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U Mass - Protein metabolism

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Protocol status: Working

We use this protocol and it's working

Created: February 14, 2019

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Keywords: Protein metabolism, type 2 diabetes, insulin sensitivity, impaired insulin sensitivity, euglycemic clamp, insulin resistance, insulin action on glucose utilization, labeled glucose, glucose metabolism, glucose metabolism in individual organ, protein metabolism summary, glucose, insulin action, protein metabolism, diabetes, glucose utilization, obesity, major characteristic of obesity

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

Troubleshooting

- 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.