Organ-specific glucose uptake

Version: 1
Edited by: Jason Kim

(note that the following list should be linked to the appropriate location.)

Summary: *(This area will include a brief description of what the protocol is used for and why someone would need to use it.)*

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.

Reagents and Materials: *(This should be a comprehensive list of stock solutions and material. The reagent list for the stock solutions is included in the reagent preparation area that is included at the end of this SOP.)*

Reagent/Material	Vendor	Stock Number
Poly-prep columns pre-filled with AG 1-X8 resin	Bio Rad	731-6211
0.2 M formic acid	Sigma	F0507
0.5 M ammomium acetate	Sigma	A1542

Protocol:

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^{o}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 dH2O (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.

Reagent Preparation: *(This area may have several different preparations with the table of contents below.)*

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.