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U Mass - Basal glucose metabolism

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Protocol status: Working
We use this protocol and it's working

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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.

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

MATERIALS

MATERIALS

⊗ [3-3H] 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) Sigma-aldrich Catalog
#B4059

⊗ Zinc sulfate heptahydrate (0.3 N) 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: [3-³H] D-glucose infusion solution

Reagents and Materials

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

Procedure

1. Transfer [3-³H]glucose into a glass tube.
2. Place [3-³H]glucose solution in a vacuum oven set at room temperature for 2 days for vacuum drying of ethanol.
3. Re-suspend [3-³H]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 µl) 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 [3-³H] D-glucose at 0.05 µCi/min in awake mice. ([3-³H] D-glucose is suspended in saline solution)
- 7 Collect plasma samples (10 µl each) at 90, 100, 110, and 120 min to measure glucose levels.
- 8 Collect additional plasma samples (10 µl each) at 90, 100, 110, and 120 min to measure [3-³H] D-glucose concentrations. (10 µl plasma samples are suspended in 20 µl distilled water [dH₂O] to make 30 µl sample solutions.)
- 9 At the end of experiment, mice are euthanized using pentobarbital.

- 10** Biochemical assay is conducted using plasma samples to measure [3-³H] D-glucose 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) Transfer 20 µl of supernatant into scintillation vials and place into vacuum oven set at room temperature for overnight drying.
 - f) Following overnight drying, add 80 µl dH₂O and vortex thoroughly.
 - g) Add 3 ml of Ultima scintillation cocktail and vortex sample.
 - h) Measure [3-³H] D-glucose using Beckman Coulter Scintillation Counter.
- 11** Basal rate of whole body glucose turnover is calculated as the ratio of the [³H]glucose infusion rate to the specific activity of plasma glucose averaged for 90~120 min of experiment.