A Comprehensive Compartmental Model for the Assessment of Net Whole Body Protein Breakdown, Using a Pulse of Phenylalanine and Tyrosine Stable Isotopes in Humans

Mason A[1,2], Engelen MPKJ[1], Toffolo G[2], Ivanov I[3], Deutz NEP[2]
[1] Center for Translational Research in Aging Longevity, Health and Kinesiology, Texas A&M University, College Station, TX
a.mason@ctral.org, mpkj.engelen@ctral.org, nep.deutz@ctral.org
[2] Department of Information Engineering, University of Padova, Italy
alvise.mason@studenti.unipd.it, toffolo@dei.unipd.it
[3] Department of Veterinary Physiology and Pharmacology, Texas A&M University, College Station, TX
IIvanov@cvm.tamu.edu

Keywords: Phenylalanine, Tyrosine, stable isotopes, tracer kinetics, compartmental model

Rationale: We recently developed a novel and easy-to-use approach to measure net whole body protein breakdown (net PB), using non-compartmental modeling after single pulse injection of the stable isotopes phenylalanine (Phe) and tyrosine (Tyr). We successively fine-tuned this approach by developing a minimal compartmental model to add small structural information to amino acids kinetics in PB. Lately, we further refined our innovative approach by developing a comprehensive compartmental model to add exhaustive and more physiologically relevant structural information regarding the Phe-Tyr pathway with high accuracy.

Method: Healthy human subjects (6 male, 5 female, age: 56.6 ± 8.3 years) were given in the postabsorptive state a single pulse (8ml) injection of L-[ring-$^{13}C_6$]-Phe (6.44 mg/ml) and L-[ring-$^{2}H_4$]-Tyr (0.46 mg/ml). Multiple plasma samples were collected for 120 min and tracer-tracee ratio of Phe (mass 6) and Tyr (mass 4 and 6) were measured by LC-MS/MS. A six compartment model was developed to describe the kinetics of Phe and Tyr and the Phe to Tyr hydroxylation. The model is a priori identifiable from the Phe+6, Tyr+6 and Tyr+4 data measured following the Phe+6 and Tyr+4 injections, provided that the Phe and Tyr accessible pool sizes are known. Since these pools represent the free amino acid extracellular fluid pools for Phe and Tyr (respectively), their size was estimated from plasma concentrations of the two amino acids and fat free mass in each subject. The model provides estimates of compartmental fluxes, including the

de novo productions of Phe and Tyr in the slow tissues' intracellular pools and the Phe to Tyr interconversion. These fluxes were compared to reference values available in the literature in order to assess the physiological relevance of the estimated variables.

Results: The model, identified by using SAAM, was able to reproduce the experimental data of all individuals and all its parameters were estimated with high precision (VC: 12% ± 5%). Estimated values for intercompartmental fluxes are physiologically plausible [1]: Net-PB: 4.04 ± 0.93 μmol·FFM kg^{-1}·h^{-1}; *de novo* production of Phe in the slow tissues' intracellular pool: 95.52 ± 14.76 μmol·FFM kg^{-1}·h^{-1}; *de novo* production of Tyr in the slow tissues' intracellular pool: 75.57 ± 14.34 μmol·FFM kg^{-1}·h^{-1}; molar ratio of the fluxes of Phe and Tye arising from protein catabolism: 0.79. By using the model to simulate a continuous infusion experiment previously done on pigs, some of the structural assumptions made in the design phase were validated.

Conclusion: Our data reveal that after single pulse injection of Phe and Tyr stable isotopes, a comprehensive compartmental model analysis estimates net PB with low variability when the physiology of Phe to Tyr conversion is included in the model estimations. Furthermore, our latest model can provide a more exhaustive, detailed and physiologically relevant structural insight in the metabolism of these amino acids, thus improving the quality of the tracer data analysis as performed by our previous minimal model.

References

[1] Dwight E Matthews, *An overview of phenylalanine and tyrosine kinetics in humans*, The Journal of nutrition **137(6)**: 1549S–1555S, 2007.