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 [methyl-$^2H_3$]methionine enrichment. The intracellular [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 ($Ep_{2H\text{-}Met}$) to approximate the intracellular [$^2H_3$]methionine enrichment ($Ep'_{2H\text{-}Met}$).

$$Ep'_{2H\text{-}Met} = Ep_{2H\text{-}Met} \cdot (Ep_{13C\text{-}Hcy} / Ep_{13C\text{-}Met})$$

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

$$Q_M = I_{2H\text{-}Met} \cdot ((E_{2H\text{-}Met} / Ep'_{2H\text{-}Met}) - 1)$$

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 $\mu mol \cdot h^{-1} \cdot kg^{-1}$ body weight) and the rate of transsulfuration (TS, $\mu mol \cdot h^{-1} \cdot kg^{-1}$ 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 CO_2 enrichment plateau, FCO_2 is the rate of total CO_2 production, and 0.81 is the assumed fraction of 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:

MacCoss MJ, Fukagawa NK, Matthews DE. Measurement of intracellular sulfur amino acid metabolism in humans. Am J Physiol Endocrinol Metab. 2001 Jun;280:E947-55.

Robert JJ, Bier DM, Zhao XH, Matthews DE, Young VR. Glucose and insulin effects on the novo amino acid synthesis in young men: studies with stable isotope labeled alanine, glycine, leucine, and lysine. Metabolism. 1982 Dec;31:1210-8.