Protein metabolism

Version: 1
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(note that the following list should be linked to the appropriate location.)

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

Hyperinsulinemic-euglycemic clamp is the gold-standard method to assess insulin sensitivity. The hyperinsulinemic-euglycemic clamp is widely used in clinics and laboratories to measure insulin action on glucose utilization in humans and animals for clinical and basic science research. Incorporation of radioactive-labeled glucose during hyperinsulinemic-euglycemic clamps makes it possible to measure glucose metabolism in individual organs in awake mice. Impaired insulin sensitivity (insulin resistance) is a major characteristic of obesity and an early requisite event in 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
HelixMark Standard Silicone Tubing	Helix Medical, Inc.	0.012'' ID / 0.025'' OD
[3-^{3}H] D-glucose	Perkin Elmer	NET331C005MC
2-[1-^{14}C] Deoxy-D-glucose	Perkin Elmer	NEC495001MC
0.9 % Sodium Chloride, Injection, USP	B.Braun Medical Inc	NDC0264-4001-55
Pentobarbital	Oak Pharmaceuticals, Inc.	NDC76478-501-50
Microdialysis pumps	CMA/Microdialysis	
Analox GM7 Micro-stat Rapid Multi-assay Analyser	Analox Instruments Ltd.	GM7
Insulin	Norvolin	Regular human insulin, U-100
20 % Dextrose, injection, USP	Hospira	NDC0409-7935-19
0.9 % Sodium Chloride, Injection, USP	B.Braun Medical Inc	NDC0264-4001-55
1 ml tuberculin syringes		
Microhematocrit capillary		

tubes		
Heparin-coated blue polyethylene open-top tubes		
Microcentrifuge tubes (1.5 ml)		

Protocol:

1. Mice are fasted overnight (~15 hours) prior to the start of experiment.
2. Chronic indwelling catheter is placed 5~6 days prior to experiment for intravenous infusion. (methods can be referred to M1023: Surgery-jugular vein cannulation)
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 μl) 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 μl 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 μl 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.