Supplement - Example calculation.

DG clearance from the blood

1. Calculate the tissue-specific DPM counts as the net [^{3}H]-2DG-6-P accumulation in the muscle tissue by subtracting [^{3}H]-2DG DPM counts measured in the supernatant of the $BaSO_4$ precipitate from the [^{3}H]-2DG + [^{3}H]-2DG-6-P DPM counts measured in the PCA supernatant.

$$[\,^3\text{H}]\text{-2DG-6-P}_{\text{muscle}}\ (\text{DPM}) = \underbrace{[\,^3\text{H}]\text{-2DG-6-P}_{\text{muscle}} + [\,^3\text{H}]\text{-2DG}}_{PCA} - \underbrace{[\,^3\text{H}]\text{-2DG}}_{BaOH+ZnSO_4}$$

Fx:

$$[\,^3\text{H}]\text{-2DG-6-P}_{\text{muscle}}\ (\text{DPM}) = \underset{PCA}{2822\,DPM} - \underset{BaOH+ZnSO_4}{2486DPM} = 336DPM$$

2. Relate the net [^{3}H]-2DG-6-P to mg tissue analyzed and to the 20 minutes experimental period to get [^{3}H]-2DG-6-P DPM g^{-1} min^{-1}.
Caution: *Remember to account for dilution factors in the calculation.*

$$[\,^3\text{H}]\text{-2DG-6-P}_{\text{muscle}}\ (\text{DPM} \times \text{g}^{-1} \times \text{min}^{-1}) = \frac{[\,^3\text{H}]\text{-2DG-6-P}_{\text{muscle}}}{\text{Tissue weight} \times \text{Time}} \times \text{Dilution factors}$$

Fx:

$$[\,^3\text{H}]\text{-2DG-6-P}_{\text{muscle}}\ (\text{DPM} \times \text{g}^{-1} \times \text{min}^{-1}) = \frac{(((400+19.3\text{mg}/150)\times(750/600) \times 336\text{DPM}/\ (19.3\text{mg}/1000)}{20\ min}$$
$$= 3042\ \text{DPM} \times \text{g}^{-1} \times \text{min}^{-1}$$

3. Extrapolate blood [^{3}H]-2DG DPM per 4 μL to 1 mL to yield blood [^{3}H]-2DG DPM mL^{-1}.
Note: *It is important to check whether tracer appearance is similar between groups because a higher [^{3}H]-2DG availability per se could result in an increased uptake of [^{3}H]-2DG. Therefore, a difference in tracer availability between groups should be taken into account in the interpretation of the data (Fig. 2B-C).*

$$[\,^3\text{H}]\text{-2DG}_{\text{blood}}\ (\text{DPM} \times \text{mL}^{-1}) = \frac{[\,^3\text{H}]\text{-2DG}_{\text{blood}}}{\text{Blood volume}} \times 1000$$

Fx:

$$[\,^3\text{H}]\text{-2DG}_{\text{blood}}\ (\text{DPM} \times \text{mL}^{-1}) = \frac{1103\ \text{DPM}}{0.004\ \text{mL}} = 275750\ \text{DPM}$$

4. Calculate the time-weighted average of blood [^{3}H]-2DG appearance.
Tip: *Assuming that blood [^{3}H]-2DG appearance is increasing linearly in the first 5 minutes after intraperitoneal injection, a value of 0 DPM mL^{-1} at time 0 should be included in the weighted average).*

$$[\,^3\text{H}]\text{-2DG}_{\text{blood\_average}}\ (\text{DPM} \times \text{mL}^{-1}) = \frac{\frac{([\,^3\text{H}]\text{-2DG}_{\text{blood\_0 min}} + [\,^3\text{H}]\text{-2DG}_{\text{blood\_5 min}})}{2} \times 5\,min + \frac{([\,^3\text{H}]\text{-2DG}_{\text{blood\_5 min}} + [\,^3\text{H}]\text{-2DG}_{\text{blood\_10 min}})}{2} \times 5\,min + \frac{([\,^3\text{H}]\text{-2DG}_{\text{blood\_10 min}} + [\,^3\text{H}]\text{-2DG}_{\text{blood\_20 min}})}{2} \times 10\,\text{min}}{20}$$

Fx:

$$[\,^3\text{H}]\text{-2DG}_{\text{blood\_average}}\ (\text{DPM} \times \text{mL}^{-1}) = \frac{\frac{(0 + 275750\ \text{DPM} \times \text{mL}^{-1})}{2} \times 5\,min + \frac{(275750\ \text{DPM} \times \text{mL}^{-1} + 621250\ \text{DPM} \times \text{mL}^{-1})}{2} \times 5\,min + \frac{(621250\ \text{DPM} \times \text{mL}^{-1} + 556750\ \text{DPM} \times \text{mL}^{-1})}{2} \times 10\,\text{min}}{20\text{min}}$$

$$= 441094\ \text{DPM} \times \text{mL}^{-1}$$

5. Normalize tissue-specific DPMs to the time-weighted average of blood $[^3H]$-2DG appearance to calculate the proportion of available $[^3H]$-2DG cleared from the blood into the tissue (glucose clearance, Fig. 3B). Data should be expressed per unit weight of the analyzed tissue and per hour (mL g^{-1} h^{-1}).

$$\text{2DG clearance}\ (\text{mL} \times \text{g}^{-1} \times \text{h}^{-1}) = \frac{[\,^3\text{H}]\text{-2DG-6-P}_{\text{muscle}}}{[\,^3\text{H}]\text{-2DG}_{\text{blood\_average}}} \times 60$$

Fx:

$$\text{2DG clearance}\ (\text{mL} \times \text{g}^{-1} \times \text{h}^{-1}) = \frac{3042\ \text{DPM} \times \text{g}^{-1} \times \text{min}^{-1}}{441094\ \text{DPM} \times \text{mL}^{-1}} \times 60 = 0.414\ \text{mL} \times \text{g}^{-1} \times \text{h}^{-1}$$

Note: *This calculation assumes that the $[^3H]$-2DG tracer measured by a tail bleed is indicative of the tracer available in the interstitial space for uptake into the tissue.*

Glucose uptake index

6. Calculate the time-weighted average of blood glucose during the experimental period (Fig. 2E-G).

$$[\text{Glucose}]_{\text{blood\_average}}\ (\text{mmol} \times \text{L}^{-1}) = \frac{(\frac{([\text{Glucose}]_{\text{blood\_0 min}} + [\text{Glucose}]_{\text{blood\_5 min}})}{2} \times 5\,min + \frac{([\text{Glucose}]_{\text{blood\_5 min}} + [\text{Glucose}]_{\text{blood\_10 min}})}{2} \times 5\,min + \frac{([\text{Glucose}]_{\text{blood\_10 min}} + [\text{Glucose}]_{\text{blood\_20 min}})}{2} \times 10\,min\,)}{20\text{min}}$$

Fx:

$$[\text{Glucose}]_{\text{blood\_average}}\ (\text{mmol} \times \text{L}^{-1}) = \frac{\left(\frac{(6.2\,mM + 9.6\,mM)}{2} \times 5\,min + \frac{(9.6\,mM + 10.3\,mM)}{2} \times 5\,min + \frac{(10.3\,mM + 10.6\,mM)}{2} \times 10 min\right)}{20\text{min}} = 9.7 mM$$

7. Calculate the specific activity (SA) of [^{3}H]-2DG in the blood as the ratio between the time-weighted average of blood [^{3}H]-2DG appearance and the weighted average of blood glucose.

$$\text{SA [}^3\text{H]-2DG}_{\text{blood}}\ (\text{DPM} \times \mu\text{mol}^{-1}) = \frac{[^3\text{H]-2DG}_{\text{blood\_average}}}{[\text{Glucose}]_{\text{blood\_average}}}$$

Fx:

$$\text{SA [}^3\text{H]-2DG}_{\text{blood}}\ (\text{DPM} \times \mu\text{mol}^{-1}) = \frac{441094\ \text{DPM} \times \text{mL}^{-1}}{9.7\,mM} = 45532\ \text{DPM} \times \mu\text{mol}^{-1}$$

8. Calculate an approximation of glucose uptake by calculating the glucose uptake index as the ratio between tissue-specific DPMs and SA. Data should be expressed per unit weight of the analyzed tissue and per hour (μmol g^{-1} h^{-1}).

$$\text{Glucose uptake index}\ (\mu\text{mol} \times \text{g}^{-1} \times \text{h}^{-1}) = \frac{[^3\text{H]-2DG-6-P}_{\text{muscle}}}{\text{SA [}^3\text{H]-2DG}_{\text{blood}}} \times 60\ min$$

Fx:

$$\text{Glucose uptake index}\ (\mu\text{mol} \times \text{g}^{-1} \times \text{h}^{-1}) \frac{3042\ \text{DPM} \times \text{g}^{-1} \times \text{min}^{-1}}{45532\ \text{DPM} \times \mu\text{mol}^{-1}} \times 60\text{min} = 4.008\ \mu\text{mol} \times \text{g}^{-1} \times \text{h}^{-1}$$