



Hyperglycemic clamp

Version: 1
 Edited by: Jason Kim

(note that the following list should be linked to the appropriate location.)

- [Summary](#)
- [Reagents and Materials](#)
- [Protocol](#)
- [Reagent Preparation](#)
 - [Reagent 1](#)
 - [Reagent 2](#)
 - [Reagent 3](#)

Summary: *(This area will include a brief description of what the protocol is used for and why someone would need to use it.)*

Hyperglycemic clamp measures insulin secretion and pancreatic β -cell function in awake mice. Islet function is affected by obesity and insulin resistance, and abnormal islet function predisposes to the development of type 2 diabetes.

Reagents and Materials: *(This should be a comprehensive list of stock solutions and material. The reagent list for the stock solutions is included in the reagent preparation area that is included at the end of this SOP.)*

Reagent/Material	Vendor	Stock Number
20 % Dextrose, injection, USP	Hospira	NDC0409-7935-19

Protocol:

1. Survival surgery is performed to establish a chronic indwelling catheter at 5~6 days prior to experiment for intravenous infusion. (refer to M1023: Surgery-jugular vein cannulation)
2. Mice are fasted overnight (~15 hours) or for 5 hours prior to the start of experiment.
3. Place a mouse in a rat-size restrainer with its tail tape-tethered at one end.
4. Expose and flush the intravenous catheter using saline solution. Then, connect the catheter to the CMA Microdialysis infusion pump.
5. Collect plasma sample (20 μ l) before the start of infusion (basal-0 min) to measure basal glucose and insulin levels.
6. Start infusion of 20% dextrose to quickly reach a target hyperglycemia (~300 mg/dl glucose level) and maintain hyperglycemia by adjusting glucose infusion rates.
7. Collect plasma samples (10 μ l each) at 10, 20, 30, 45, 60, 90, and 120 min to measure glucose levels. Adjust glucose infusion rates based on instantaneous glucose levels to maintain at target hyperglycemia.
8. Collect additional plasma samples (10 μ l each) at 10, 20, 30, 45, 60, 90, and 120 min to measure insulin concentrations.

9. At the end of experiment, mice are euthanized, and pancreas may be collected for further studies.
10. For data analysis, plasma insulin concentrations may be plotted during the 120-min hyperglycemic clamp experiment, and area-under-curve may be calculated. Area-under-curve of insulin levels during hyperglycemic clamps may be directly correlated with insulin secretion and pancreatic β -cell function assuming there are no effects on insulin clearance rates.
11. Additional plasma samples may be collected to measure serum c-peptide concentrations which may further reflect glucose-induced insulin secretion and pancreatic β -cell function in awake mice.