Intraperitoneal glucose tolerance test (IP GTT)

Version: 1
Replaced by version: N/A
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Summary:

The glucose tolerance test measures the clearance of an intraperitoneally injected glucose load from the body. It is used to detect disturbances in glucose metabolism that can be linked to diabetes or metabolic syndrome. Animals are fasted for 5 hours, fasted blood glucose levels are determined before a solution of glucose is administered by intra-peritoneal (IP) injection. Subsequently, the blood glucose level is measured at different time points during the following 90 minutes.

Reagents and Materials:

Reagent/Material	Vendor	Stock Number
20% Dextrose		
Glucometer and glucose strips	Accu-Check	
Syringes for IP injections	BD	
Timer		
Microvettes for blood collection	Sarstedt	16.444.100
Surgical scissors		

Protocol:

1. Weigh the mice. For mice of differing fat mass, we recommend dosing the bolus of glucose on lean mass. This requires body composition.

2. Fast mice for 5 hours by transferring mice to individual cages or containers.

3. Prepare an experiment record sheet, sticks for glucose measurement and syringe for IP injection of the Dextrose solution.

4. Calculate and record the volume of 20% Dextrose solution required for each individual mouse for IP injection as follows:

    a. To inject 2g of dextrose/kg body mass, the volume of the IP glucose injection is:

20% Dextrose (µl) = 10 x body weight (g)

5. Cut the tip of the tail using clean surgical scissors. A small drop of blood (<5µl) is placed on the test strip of the blood glucose meter. This is the baseline glucose level (t = 0) and is recorded in the experiment record sheet.

6. Collect 40µL of whole blood in a tube for measurement of insulin. This is greatly facilitated by use of a collection tube such as Sarstedt's Microvette® CB 300 (cat # 16.443.100 for Heparin or cat # 16.444.100 for EDTA). Spin the blood and collect plasma in a clean tube (20µL). Store plasma on ice. Spin the blood 1min at 13k rpm and collect plasma in a new tube (25µL).

7. Inject the mouse intraperitoneally with the appropriate amount of glucose solution, as previously determined (point 4) and start the timer.

8. The blood glucose levels are measured at 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes after glucose gavage. Samples for insulin determination (20µl plasma) are collected at 10, 30, 60 and 120min (**Table 1**). For each sample, start the bleeding again by removing the clot from the first incision, massage the tail if blood flow is inadequate. Place a small drop of blood on a new test strip. Results are recorded in the record sheet.

9. Ensure that further blood loss from the incision is minimal by briefly applying pressure to the incision after each measurement.

10. At the end of the experiment, mice are euthanized for tissue collection or placed in a clean cage with water and food available ad libitum for recovery. Monitor the mice carefully to observe any abnormal behavior. Plasma samples are stored in -20 or -80°C freezer until analysis.

Table 1: IPGTT Record sheet

Mouse ID: Body Weight:			
Time (min)		**Blood Glucose (mg/dL)**	**Comments**
-5	**G, I**		
0	*Glucose injection (2g/kg body weight)*		
5	**G**		
10	**G, I**		
15	**G**		
20	**G**		
30	**G, I**		
45	**G**		
60	**G, I**		
90	**G**		
120	**G, I**		

G: glucose reading
I: Sample for Insulin (20µL plasma).