

## Glucose Tolerance Test Protocol (GTT)

### Preparation

-Prepare to do the GTT in a quiet room and keep handling down to a minimum during the procedure.

- 1) Bring your mice up to the lab late in the day and fast overnight, starting around 6pm, ON HARDWOOD BEDDING-NOT CORN COB. (if you are looking for a muscle effect, fast overnight; if you are looking for liver effects, fast 6 hours. For the 6 hour fast, bring animals up to the lab between 8-9am and begin the fast. Animals should be kept on the same bench where you will be performing the experiments so that they can be accustomed to the area to reduce stress during the procedure.)
- 2) Before starting the fast, prepare your mice (doing this before the fast reduces their stress later when it is time for the procedure):
  - Have an extra mouse or two in order to take insulin levels by mandible bleed at the 30 minutes time point (Optional)
  - 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

3) Set up a table that you can use to record your data, it should look like this: A sample data table is also attached to the back of this protocol.

#	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

- 3) In the morning before the procedure, make your glucose solution:
  - **10% glucose : 9 mL H<sub>2</sub>O + 1g D-glucose. Then QS to 10 mL with H<sub>2</sub>O.**

- 4) Set up your bench with the following:
- chux
  - 2 glucometers (glucose readers)
  - one or more canister of cuvettes, depending on how many mice you are testing (located in drawer marked “cuvettes” in cold room)
  - two U300 insulin syringe
  - razor blades
  - timer
- 5) When you are completely set up and ready to begin, measure blood glucose then inject the mice with glucose solution following the steps below:

-fill the syringe with the glucose solution:

**1 unit to 1 gram of weight → 24.5 grams = 24.5 units of glucose**

- 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 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 over 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 mice 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*
- then immediately inject that mouse with glucose as described in (6).

- 6) Inject mouse with glucose:

- while you are still holding the mouse, turn it so that its abdomen is facing you and pull the tail tightly back so that its legs cannot move easily
- pick up your syringe with the appropriate amount of glucose, insert the needle into the peritoneal cavity about 1cm inside of the mouse's right thigh at a shallow angle → you do not want to go straight in or you will puncture their inferior vena cava and you do not want to go too shallow or you will be injecting the glucose subcutaneously and will see a bubble form under the skin (**please ask someone to show you the correct technique 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 glucose solution 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**

-put all used cuvettes and syringes in a bio hazard sharps container

7) Take second blood glucose:

-set out all new cuvettes

-exactly 30 minutes from the time of injection, 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

-It is not necessary to re-cut the tail. The original wound should still bleed. Drag your fingers down the mouse's tail a few times while holding the end of the tail directly on the cuvette → make sure you wipe both sides of the cuvette

**-make sure you record the blood glucose for each mouse**

8) Take third blood glucose:

-set out all new cuvettes

-wait 60 minutes from the time of injection to take the third blood glucose → collect blood from the first mouse you injected when your timer reads 60 minutes, and if you injected the second mouse at 1 minute, collect blood from the second mouse when timer reads 61 minutes, and so on for the rest of the mice

-to get enough blood to fill the cuvette you may need to drag your fingers down the mouse's tail a few times while holding the end of the tail directly on the cuvette → make sure you wipe both sides of the cuvette

**-make sure you record the blood glucose for each mouse**

9) Take fourth and final blood glucose:

- set out all new cuvettes

-wait 120 minutes from the time of injection to take the third blood glucose → collect blood from the first mouse you injected when your timer reads 2 hours, and if you injected the second mouse at 1 minute, collect blood from the second mouse when timer reads 2 hours and 1 minute, and so on for the rest of the mice

-to get enough blood to fill the cuvette you may need to drag your fingers down the mouse's tail a few times while holding the end of the tail directly on the cuvette → make sure you wipe both sides of the cuvette

**-make sure you record the blood glucose for each mouse**

-put all used cuvettes in a bio hazard sharps container

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

