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Working

U Mass - Protein metabolism

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Mouse Metabolic Phenotyping Centers

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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.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=146&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR	CAS NUMBER	RRID
HelixMark Standard Silicone Tubing	0.012" ID / 0.025" OD	Helix Medical, Inc.		
[3-3H] D-glucose	NET331C005MC	Perkin Elmer		
2-[1-14C] Deoxy-D-glucose	NEC495001MC	Perkin Elmer		
0.9 % Sodium Chloride Injection USP	NDC0264-4001-55	B.Braun Medical Inc		
Pentobarbital	NDC76478-501-50	Oak Pharmaceuticals, Inc.		
Microdialysis pumps		CMA/Microdialysis		
Analox GM7 Micro-stat Rapid Multi-assay Analyser	GM7	Analox		
Insulin	Regular human insulin, U-100	Novolin		
20 % Dextrose injection USP	NDC0409-7935-19	Hospira(Pfizer)		
1 ml tuberculin syringes				
Microhematocrit capillary tubes				
Heparin-coated blue polyethylene open-top tubes				
Microcentrifuge tubes (1.5 ml)				

MATERIALS TEXT

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 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-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.