In vivo tissue glucose transport during a glucose tolerance test

1. Mix 2-deoxy-D-[1, 2-^{3}H]-glucose (Amersham) with 20% dextrose.
2. Inject intraperitoneally (2 g/kg body weight; 10 uCi/mouse) into mice.
3. Obtain blood samples (20 ul each) from tail veins at 0, 30, 60 and 120 min and measure glucose using an Elite glucometer (Bayer) at the same time.
4. Kill the mice at 120 min and freeze the skeletal muscle, heart, brain, liver and fat tissues in liquid nitrogen.

Determination of glucose specific activity:

1. Deproteinize 3 ul plasma with 200 ul 3.5% ice-cold perchloric acid. Centrifuge and neutralize with 45 ul 2.2 M $KHCO_3$, and measure radioactivity in a scintillation counter.
2. Calculate the glucose-specific activity (in degenerations per minute per micromole) by dividing sample radioactivity by the glucose concentration, and the area under the curve was integrated for the duration of the experiment (120 min).

Determination of tissue accumulation of 2-DOG:

1. Homogenize 100−500 mg tissue in 2 ml distilled water, and transfer 1.6 ml of the homogenate to 1.6 ml 7% ice-cold perchloric acid.
2. Centrifuge the sample to remove precipitated protein.
3. Neutralize 2.5 ml of the supernatant for 30 min with 625 ul 2.2 M $KHCO_3$.
4. Remove the precipitate by centrifugation, and divide the supernatant in 800-ul aliquots.
5. Determine total ^{3}H radioactivity in one liquot.
6. Pass another aliquot through an anion exchange column (AG 1-X8 resin; Bio-Rad, Hercules, California) to remove labeled 2-DOG-6-phosphate.
7. Wash the column with 3 ml distilled water, and measure the radioactivity in the eluted volume in a scintillation counter.
8. The difference between total and eluted ^{3}H radioactivity represents accumulated 2-deoxy-D-[1, 2-^{3}H]-glucose-6-phosphate.
9. Digest the protein pellet for 20 min at 55 °C with 1 N KOH, and determine protein concentration by the Bradford assay (Bio-Rad, Hercules, California).
10. Calculate the 2-DOG uptake by the dividing counts (degenerations per minute) by the integrated glucose-specific activity area under the curve and the sample protein content.