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Working

U Mass - Organ-specific glucose uptake

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ABSTRACT

Summary:

Glucose uptake in individual organs can be measured using a bolus injection of 2-deoxy-D-[1-^{14}C] 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.

EXTERNAL LINK

https://mmpc.org/shared/document.aspx?id=138&docType=Protocol

MATERIALS

NAME	CATALOG #	VENDOR	CAS NUMBER	RRID
Poly-prep columns prefilled with AG 1-X8 resin	731-6211	Bio-rad Laboratories		
0.2 M formic acid	F0507	Sigma Aldrich		
0.5 M ammomium acetate	A1542	Sigma Aldrich		

MATERIALS TEXT

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 dH_2O, 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 dH_2O.
3. Add dH_2O to make a final solution volume of 1,000 ml.

Note:

Bio-Rad Laboratories RRID:SCR_008426
Sigma-Aldrich RRID:SCR_008988

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 μCi of 2-deoxy-D-[1-^{14}C] glucose (2-[^{14}C]DG) in awake mice. Alternatively, intraperitoneal injection of 10 μCi of 2-[^{14}C]DG may be used in awake mice.

5 After 30 min, rapidly freeze-clamp the tissues in liquid N_2, 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 dH_2O.

c) Homogenize 50–100 mg of frozen tissue samples by adding ten times the volume of dH_2O (50 mg of tissue in 500 μl of dH_2O) 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 × g for 5 min.

f) Add 33 μl of homogenate (supernatant) to 467 μl dH_2O in a scintillation vial labeled “total” sample.

g) Add 5 ml of scintillation cocktail, vortex, and count the samples for ^{14}C using a liquid scintillation counter (total ^{14}C samples).

h) Transfer 333 μl of homogenate (supernatant) to the anionexchange columns for the separation of 2-[^{14}C]DG-6-P from 2-[^{14}C] DG.

i) Wash the columns with 2 ml of dH_2O three times and collect the samples into a scintillation vial labeled “wash” sample.

j) Vortex the “wash” samples, and transfer 500 μl of “wash” samples to another set of scintillation vials to be counted for ^{14}C using a liquid scintillation counter (wash samples containing 2-[^{14}C] 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 μl of “eluate” samples to another set of scintillation vials to be counted for ^{14}C using a liquid scintillation counter (eluate samples containing 2-[^{14}C] DG-6-P).

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