Regular Glucose Tolerance Test

Protocol used in Animal Characterization Core at UVA
Susanna R. Keller

Short Protocol: Mice are fasted overnight (16 hours) before the test. Glucose is injected intraperitoneally (1-2g/kg) and blood samples are taken before and at 10, 20, 30, 60, 90 and 120 min after the injection of glucose for the measurement of glucose with a glucometer.

Material:

- Glucometer and glucose strips
  (OneTouch Ultra from Lifescan works very well as it only requires 5 µl of blood and gives you the result in five seconds).

- D-Glucose (Sigma Cat. No. G-7528)

- 1 ml syringes with 27 or 30 gauge needles
  (or use syringes with attached needles that are used for insulin injections in patients)

- Gauze

- Timer and Pen

- Table of mice for record keeping of glucose values

Preparations before the test

Weigh the animals the afternoon before the test. The animals are fasted overnight (approximately 16 hours: 5 pm to 9 am). Place each of the animals in a separate fresh cage with no food, but make sure they have water bottles. Use cages with

woodchip bedding and NOT corncob bedding. The mice eat the corncob (containing starch) when they are hungry and are then not fasted. Make sure that there is no activity in the animal room before and while you are performing the test. Prepare the glucose solution the night before the test (for details see below).

Determine the glucose to be given from the weights of the mice (1-2 g/kg body weight).
For 1 g/kg body weight inject the following amount of glucose solution (for preparation see below) in µl: multiply the weight of the mouse in g by 4. For example for a 25 g mouse inject 100 µl of the glucose solution.
For 2 g/kg body weight inject the following amount of glucose solution (for preparation see below) in µl: multiply the weight of the mouse in g by 8. For example for a 25 g mouse inject 200 µl of the glucose solution. mult. weight by 8

Procedure

After the overnight fast, determine blood glucose (time 0) in a drop of blood as following: Place the mice on top of the cage (let it hold onto the grid). Cut the tail tip with a pair of scissors, wipe off the tail tip with gauze, use the next drop of blood for the determination of glucose with a glucometer. Inject glucose solution intraperitoneally (for details on intraperitoneal injections see ITT protocol). Determine blood glucose at 10, 20, 30, 60, 90 and 120 min after the injection of glucose. For the determination of glucose at each of these time points collect a drop of blood as following: wipe the cut end of the tail to break any blood clot that has formed and collect the next drop of blood. If the blood does not appear spontaneously "milk" the tail by holding the tail between your thumb and index finger

and move along the tail from the base of the tail to the tip of the tail while applying gentle pressure.

The number of animals that can be used in one test is about 12-16 (depending on experience with intraperitoneal injections). To perform the test with this number of mice proceed as following:
First batch of 6-8 mice: Start the timer when you inject the first mouse (time 0). Take the next mouse measure blood glucose and inject it with the glucose (check the timer when you are done with the injection on this mouse, this will be time 0 for this mouse) etc. As soon as you reach the 10 min time point after injection of the first mouse measure blood glucose in the first mouse. For the second mouse wait until it reaches its 10 min time point etc.

Second batch of 6-8 mice: Start the second batch after you have taken the 30 min time point for the first batch of mice and proceed as done with the first batch of mice. You will get into a crunch when both batches of mice reach a time point of measurement simultaneously, at the 60 min time point for the first batch of mice and the 20 min time point for the second batch of mice. But if you are well organized it works. If you are inexperienced do fewer mice or do the tests together with another person.

To compare two groups of mice (for example: knockout vs wild type or transgenic versus non-transgenic or treated vs non-treated groups) under as much as possible identical conditions use the different groups of mice in the same test and proceed with mice in alternating order.
For example: wild type - knockout - wild type - knockout and so on.

At the end of the test be sure to fill the feeders with food.

Evaluation of GTT: The mean blood glucose values +/-SEM (mg/dl) for each group of mice are plotted versus time.

Preparation of glucose solution

D-glucose

Dissolve 2.5 g D-glucose (Sigma Cat. No. G-7528) in 7.5 ml of milliQ water and bring the final volume up to 10 ml.
-> 0.25 g/1 ml

Prepare the solution the day before the test and leave it at RT overnight (see remarks below *)

filter sterilize next morning

D-glucose characteristics

Solubility of glucose: 82 g/100 ml at 25°C (-> total volume approximately 182 ml).

* The crystallized glucose is in the α-form. To obtain the β-form, which is the form which is transported better than the α-form (about 2-fold more rapidly), leave the solution overnight at RT. An equilibrium between the α-form and the β-form will be reached during this time. The β-form is the predominant form in an aequous solution (about 2/3 β-form and 1/3 α-form).