200 mg/dl) confirms a diagnosis of diabetes.

## Materials and reagents

1. Mice (~20 C57BL/6J (B6) males of 2-3 months old)
2. 70% ethanol
3. Beta-D(+)-glucose (Sigma-Aldrich, catalog number: G8270)
4. NaCl
5. KCl
6. Sodium phosphate
7. Phosphate buffered saline (PBS) (see Recipes)

## Equipment

1. ACCU-CHEK comfort curve glucometer (Roche Diagnostics, catalog number: 03537536001) (this product has been discontinued. Any new product of ACCU-CHEK should work fine as well)
   Such device quantifies glucose amperometrically by measuring the current produced upon oxidation of glucose to gluconic acid by glucose oxidase, or to gluconolactone by dehydrogenase.

2. 27 gauge needle (Single-Use Needles, supplied by VWR, BD Medical, catalog number: BD305109)
3. Microvette CB300 Z serum separator (SARSTEDT, catalog number: 16.440.100)
4. Acrodisc 25 mm syringe filters w/ 0.2 μM HT Tuffryn membrane (Pall Corporation, catalog number: 4192)

## Procedure

*Note: All the following experimental procedures that involve animals (rodents) should receive approval from IACUC or equivalent committee. Humane treatment of animals should be practiced all the time.*

In the Cavener lab, we used glucose tolerance test extensively in the characterization of mice lacking *Pek/Perk*, encoding an eIF2alpha kinase that is crucial to translational control and ER homeostasis (Zhang *et al.,* 2002).

1. Obtain ~20 C57BL/6J (B6) males of 2-3 months old from Jackson Laboratory or Harlan Teklad and raise them on a regular diet for about 2 weeks.

   *Tip 1*: Males, if not from the same litter, tend to fight with each other. Raise only 3-4 animals per cage and monitor any aggressive behavior.
2. Fast animals O/N in fresh cages with water supply for ~16 h the day before experiment.
3. Monitor body weight as well as baseline blood glucose level for each animal.

   *Tip 2*: Clean tail with 70% ethanol and rub it for better circulation before making a clip with sterile scissors.

   In *non*-terminal procedures, collect sufficient amount of blood sample (*i.e.*, ~30 μl) from saphenous vein for serum isolation and insulin determination by radioimmunoassay (RIA). To reduce lysis of red blood cells, make multiple punctures on the saphenous vein with a small needle and collect blood using Microvette CB300 Z serum separator.
4. Prepare 10% glucose in 1x PBS and sterilize the solution by 0.2 μm-filtration.
5. Prepare injection solution in a 0.5 ml eppendorf tube for each animal (2 mg glucose/g body weight). Volume (μl) = Body Weight (g) x 20. Use 20% glucose instead, should the body weight be greater than 30 g.
6. Sterilize abdominal region of animal with 70% ethanol, and clean up with dry cotton ball. Hold the back of the animal firmly, and inject glucose intraperitoneally into recipient subject with a 27-gauge sterile needle.

   *Tip3:* Remove air bubble before injection and do NOT stick the needle into the body *too* deeply. Perform injection slowly with an angle of 30 to 45 °C to avoid subcutaneous injection. Pick the injection site away from the liver and close to the ventral axis to avoid kidney damage.

7. Put the animal back into the cage and measure blood glucose levels at 15, 30, 60, 90 and 120 min (or 30, 60, 90, 120 and 240 min). At least 3-4 replicate animals should be used for each time-point.

## Representative data (optional)

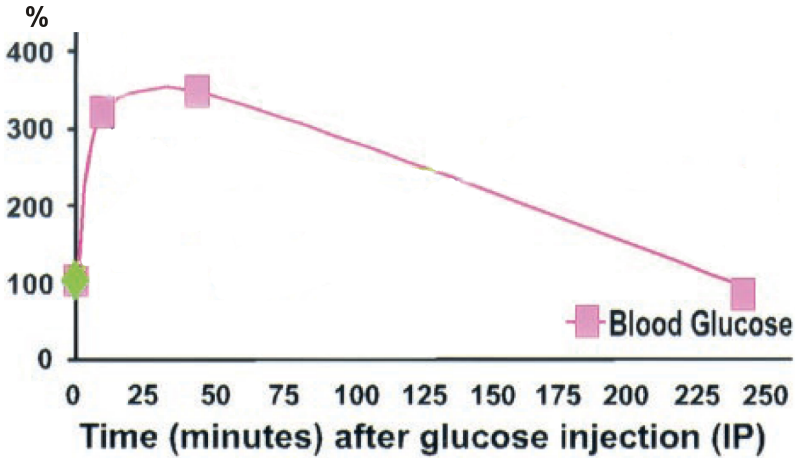


**Figure 1. This figure is adapted from the original (Zhang *et al.*, 2002).** Fasted B6 mice were injected at time zero with glucose, and blood glucose levels were monitored and normalized to the value of time zero. Each data point represents the average of three to five individual mice. The baseline value of blood glucose is typically ~100 mg/dl. Representative data are shown here.

## Notes

Please note that mice of different genders, ages, and inbred backgrounds can show different responses to glucose. Certain strain-specific phenotype data can be found at http://phenome.jax.org/db/q?rtn=meas/methodologies.

## Recipes

1. PBS (pH 7.4)
   0.8% NaCl
   0.02% KCl
   12 mM sodium phosphate

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**References**

1. Zhang, P., McGrath, B., Li, S., Frank, A., Zambito, F., Reinert, J., Gannon, M., Ma, K., McNaughton, K. and Cavener, D. R. (2002). The PERK eukaryotic initiation factor 2 alpha kinase is required for the development of the skeletal system, postnatal growth, and the function and viability of the pancreas. *Mol Cell Biol* 22(11): 3864-3874.

 