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U Mass - Organ-specific glucose uptake

Jason Kim¹

¹University 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:

Glucose uptake in individual organs can be measured using a bolus injection of 2-deoxy-D-[1-¹⁴C] glucose, a non-metabolizable glucose analog, and by determining labeled metabolite levels in select tissues. Insulin resistance is characterized by reduced glucose metabolism and develops in obese mice.

MATERIALS

MATERIALS

⊗ Poly-prep columns prefilled with AG 1-X8 resin Bio-rad Laboratories Catalog #731-6211

⊗ 0.2 M formic acid Sigma Aldrich Catalog #F0507

⊗ 0.5 M ammonium acetate Sigma Aldrich Catalog #A1542

Reagent Preparation:

Reagent 1: 0.2 M formic acid/0.5 M ammonium acetate

Reagents and Materials: formic acid, ammonium acetate, deionized water

Procedure:

1. Prepare 900 ml of dH₂O, and add 7.69 ml of formic acid.
2. Add 38.84 g of ammonium acetate, and adjust pH to 4.9±0.05 using dH₂O.
3. Add dH₂O to make a final solution volume of 1,000 ml.

Note:

Bio-Rad Laboratories [RRID:SCR_008426](https://pubmed.ncbi.nlm.nih.gov/308426/)

Sigma-Aldrich [RRID:SCR_008988](https://pubmed.ncbi.nlm.nih.gov/308988/)

Keywords: Glucose uptake,
obese mice

- 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 Administer an intravenous bolus injection of 10 μ Ci of 2-deoxy-D-[1- 14 C] glucose (2-[14 C]DG) in awake mice. Alternatively, intraperitoneal injection of 10 μ Ci of 2-[14 C]DG may be used in awake mice.
- 5 After 30 min, rapidly freeze-clamp the tissues in liquid N₂, and store tissue samples in -80°C freezer for biochemical assay.
- 6 Biochemical assay is conducted using frozen tissue samples (e.g., skeletal muscle, adipose tissue, heart) to measure tissue levels of 2-[14 C]DG-6-phosphate.
 - a) Prepare a heat block set to ~ 100°C.
 - b) Prepare anion-exchange columns by washing with 5 ml of dH₂O.
 - c) Homogenize 50–100 mg of frozen tissue samples by adding ten times the volume of dH₂O (50 mg of tissue in 500 μ l of dH₂O) in glass tubes using a tissue homogenizer.
 - d) Following homogenization, place the glass tubes in the heat block for 10 min, vortex for 2 sec, and then cool to room temperature.
 - e) Transfer the homogenized samples to microcentrifuge tubes using transfer pipettes and centrifuge at 16,000 $\times$ g for 5 min.

- f) Add 33 μ l of homogenate (supernatant) to 467 μ l dH₂O in a scintillation vial labeled “total” sample.
- g) Add 5 ml of scintillation cocktail, vortex, and count the samples for ¹⁴C using a liquid scintillation counter (total ¹⁴C samples).
- h) Transfer 333 μ l of homogenate (supernatant) to the anionexchange columns for the separation of 2-[¹⁴C]DG-6-P from 2-[¹⁴C] DG.
- i) Wash the columns with 2 ml of dH₂O three times and collect the samples into a scintillation vial labeled “wash” sample.
- j) Vortex the “wash” samples, and transfer 500 μ l of “wash” samples to another set of scintillation vials to be counted for ¹⁴C using a liquid scintillation counter (wash samples containing 2-[¹⁴C] DG).
- k) Elute the columns with 2 ml of 0.2 M formic acid/0.5 M ammonium acetate three times, and collect the samples into a scintillation vial labeled “eluate” sample.
- l) Vortex the “eluate” samples, and transfer 500 μ l of “eluate” samples to another set of scintillation vials to be counted for ¹⁴C using a liquid scintillation counter (eluate samples containing 2-[¹⁴C] DG-6-P).

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The rate of glucose uptake in individual organs is determined using 2-[¹⁴C] DG. 2-[¹⁴C] DG is taken up by cells, phosphorylated by glucokinase to become 2-[¹⁴C] DG-6-P, and not further metabolized. Thus, organ-specific accumulation and level of 2-[¹⁴C] DG-6-P following a bolus injection of 2-[¹⁴C] DG reflect glucose uptake in individual organs.