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## U Mass - Basal glucose metabolism

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Jason Kim<sup>1</sup>

<sup>1</sup>University of Massachusetts

Mouse Metabolic Phenotyping Centers  
Tech. support email: [info@mmpc.org](mailto:info@mmpc.org)



Lili Liang

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**Protocol status:** Working

**We use this protocol and it's working**

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**Keywords:** body glucose turnover, hepatic glucose production, obesity, basal glucose level



## Abstract

### Summary:

Whole body glucose turnover and hepatic glucose production rates are measured at basal state using an intravenous infusion of labeled glucose in awake mice. Whole body glucose turnover and hepatic glucose production regulate basal glucose levels and are altered in obesity.

## Materials

### MATERIALS

☒ [3-<sup>3</sup>H] D-glucose **Perkin Elmer Catalog #NET331C005MC**

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

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

☒ Barium hydroxide mono- hydrate (0.3 N) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #B4059**

☒ Zinc sulfate heptahydrate (0.3 N) **Merck MilliporeSigma (Sigma-Aldrich) Catalog #Z2876**

☒ Scintillation cocktail **Perkin Elmer Catalog #6013329**

☒ Beckman Coulter LS6500 Multi-Purpose Scintillation Counter **Beckman Coulter Catalog #LS6500**

### Reagent Preparation:

**Reagent 1:** [3-<sup>3</sup>H] D-glucose infusion solution

### Reagents and Materials

1. [3-<sup>3</sup>H] D-glucose
2. 0.9 % Sodium Chloride, Injection, USP

### Procedure

1. Transfer [3-<sup>3</sup>H]glucose into a glass tube.
2. Place [3-<sup>3</sup>H]glucose solution in a vacuum oven set at room temperature for 2 days for vacuum drying of ethanol.
3. Re-suspend [3-<sup>3</sup>H]glucose using saline for intravenous infusion.

### Note:

**Sigma-Aldrich** **RRID:SCR\_008988**

**B Braun Medical**, Cite this (**B Braun Sharing Expertise**, **RRID:SCR\_007148**)

**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 Collect plasma sample (10  $\mu$ l) before the start of infusion (basal-0 min) to measure basal glucose levels.
- 6 Start the experiment by turning on the pump and intravenously infusing [ $3\text{-}^3\text{H}$ ] D-glucose at 0.05  $\mu\text{Ci}/\text{min}$  in awake mice. ([ $3\text{-}^3\text{H}$ ] D-glucose is suspended in saline solution)
- 7 Collect plasma samples (10  $\mu$ l each) at 90, 100, 110, and 120 min to measure glucose levels.
- 8 Collect additional plasma samples (10  $\mu$ l each) at 90, 100, 110, and 120 min to measure [ $3\text{-}^3\text{H}$ ] D-glucose concentrations. (10  $\mu$ l plasma samples are suspended in 20  $\mu$ l distilled water [ $\text{dH}_2\text{O}$ ] to make 30  $\mu$ l sample solutions.)
- 9 At the end of experiment, mice are euthanized using pentobarbital.
- 10 Biochemical assay is conducted using plasma samples to measure [ $3\text{-}^3\text{H}$ ] D-glucose concentrations.
  - a) Transfer 15  $\mu$ l of plasma sample solutions into microcentrifuge tubes with sample time clearly labeled.
  - b) Add 25  $\mu$ l BaOH and vortex samples.
  - c) Add 25  $\mu$ l  $\text{Zn}(\text{SO})_2$  and vortex samples.
  - d) Centrifuge samples for 5 min at 12,000g (~14,000 rpm).

- e) Transfer 20  $\mu$  of supernatant into scintillation vials and place into vacuum oven set at room temperature for overnight drying.
  - f) Following overnight drying, add 80  $\mu$ l dH<sub>2</sub>O and vortex thoroughly.
  - g) Add 3 ml of Ultima scintillation cocktail and vortex sample.
  - h) Measure [3-<sup>3</sup>H] D-glucose using Beckman Coulter Scintillation Counter.
- 11 Basal rate of whole body glucose turnover is calculated as the ratio of the [<sup>3</sup>H]glucose infusion rate to the specific activity of plasma glucose averaged for 90~120 min of experiment.