Supplemental Methods: Kinetic Principles, Calculations and Interpretation

According to MacCoss et al. (2001), for infusion of the \(^{13}C_1\)methionine tracer, extracellular enrichment is represented by plasma \(^{13}C_1\)methionine (Ep\(_{13C\text{-Met}}\)) and intracellular enrichment is represented by plasma \(^{13}C_1\)homocysteine (Ep\(_{13C\text{-Hcy}}\)). The flux of the \(^{13}C_1\)methionine tracer was calculated as:

\[
Q_C = I_{13C\text{-Met}} \cdot ((E_{13C\text{-Met}} / Ep_{13C\text{-Hcy}}) - 1)
\]

As the labeled methyl-group is lost during methyltransferase reactions, the intracellular surrogate homocysteine cannot be used for estimation of intracellular \([\text{methyl}^2H_3]\)methionine enrichment. The intracellular \([\text{methyl}^2H_3]\)methionine enrichment is therefore estimated on the basis of the measured methionine intracellular/extracellular gradient determined from the \(^{13}C_1\)methionine tracer (Ep\(_{13C\text{-Hcy}}\) / Ep\(_{13C\text{-Met}}\)), which is used to adjust the plasma \([^2H_3]\)methionine enrichment (Ep\(_{[^2H_3}\text{Met}}\)) to approximate the intracellular \([^2H_3]\)methionine enrichment (Ep\(_{[^2H_3}\text{Met}}\)).

\[
Ep'_{[^2H_3}\text{Met}} = Ep_{[^2H_3}\text{Met}} \cdot (Ep_{13C\text{-Hcy}} / Ep_{13C\text{-Met}})
\]

With this corrected value for intracellular \([^2H_3]\)methionine enrichment, the flux of methyl-labeled methionine is calculated as:

\[
Q_M = I_{[^2H_3}\text{Met}} \cdot ((E_{[^2H_3}\text{Met}} / Ep'_{[^2H_3}\text{Met}}) - 1)
\]

The overall rate of homocysteine remethylation (RM) is then calculated as the difference between the fluxes of the methionine carboxyl and methyl groups:

\[
RM = Q_M - Q_C.
\]

The rate of production of \(^{13}CO_2\) provided a direct and specific measurement of the in vivo whole body flux through amino acid oxidation reactions; in the case of methionine, the release of the \([1-^{13}C]\)atom reflects the rate of transsulfuration. The rate of \(^{13}CO_2\) release (F\(_{^{13}CO_2}\), in units of \(\mu\text{mol} \cdot \text{h}^{-1} \cdot \text{kg}^{-1}\) body weight) and the rate of transsulfuration (TS, \(\mu\text{mol} \cdot \text{h}^{-1} \cdot \text{kg}^{-1}\) body weight) were calculated as follows:

\[
F_{^{13}CO_2} = E_{^{13}CO_2} \cdot (FCO_2 / 0.81) \cdot (1 / W)
\]

where: \(E_{^{13}CO_2}\) is breath \(^{13}CO_2\) enrichment plateau, FCO\(_2\) is the rate of total \(^{13}C\) release, and 0.81 is the assumed fraction of \(^{13}CO_2\) release from the body pool of bicarbonate and W is body weight (Robert et al. 1982).

\[
TS = F_{^{13}CO_2} / Ep_{13C\text{-Hcy}}
\]

where: F\(_{^{13}CO_2}\) is the rate of \(^{13}CO_2\) release and Ep\(_{13C\text{-Hcy}}\) is the plateau enrichment of \([^{13}C]\)homocysteine in plasma.

The rate of methionine uptake for protein synthesis (S) was calculated as: S = \(Q_C - TS\)

The rate of transmethylation (TM) is calculated from TS and RM: TM = TS + RM

**References:**
