May 10, 2019

Working

U Mass - Hyperinsulinemic-euglycemic clamp

Jason Kim[1]

[1]University of Massachusetts

dx.doi.org/10.17504/protocols.io.xzefp3e

Mouse Metabolic Phenotyping Centers

Tech. support email: **info@mmpc.org**

Lili Liang

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=136&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-glucos	NEC495001MC	Perkin Elmer		
Pentobarbital	NDC76478-501-50	Oak Pharmaceuticals, Inc.		
Microdialysis pumps	CMA 402	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)		
0.9 % Sodium Chloride Injection USP	NDC0264-4001-55	B.Braun Medical Inc		
1 ml tuberculin syringes	REF 309659	BD Biosciences		
Microhematocrit capillary tubes	22-362-566	Fisher Scientific		
Heparin-coated blue polyethylene open-top tubes	652825	Beckman Coulter		
Microcentrifuge tubes (1.5 ml)	C-2170	Denville Scientific Inc.		

MATERIALS TEXT

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 -80°C 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 ^3H_2O 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.