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U Mass - Hyperinsulinemic-euglycemic clamp

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Jason Kim[1]

[1]University of Massachusetts

Mouse Metabolic Phenotyping Centers
Tech. support email: **info@mmpc.org**


Lili Liang

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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-glucos **Perkin Elmer** **Catalog #**NEC495001MC
- Pentobarbital **Oak Pharmaceuticals, Inc.** **Catalog #**NDC76478-501-50
- Microdialysis pumps **CMA/Microdialysis** **Catalog #**CMA 402
- 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
- 0.9 % Sodium Chloride Injection USP **B.Braun Medical Inc** **Catalog #**NDC0264-4001-55
- 1 ml tuberculin syringes **Becton Dickinson (BD)** **Catalog #**REF 309659
- Microhematocrit capillary tubes **Fisher Scientific** **Catalog #**22-362-566
- Heparin-coated blue polyethylene open-top tubes **Beckman Coulter** **Catalog #**652825
- Microcentrifuge tubes (1.5 ml) **Denville Scientific Inc.** **Catalog #**C-2170

Note:

Hospira, RRID:SCR_003985
Fisher Scientific, RRID:SCR_008452
BD Biosciences, RRID:SCR_013311
Beckman Coulter, RRID:SCR_008940

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 During the 2-hour acclimation period, infuse D-[3-^{3}H] glucose at 0.05 µCi/min to measure the basal rate of whole body glucose turnover.

6 Collect a plasma sample (30 µl) at the end for the measurement of plasma glucose, insulin, and [^{3}H] glucose concentrations (basal parameters).

7 Following the basal period, start a 2-hour hyperinsulinemic-euglycemic clamp with a primed (150 mU/kg body weight) and continuous infusion of human insulin at 2.5 mU/kg/min to raise plasma insulin levels.

8 Infuse 20% dextrose at variable rates to maintain plasma glucose at basal concentrations (euglycemia) throughout the 2-hour clamp.

9 Insulin-stimulated whole body glucose turnover rates are estimated with a continuous infusion of [3-^{3}H] glucose at 0.1 µCi/min throughout the clamp.

10 Collect plasma samples (10µl) at 20, 40, 60, 70, 90, 100, 110, and 120 min to measure plasma glucose concentrations.

11 Adjust glucose infusion rates based on the instantaneous glucose levels to maintain euglycemia.

12 To estimate insulin-stimulated glucose uptake in individual organs, administer a bolus injection of 10 µCi of 2-deoxy-D-[1-^{14}C] glucose (2-[^{14}C]DG) at 75 minutes after the start of the clamp.

13 Collect plasma samples (10 µl) at 80, 85, 90, 100, 110, and 120 min for the measurement of plasma [^{3}H] glucose, $^{3}H_2O$, and 2-[^{14}C] DG concentrations. (10 µl plasma samples are

suspended in 20 µl distilled water [dH_2O] to make 30 µl sample solutions.)

14 Collect additional plasma sample (10 µl) at the end of the clamp (at 120 min) to measure plasma insulin concentrations (clamp parameter).

15 At the end of hyperinsulinemic-euglycemic clamp, anesthetize mice using pentobarbital and quickly dissect and collect tissues including skeletal muscles (gastrocnemius and quadriceps) from both hindlimbs, white and brown adipose tissues, liver, and heart.

16 Rapidly freeze-clamp the tissues in liquid N_2, and store tissue samples in -80ºC freezer for biochemical analysis.

17 Biochemical assay is conducted using plasma samples to measure [3-^{3}H] D-glucose, ^3H_2O, and 2-[^{14}C] DG concentrations.

a) Transfer 15 µl of plasma sample solutions into microcentrifuge tubes with sample time clearly labeled.
b) Add 25 µl BaOH and vortex samples.
c) Add 25 µl $Zn(SO)_2$ and vortex samples.
d) Centrifuge samples for 5 min at 12,000g (~14,000 rpm).
e) Prepare 2 sets of scintillation vials labeled Dry and Non-Dry for each sample.

Non-Dry samples
i. Prepare 60 µl of dH_2O in NON-DRY labeled scintillation vials for each sample.
ii. Transfer 20 µl of supernatant from step (d) into respective scintillation vials and vortex samples.
iii. Add 3 ml of Ultima scintillation cocktail and vortex thoroughly.
iv. Measure radioactive labeling using Beckman Coulter Scintillation Counter.

Dry samples
i. Transfer 20 µl of supernatant from step (d) into respective scintillation vials and place into vacuum oven set at room temperature for overnight drying.
ii. Following overnight drying, add 80 µl dH_2O and vortex thoroughly.
iii. Add 3 ml of Ultima scintillation cocktail and vortex samples.
iv. Measure radioactive labeling using Beckman Coulter Scintillation Counter.

18 Plasma concentrations of ^3H_2O will be calculated as the difference in ^{3}H counts between Dry and Non-Dry samples and will be used to calculate the rate of whole body glycolysis.

19 For biochemical assay to measure glucose uptake in individual organs, refer to M1003: Organ-specific glucose uptake experiment.

20 Basal rate of hepatic glucose production (HGP) or glucose turnover can be determined as the ratio of the basal [^{3}H] glucose infusion rate (dpm/min) to the specific activity of plasma glucose (dpm/μmol) at the end of the basal period (0 min sample before the start of hyperinsulinemic-euglycemic clamp).

21 Insulin-stimulated whole body glucose turnover is determined as the ratio of the clamp [^{3}H] glucose infusion rate (dpm/min) to the specific activity of plasma glucose (dpm/μmol) during the final 30 min of the clamp (90~120 min of clamp).

22 Insulin-stimulated HGP (during the clamp) is determined by subtracting the glucose infusion rate from the whole body glucose turnover rate. The difference between insulinstimulated and basal rates of HGP reflects hepatic insulin action (insulin-mediated suppression of HGP).

23 Whole body glycolysis is calculated from the rate of increase in plasma $^{3}H_{2}O$ concentrations, determined by linear regression of the measurements at 80, 85, 90, 100, 110, and 120 min of clamp.

24 Whole body glycogen plus lipid synthesis are estimated by subtracting whole body glycolysis from whole body glucose turnover.