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U Mass - Protein metabolism

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Protocol status: Working

We use this protocol and it's working

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Abstract

Summary:

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.

Materials

MATERIALS

⊗ HelixMark Standard Silicone Tubing **Helix Medical, Inc. Catalog #0.012" ID / 0.025" OD**

⊗ [3-3H] D-glucose **Perkin Elmer Catalog #NET331C005MC**

⊗ 2-[1-14C] Deoxy-D-glucose **Perkin Elmer Catalog #NEC495001MC**

⊗ 0.9 % Sodium Chloride Injection USP **B.Braun Medical Inc Catalog #NDC0264-4001-55**

⊗ Pentobarbital **Oak Pharmaceuticals, Inc. Catalog #NDC76478-501-50**

⊗ Microdialysis pumps **CMA/Microdialysis**

⊗ Analox GM7 Micro-stat Rapid Multi-assay Analyser **Analox Catalog #GM7**

⊗ Insulin **Novolin Catalog #Regular human insulin, U-100**

⊗ 20 % Dextrose injection USP **Pfizer (Hospira) Catalog #NDC0409-7935-19**

⊗ 1 ml tuberculin syringes

⊗ Microhematocrit capillary tubes

⊗ Heparin-coated blue polyethylene open-top tubes

⊗ Microcentrifuge tubes (1.5 ml)

Note:

B Braun Medical, Cite this (B Braun Sharing Expertise, RRID:SCR_007148**)**

Hospira, RRID:SCR_003985

- 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 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-undercurve 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.