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APPLICATION NOTE 30

Determination of Nitric Oxide Production and *de novo* Arginine Production with Stable Isotopes

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Arginine is a semi-essential amino acid involved in many physiological and pathophysiological processes. The endogenous synthesis of arginine depends on the production of its precursor, citrulline, by the small intestine. Citrulline can be utilized by many cell types to produce arginine, but quantitatively the kidney is the main site for citrulline utilization and arginine production.

One of the products of arginine metabolism, nitric oxide (NO), is an important signaling molecule involved in the regulation of blood pressure, post-translational regulation of proteins and in the modulation of the immune response. Because of its high reactivity and short life the measurement of NO depends, for the most part, on indirect methods of quantitation. For each NO generated from arginine, a citrulline molecule is produced (see figure below). Thus, citrulline can be both the precursor and a product of arginine metabolism.

The whole body quantification of these processes can then be accomplished by utilizing stable isotopes to determine the entry rate of arginine and citrulline and their rates of interconversions (Figure 2).

Because *only* a small proportion of the entry rate of arginine (~1%) is converted into NO (and citrulline) the choice of tracers and method of analysis is crucial for an optimal quantitation. The possible recycling of the tracer through ornithine, also a product of arginine and the precursor for citrulline synthesis, further limits the choice of tracers. For this reason the arginine tracer of choice is labeled in the guanidino group (although additional labeled atoms may also be present). The use of L-Arginine•HCl (guanido-$^{15}N_2$, 98%+) (NLM-395) to determine the rate of appearance of arginine (or more appropriately of its guanidino group) and its conversion into L-Citrulline (ureido-$^{15}N_1$, 98%) (NLM-6850) has become the protocol of choice for the determination of NO production. To determine the rate of appearance of citrulline and the rate of conversion to arginine a citrulline tracer is employed (Figure 3).

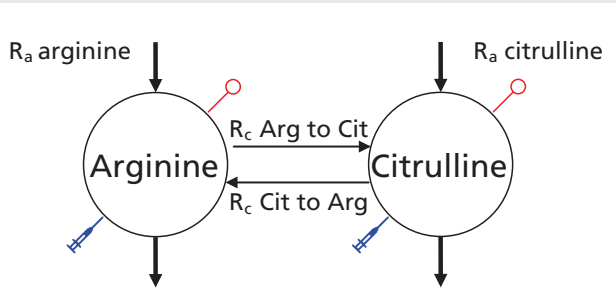

Figure 2. Arginine and citrulline rate of appearance (Ra) and rate of interconversions (Rc) model. Two different tracers are infused ( ) and the arginine and citrulline pool sampled ( ).

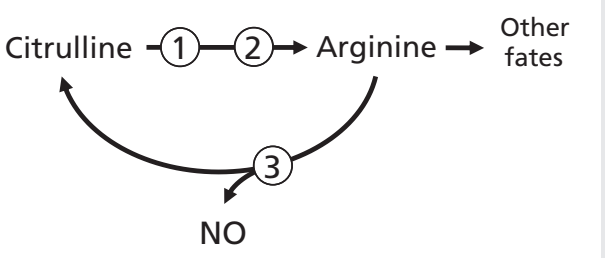

Figure 1. Arginine is synthesized from citrulline by action of argininosuccinate synthase (1) and argininosuccinate lyase (2). In turn, citrulline is a byproduct of the synthesis of NO by action of NO synthase (3).

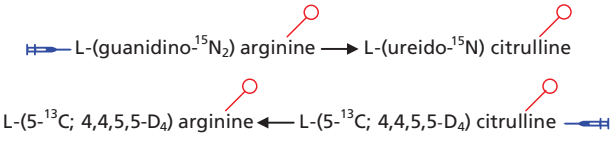

Figure 3. Tracer protocol for the determination of aginine and citrulline rate of appearance and rate of interconversions. After L-(guanido-$^{15}N_2$) arginine and L-(5-^{13}C; 4,4,5,5-D_4) citrulline (CDLM-7139) infusion ( ), the arginine and citrulline pools are sampled ( ) and analyzed for the isotopologues shown in the figure.

(continued)

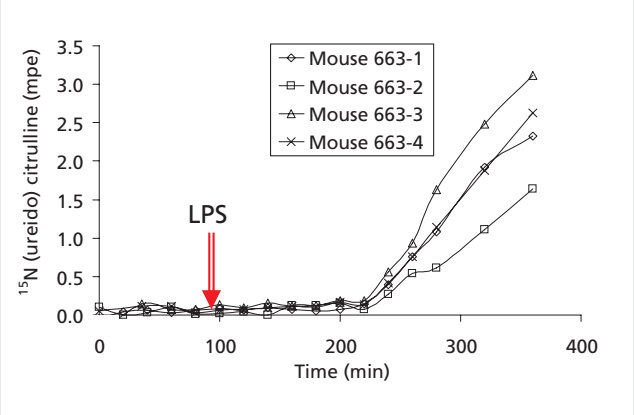

Figure 4. (Ureido-^{15}N) citrulline enrichment in mice challenged with endotoxin. The arrow denotes the time of endotoxin challenge.

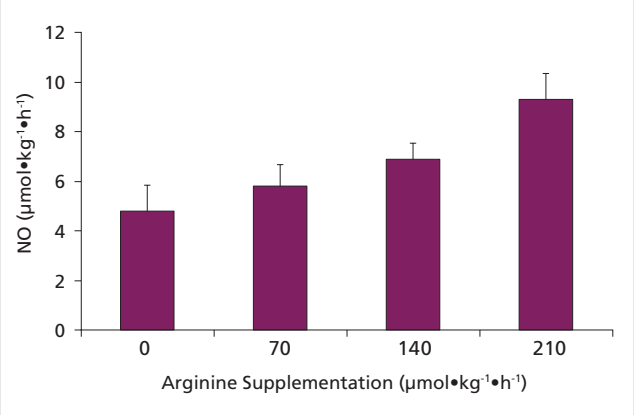

Figure 5. Nitric oxide production in mice supplemented with arginine four hours after endotoxin challenge. A linear relationship between arginine availability and NO response (R^2 = 0.57; P <0.0001) was observed.

The loss of the ureido nitrogen of citrulline during fragmentation in LC/MS/MS analysis results in a reduced natural (background) enrichment (~0.4 mp), and thus in the ability to reliably detect small enrichments above background (Figure 4). In addition, the derivatization of citrulline (e.g. dansylation) increases sensitivity and improves chromatography.

This isotopic approach has allowed us to study the NO time-dependent response after endotoxin (LPS) challenge and the effect of arginine supplementation on NO production during endotoxemia. Under basal conditions very little NO is produced as shown by the reduced (ureido-^{15}N) citrulline enrichment before LPS challenge (Figure 4). Following a ~2h lag period after endotoxin administration a dramatic increase in NO production can be detected. In another set of mice, the intravenous arginine supplementation in endotoxin-challenged mice resulted in a linear increase in NO production at 4h post LPS administration (Figure 5).

For over the past 15 years[1] these methods have proven to be useful in the determination of whole body nitric oxide production and *de novo* arginine production in various species. For an in depth discussion on the tracer methodology for the determination of NO see Van Eijk, et al.[2] and for a comprehensive review on the human data, Siervo, et al., 2011.[3]

References

1. Castillo, L. **1996**. Whole body nitric oxide synthesis in healthy men determined from [^{15}N] arginine-to-[^{15}N] citrulline labeling. *Proc Natl Acad Sci USA, 93*, 11460-11465.
2. van Eijk, H.M.H.; Luiking, Y.C.; and Deutz, N.E.P. **2007**. Methods using stable isotopes to measure nitric oxide (NO) synthesis in the L-arginine/NO pathway in health and disease. *J Chromatogr, B: Anal Technol Biomed Life Sci, 851*, 172-185.
3. Siervo, M. **2011**. Measurement of *in vivo* nitric oxide synthesis in humans using stable isotopic methods: A systematic review. *Free Radical Biol Med, 51*, 795-804.

Related Products

Catalog No.	Description
NLM-395	L-Arginine•HCl (guanido-$^{15}N_2$, 98%+)
NLM-6850	L-Citrulline (ureido-$^{15}N_1$, 98%)
CDLM-7139	L-Citrulline (5-^{13}C, 99%; 4,4,5,5-D_4, 95%)