

Insulin Tolerance Test Protocol (ITT)

Preparation

- 1) Bring your mice up to the lab first thing in the morning (8-9am), place them on the bench where the procedure will occur and leave them there for the duration to reduce the amount of stress. Weigh each mouse (weight determines amount of glucose to inject) and mark their tails in the order in which you will inject them, for example:
 - 1: 1 line across tail
 - 2: 2 lines across tail
 - 3: 3 lines across tail
 - 4: 4 lines across tail
 - 5: 1 long mark down tail
 - 6: 1 long mark down tail and 1 line across tail
 - 7: 1 long mark down tail and 2 lines across tail
 - 8: 1 long mark down tail and 3 lines across tail
 - 9: 1 long mark down tail and 4 lines across tail
 - 10: 2 long marks down tail = #10*start over from 1 at #11*
- 2) Give the mice a clean cage (HARDWOOD BEDDING), and begin fasting them (6 hour fast) with water ad lib.
- 3) Set up a data collection table to record your data. A sample is shown:

#	Mouse ID	PCR	Sex	Body Weight	Start Time	Time 0	30 min	60 min	120 min
1	UCPK 29- 12	-	F	25.8 g	:00	103	176	157	124
2	UCPK 29-13	+	F	25.3 g	1:02	125	215	187	141

- 4) Insulin can be obtained from the pharmacy located in the basement of CSRB:
 - you will want the 100 unit bottle of human insulin (Humalin)
 - dilute insulin 1:1000 (5 λ /5ml) in sterile PBS to prepare for injection
- 5) Set up your bench with the following:
 - chux
 - 2 glucometers
 - one or more canister of cuvettes, depending on how many mice you are testing (located in drawer marked "cuvettes" in cold room)
 - two U100 insulin syringes (Terumo 3/10 CC)
 - razor blades
 - timer

- 6) Calculate the dose of insulin required for each mouse:
-multiply the body weight in kg by 7500 to obtain a dose of 0.75U/kg,

$$30\text{g} = 0.030\text{kg}$$

$$0.030\text{kg} \times 7500 = 112.5\lambda$$

$$112.5\lambda = \sim 11\text{units on the } 3/10\text{cc syringe}$$

Note: Depending on the insulin sensitivity of your strain, the dose may vary. The range in the literature is typically between 0.75 and 1U/kg although we have had some luck with 0.375U/kg as well.

- 7) At the end of the 6 hour fast, when you are completely set up and ready to begin, measure blood glucose and then inject the mouse with the diluted insulin following the steps below:
- fill the syringe with the correct dose of diluted insulin (see step #6)
 - pick up mouse by back of neck, stroke tail a few times to make the blood flow to the bottom, use razor blade to cut off a small piece of the tip of the tail and squeeze the blood onto a cuvette
 - blood should fill up the cuvette, if it doesn't, drag your fingers down the tail while it is directly on the cuvette to try and get more blood out
 - wipe both sides of the cuvette and put it into the glucose reader
 - *put odd numbered samples in the #1 reader and put even numbered samples in the #2 reader so that you don't get mixed up as to which cuvette is from which mouse*
 - immediately inject that mouse with insulin as described in (7)
- 8) Inject mouse with insulin:
- immediately following blood collection, inject the correct dose of diluted insulin by an intraperitoneal (i.p.) injection (**ask someone to show you the correct technique for i.p. injection before attempting for the first time**)
 - as soon as you inject the mouse start your timer counting up from 0:00 and record the time and blood glucose
 - fill your syringe and grab your second mouse → cut tail, collect blood in cuvette, wipe cuvette and put it in glucose reader → inject second mouse with insulin and record the time that you injected the mouse and the original blood glucose
 - continue these steps until you have taken blood glucose and injected all of your mice
 - make sure you record all injection times and the blood glucose for each mouse**
 - collect blood glucose data exactly 30minutes, 1 hour, and 2 hours after insulin injection
 - put all used cuvettes and syringes in a bio hazard sharps container

***the insulin injection can sometimes cause the blood glucose to go so low that the mice can show signs of hyperglycemic shock such as convulsions or coma, this may occur if blood glucose goes below 20 mg/dl. If this happens, you can give the mouse an injection of dextrose to keep it from dying*

9) Take second blood glucose:

-it is not necessary to re-cut the tail. The original wound should still bleed.

-exactly 30 minutes from the time of injection to take the second blood glucose → collect blood from the first mouse you injected when your timer reads 30 minutes, and if you injected the second mouse at 1 minute, collect blood from the second mouse when your timer reads 31 minutes, and so on for the rest of the mice

10) Take third blood glucose:

-exactly 60 minutes from the time of injection to take the third blood glucose

11) Take fourth and final blood glucose:

-exactly 120 minutes from the time of injection to take the third blood glucose

12) After all blood glucose readings have been recorded, throw away chux, and razor blades/cuvettes (in red sharps container) and put glucometers away. Put food back in mouse cages (they have been fasting and are hungry!) Graph out data on Excel.