The intrinsically labeled protein approach is the preferred method to quantify the release of dietary protein-derived amino acids into the circulation

Am J Physiol Endocrinol Metab 317: E433–E434, 2019;
doi:10.1152/ajpendo.00155.2019.

LETTER TO THE EDITOR

The intrinsically labeled protein approach is the preferred method to quantify the release of dietary protein-derived amino acids into the circulation

Jorn Trommelen,[1] Andrew M. Holwerda,[1] Jean Nyakayiru,[1] Stefan H. M. Gorissen,[1] Olav Rooyackers,[2] Nicholas A. Burd,[3] Yves Boirie,[4] and Luc J. C. van Loon[1]

[1]*Department of Human Biology, NUTRIM School of Nutrition and Translational Research in Metabolism, Maastricht University Medical Centre, Maastricht, The Netherlands;* [2]*Division of Anaesthesia and Intensive Care, Department of Clinical Science Intervention and Technology (CLINTEC), Karolinska Institutet, Stockholm, Sweden;* [3]*Department of Kinesiology and Community Health, Division of Nutritional Sciences, University of Illinois, Urbana, Illinois; and* [4]*Université Clermont Auvergne, INRA, UNH, Unité de Nutrition Humaine, CHU Clermont-Ferrand, Service de Nutrition Clinique, CRNH Auvergne Clermont-Ferrand, France*

Submitted 17 April 2019; accepted in final form 7 July 2019

TO THE EDITOR: We enjoyed reading the critical work by Wolfe et al. (12) on the use of the intrinsically labeled protein method to quantify postprandial protein kinetics and its impact on whole body protein metabolism. We routinely combine the use of intrinsically labeled protein with contemporary stable isotope methodology to quantify the postprandial release of dietary protein-derived amino acids into the circulation (1, 4, 6, 8–10). The authors outlined their perceived assumptions and concluded that the method is "unreliable" by underestimating exogenous amino acid rate of appearance, which would result in an overestimation of postprandial (whole body) protein breakdown rates. This speculation centers on their assumption that the enrichment of the ingested protein (E_{pro}) is diluted in the gastrointestinal tract and the splanchnic bed due to high rates of endogenous protein secretion. However, they base their conclusions on an incorrect definition of E_{pro}. Specifically, the authors appear to assume that the definition of E_{pro} represents (or rather should represent) the enrichment of all available free amino acids in the gastrointestinal tract and splanchnic bed. Under this alternative definition, the ingested tracer would no longer be predictive of the ingested protein but rather of the combined amount of amino acids originating from the ingested protein, the gastrointestinal tract, and the splanchnic bed. This is clearly in contrast with the intended purpose of the oral tracer: to allow differentiation between endogenous and exogenous protein-derived amino acids (see Fig. 1). The correct definition of E_{pro} only reflects the enrichment of the ingested protein. Amino acids originating from the gastrointestinal tract and splanchnic bed are accounted for in the same manner as the release of amino acids derived from (unlabeled) protein from peripheral tissues into the circulation and, therefore, contribute to the calculation of whole body protein breakdown rate. Under the correct definition of E_{pro}, the intrinsically labeled protein method quantifies the rate of appearance of exogenous protein-derived amino acids into the circulation and, as such, by deduction the endogenous amino acid rate of appearance and thus whole body protein breakdown rate.

The authors also claim that the dietary protein-derived amino acids incorporated into muscle protein reported by Groen et al. (3) represent an underestimation. This claim is used to support their argument that the intrinsically labeled protein method underestimates exogenous protein-derived amino acid rate of appearance. However, the incorporation of exogenous protein-derived amino acids into muscle protein is directly assessed by expressing the amount of ingested tracer that is incorporated in muscle protein relative to the ingested amount of tracer. The assessment of exogenous protein-derived amino acid rate of appearance is not part of this calculation, which renders the claim invalid.

Finally, the authors propose the bioavailability method as the preferred alternative. However, dietary protein-derived amino acid bioavailability is highly dependent on the experimental conditions [e.g., duration of the postprandial assessment, dose of the ingested protein, (prior) exercise, disease, etc. (2, 5, 7, 11)]. Any deviation from the conditions in which the static bioavailability value was obtained will result in incorrect estimates. In contrast, the intrinsically labeled protein method directly assesses postprandial protein-derived amino acid bioavailability over time, within each subject and within the experimental conditions.

In conclusion, the combined use of contemporary stable isotope methodology with the ingestion of intrinsically labeled protein remains the preferred method to directly quantify the postprandial release of exogenous protein-derived amino acids into the circulation in vivo in humans.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

L.J.v.L. conceived and designed research. J.T. and L.J.v.L. prepared figures; J.T., A.M.H., J.N., S.H.G., and L.J.v.L. drafted manuscript; J.T., A.M.H., J.N., S.H.G., O.R., N.A.B., Y.B., and L.J.v.L. edited and revised manuscript; J.T., A.M.H., J.N., S.H.G., O.R., N.A.B., Y.B., and L.J.v.L. approved final version of manuscript.

Address for reprint requests and other correspondence: L. van Loon, Dept. of Human Biology, NITRIM School of Nutrition and Translational Research in Meatoblism, Maastrict University Medical Centre, PO Box 616, 6200 MD Maastricht, The Netherlands (e-mail: l.vanloon@maastrichtuniversity.nl).

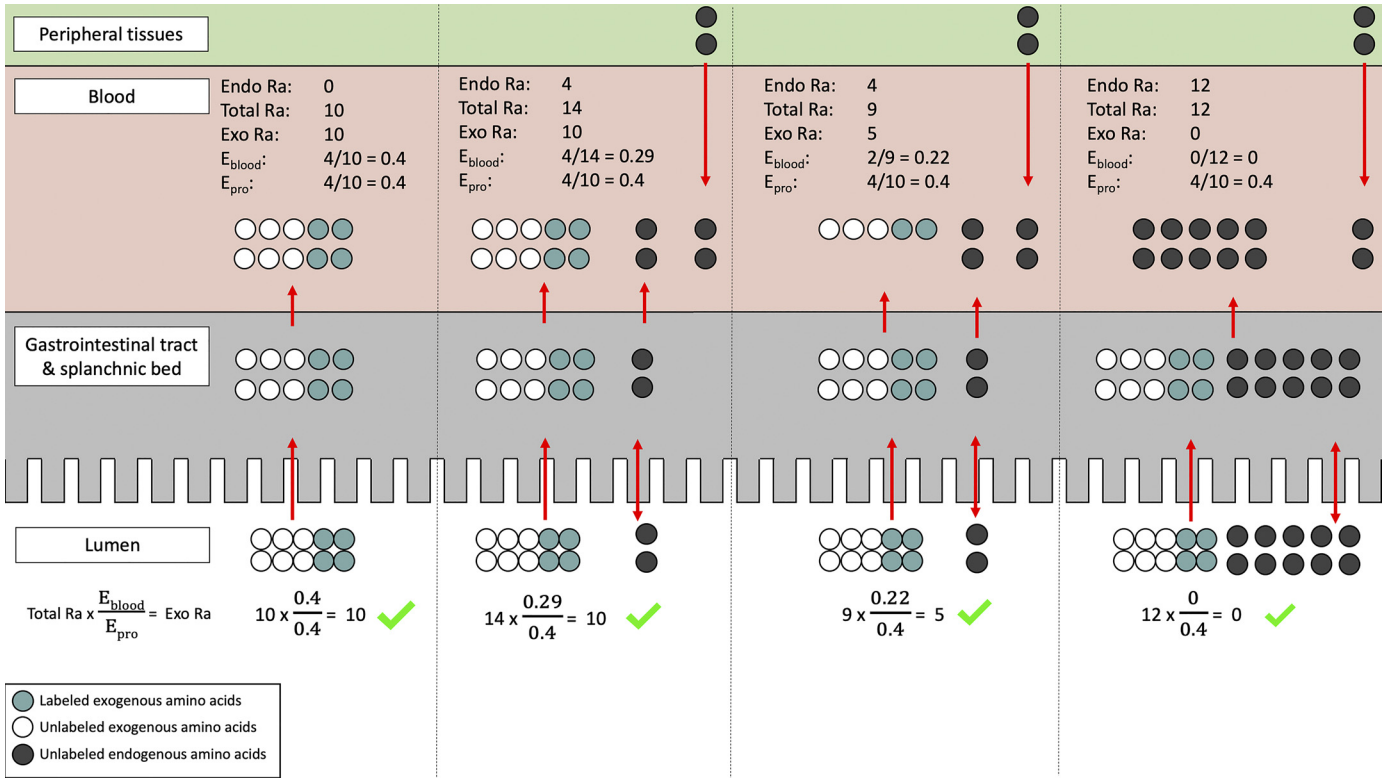

Fig. 1. Postprandial appearance rate of dietary protein-derived amino acids into the circulation is assessed appropriately when applying the intrinsically labeled protein method, regardless of the flow of unlabeled amino acids originating from the gastrointestinal tract and splanchnic bed. Total Ra, total rate of appearance; endo Ra, endogenous rate of appearance; exo Ra, exogenous rate of appearance; E_{blood}, plasma enrichment; E_{pro}, enrichment of the ingested protein.

REFERENCES

1. **Boirie Y, Dangin M, Gachon P, Vasson MP, Maubois JL, Beaufrère B.** Slow and fast dietary proteins differently modulate postprandial protein accretion. *Proc Natl Acad Sci USA* 94: 14930–14935, 1997. doi:10.1073/pnas.94.26.14930.
2. **Gorissen SHM, Burd NA, Hamer HM, Gijsen AP, Groen BB, van Loon LJC.** Carbohydrate coingestion delays dietary protein digestion and absorption but does not modulate postprandial muscle protein accretion. *J Clin Endocrinol Metab* 99: 2250–2258, 2014. doi:10.1210/jc.2013-3970.
3. **Groen BBL, Horstman AM, Hamer HM, de Haan M, van Kranenburg J, Bierau J, Poeze M, Wodzig WKWH, Rasmussen BB, van Loon LJC.** Post-prandial protein handling: you are what you just ate. *PLoS One* 10: e0141582, 2015. doi:10.1371/journal.pone.0141582.
4. **Holwerda AM, Kouw IWK, Trommelen J, Halson SL, Wodzig WK, Verdijk LB, van Loon LJ.** Physical activity performed in the evening increases the overnight muscle protein synthetic response to presleep protein ingestion in older men. *J Nutr* 146: 1307–1314, 2016. doi:10.3945/jn.116.230086.
5. **Holwerda AM, Paulussen KJM, Overkamp M, Goessens JPB, Kramer IF, Wodzig WKWH, Verdijk LB, van Loon LJC.** Dose-dependent increases in whole-body net protein balance and dietary protein-derived amino acid incorporation into myofibrillar protein during recovery from resistance exercise in older men. *J Nutr* 149: 221–230, 2019. doi:10.1093/jn/nxy263.
6. **Kouw IW, Holwerda AM, Trommelen J, Kramer IF, Bastiaanse J, Halson SL, Wodzig WK, Verdijk LB, van Loon LJ.** Protein ingestion before sleep increases overnight muscle protein synthesis rates in healthy older men: a randomized controlled trial. *J Nutr* 147: 2252–2261, 2017. doi:10.3945/jn.117.254532.
7. **Liebau F, Wernerman J, van Loon LJC, Rooyackers O.** Effect of initiating enteral protein feeding on whole-body protein turnover in critically ill patients. *Am J Clin Nutr* 101: 549–557, 2015. doi:10.3945/ajcn.114.091934.
8. **Trommelen J, Holwerda AM, Kouw IWK, Langer H, Halson SL, Rollo I, Verdijk LB, VAN Loon LJC.** Resistance exercise augments postprandial overnight muscle protein synthesis rates. *Med Sci Sports Exerc* 48: 2517–2525, 2016. doi:10.1249/MSS.0000000000001045.
9. **Trommelen J, Kouw IWK, Holwerda AM, Snijders T, Halson SL, Rollo I, Verdijk LB, van Loon LJC.** Presleep dietary protein-derived amino acids are incorporated in myofibrillar protein during postexercise overnight recovery. *Am J Physiol Endocrinol Metab* 314: E457–E467, 2018. doi:10.1152/ajpendo.00273.2016.
10. **van Vliet S, Shy EL, Abou Sawan S, Beals JW, West DW, Skinner SK, Ulanov AV, Li Z, Paluska SA, Parsons CM, Moore DR, Burd NA.** Consumption of whole eggs promotes greater stimulation of postexercise muscle protein synthesis than consumption of isonitrogenous amounts of egg whites in young men. *Am J Clin Nutr* 106: 1401–1412, 2017. doi:10.3945/ajcn.117.159855.
11. **van Wijck K, Pennings B, van Bijnen AA, Senden JMG, Buurman WA, Dejong CHC, van Loon LJC, Lenaerts K.** Dietary protein digestion and absorption are impaired during acute postexercise recovery in young men. *Am J Physiol Regul Integr Comp Physiol* 304: R356–R361, 2013. doi:10.1152/ajpregu.00294.2012.
12. **Wolfe RR, Park S, Kim IY, Starck C, Marquis BJ, Ferrando AA, Moughan PJ.** Quantifying the contribution of dietary protein to whole body protein kinetics: examination of the intrinsically labeled proteins method. *Am J Physiol Endocrinol Metab* 317: E74–E84, 2019. doi:10.1152/ajpendo.00294.2018.