

UPLC-MSMS analysis of arginine kinetics and metabolism in children with severe falciparum malaria

Haoyue Zhang¹ Donald L Granger², Matthew P Rubach^{2,3}, J Brice Weinberg³, Nicholas M Anstey⁴, Tsin W Yeo^{4,5}, Salvatore Florence⁶, Esther D Mwaikambo⁶, David S Millington¹

¹ Duke Medicine Biochemical Genetics Laboratory, Durham, North Carolina, United States

² University of Utah School of Medicine and VA Medical Center, Salt Lake City, Utah, United States

³ Duke University and VA Medical Centers, Durham, North Carolina, United States

⁴ Menzies School for Health Research and Charles Darwin University, Darwin, Australia

⁵ Lee Kong Chian School of Medicine, Nanyang Technological University, Singapore

⁶ Hubert Kairuki Memorial University, Dar es Salaam, United Republic of Tanzania

We have previously shown that nitric oxide (NO) is protective against development of severe malaria (SM). We showed that plasma arginine, NO metabolites, and PBMC NO synthase (NOS) protein and RNA are very low in children with falciparum SM compared with healthy children (HC) [1,2]. In the present study, we conducted primed infusions of ¹³C₆, ¹⁵N₄-labeled arginine in 10 children with SM and 10 HC children under an IRB-approved protocol, and then determined isotopic-enrichment of both arginine and citrulline at baseline and 90, 120, 150, 180 min post-infusion. Plasma amino acid levels were measured by an amino acid analyzer (Hitachi L-8800). Isotope ratios were determined by UPLC-MSMS with selected ion monitoring (Waters BEH Amide 1.7 μm 2.1x100 mm UPLC column; Waters Xevo TQ with Acquity UPLC System). Isotopic equilibrium in arginine was attained within 180 min. At that time point, arginine flux was determined to be higher in children with SM than in HC children. Median values were 108 in SM (range 84-144) vs 85 in HC (68-95) μmol/kg/hr. In addition, differences in the isotopic labeling patterns allowed us to distinguish between citrulline produced from arginine via the urea cycle and citrulline produced by NOS (Figure 1). The signals for A+9 citrulline derived from A+10 arginine via the NOS pathway, and A+7 citrulline derived from A+10 arginine via the urea cycle are shown relative to A and A+1 signals at baseline and at 180 min post-infusion in the example shown in Figure 2. By calculation of the isotopic enrichment and knowing the total

citrulline plasma concentration, the approximate concentrations of the enriched forms of citrulline were determined and compared. A+ 9 citrulline in children with SM was significantly lower than that in HC children (Figure 3). Overall, our results definitively demonstrate that children with severe falciparum malaria have increased arginine flux and decreased citrulline derived from actions of NOS (and thus decreased NO production).

References:

1. Anstey NM, Weinberg JB, Hassanali MY, Mwaikambo ED, Manyenga D, Misukonis MA, Arnelle DR, Hollis D, McDonald MI, Granger DL. Nitric oxide in Tanzanian children with malaria. Inverse relationship between malaria severity and nitric oxide production/nitric oxide synthase type 2 expression. *J Exp Med* 184:557-567, 1996.
2. Yeo TW, Lampah DA, Gitawati R, Tjitra E, Kenangalem E, McNeil Y, Darcy CJ, Granger DJ, Weinberg JB, Lopansri BK, Price RN, Duffull S, Celermajer Ds, Anstey NM. Impaired nitric oxide bioavailability and L-arginine-reversible endothelial dysfunction in adults with falciparum malaria. *J Exp Med* 204: 2693-2704, 2007.

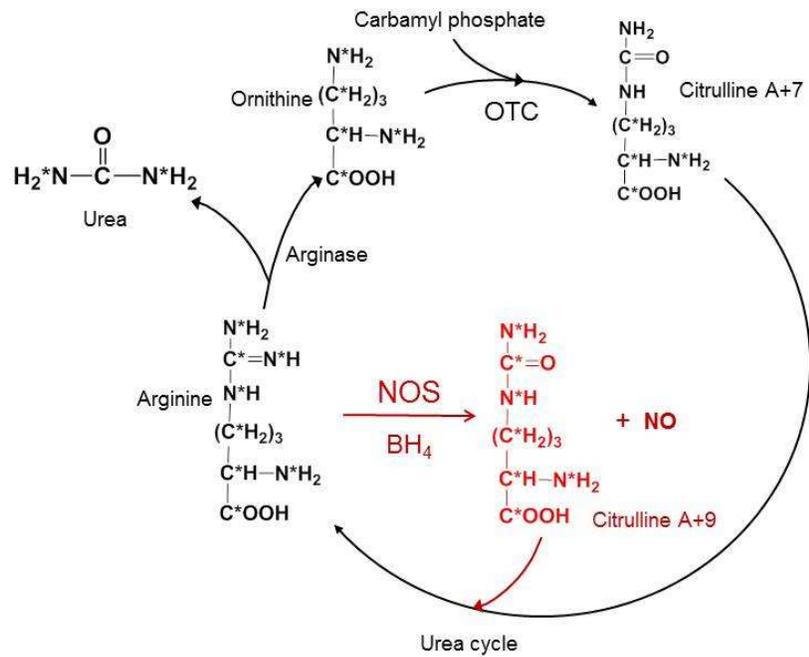


Figure 1. The pathways of isotope-labeled arginine (A+10) producing citrulline (A+9) by action of nitric oxide synthase (NOS) and citrulline (A+7) generated from the urea cycle.

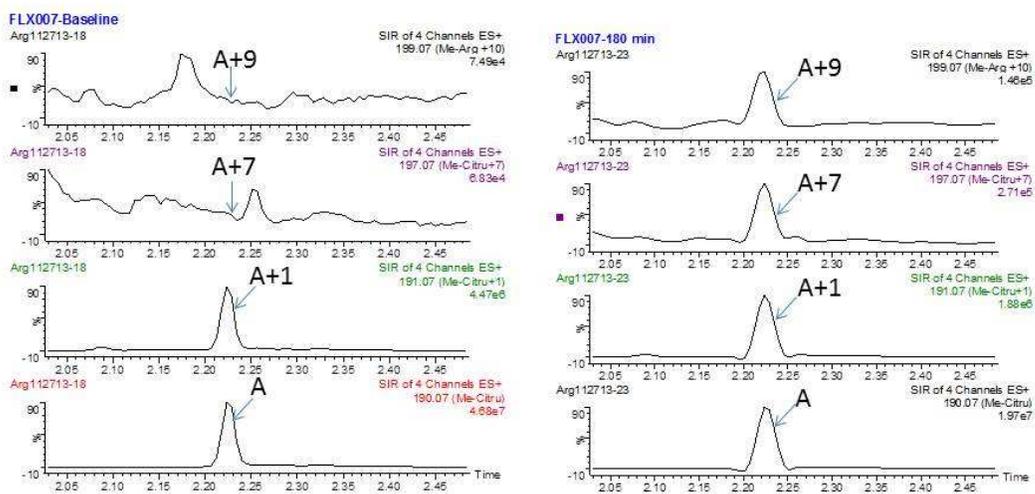


Figure 2. The selected ion monitoring (SIM) chromatograms of citrulline (A+9), citrulline (A+7), citrulline (A+1) and citrulline (A): left panel at baseline, right panel at 180 min post infusion.

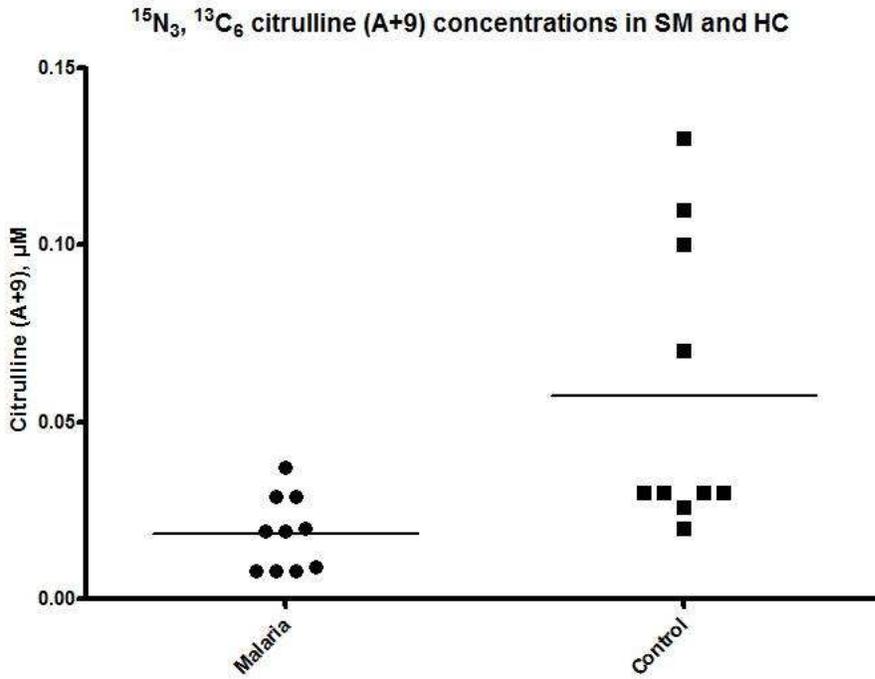


Figure 3. Citrulline (A+9) levels in severe malaria (SM) patients compared to those in healthy children (HC). The mean level of citrulline (A+9) derived from nitric oxide synthase for SM was significantly lower than that in HC (p value = 0.016, analyzed by unpaired t test with Welch's correction).