British Journal of Nutrition (2007), **97**, 814–815
doi: 10.1017/S0007114507691612

Invited Commentary

Arginine supplementation for neonatal necrotizing enterocolitis: are we ready?

Necrotizing enterocolitis (NEC) is one of the most common, deadliest and enigmatic intestinal problems encountered by premature infants (Neu, 1996, 2005). The precise pathophysiology of NEC is unclear, but major factors thought to play an important role include an immature intestine, the propensity toward an over-reactive inflammatory response to intestinal microbes, and a cascade of events that leads to ischaemic necrosis. As with sepsis, NEC advances to a systemic inflammatory response and frequently multiple organ system failure.

Metabolic abnormalities are also associated with NEC, one of which is reduced plasma arginine concentrations, as shown by Richir and colleagues in this issue (Richir *et al.* 2007). The authors confirm previous studies (Zamora *et al.* 1997; Becker *et al.* 2000) showing reduced plasma concentrations of arginine in babies with NEC, and they also evaluate plasma concentrations of the endogenous nitric oxide synthase (NOS) inhibitor asymmetric dimethylarginine (ADMA). They compare plasma concentrations of arginine and ADMA in ten premature infants with a diagnosis of NEC to concentrations in ten matched controls. Arginine and ADMA, as well as the ratio of arginine to ADMA, were lower in infants with NEC (Richir *et al.* 2007). They also found a relationship between low arginine and arginine:ADMA ratios and mortality in infants with NEC.

Based on these findings, the authors suggest that that decreased arginine availability causes diminished NO production via the NOS pathway, and that this may be involved in the pathophysiology of NEC. As stated in their discussion, they cannot, from the results of this study, rule out the possibility that NEC causes low arginine concentrations. However, in a previous study (Becker *et al.* 2000), it was demonstrated that the plasma concentrations of both arginine and glutamine were reduced 7 days before the onset of NEC, suggesting that it is not the disease that causes the low plasma arginine concentration. Based on their findings and those of a previous study of arginine supplementation (Amin *et al.* 2002), Richir *et al.* propose a multicentre trial of arginine supplementation in premature infants to determine its efficacy in the prevention of NEC.

Whereas much of the information about arginine supplementation suggests a beneficial role against NEC in premature infants, significant caution is warranted because misadventures with various agents and treatments have a strong precedent in neonatology (Robertson & Baker, 2005). Caution about arginine supplementation is found in reviews of studies in adults that show increased mortality in patients with pre-existing sepsis who were given arginine supplements (Heyland *et al.* 2003). This has prompted the Canadian Society of Critical Care to suggest a very cautious approach with arginine supplementation in adult patients with sepsis (Heyland *et al.* 2003).

Contrary to the thesis that NO, and thus substrates for NO production such as arginine, may be beneficial in the prevention of NEC, studies implicating NO in a harmful role as an inflammatory mediator cannot be ignored. Ford (2006) has shown that NO may play a role in the pathogenesis of epithelial destruction in NEC through the generation of peroxynitrite. This leads to enterocyte apoptosis and an inhibition of enterocyte proliferation and migration, which may lead to a cycle of injury and uncontrolled inflammatory response, the net effect being further tissue destruction, intestinal perforation and systemic sepsis. Recent reviews of the literature pertaining to sepsis in adults show mixed results and provide a cautionary note (Kalil & Danner, 2006). Thus, a better understanding of the basic biology of arginine during health and inflammatory conditions should be considered while designing large multicentre trials.

The article by Richir *et al.* (2007) is a step in the right direction. In addition to evaluating plasma arginine concentrations, previously known to be reduced in infants with NEC (Zamora *et al.* 1997; Becker *et al.* 2000), the authors also evaluate ADMA, an inhibitor of NOS that has been found to be increased in human adults with sepsis. Based on similarities between NEC and sepsis, the *a priori* expectation was that ADMA would also be elevated in premature infants with NEC. Contrary to their hypothesis, the authors actually found low concentrations of both arginine and ADMA in the plasma of the infants with NEC. They suggest that the low arginine concentrations are associated with a decreased capacity to produce NO because of decreased substrate for the NOS reaction. This is similar to the conjecture made by Amin *et al.* (2002) in their study of arginine supplementation of low birthweight infants, whereby it was proposed that increased NO production by increasing the substrate for NOS, namely arginine, would prevent vasoconstriction and gut injury. Could there be alternative explanations? A brief review of basic arginine metabolic pathways may be helpful in this regard.

Although considerable attention is placed on arginine metabolism through NOS, other arginine-related pathways should be considered. One is the arginase pathway, wherein arginine is converted to L-ornithine, proline, glutamate and polyamines, which are responsible for cell growth, differentiation and connective tissue formation. A relatively large percentage of plasma arginine is used for protein synthesis. Arginine also stimulates the secretion of several hormones and is a metabolic precursor of creatine and creatinine. In the neonate, the basic metabolism of arginine also differs from that of the more mature infant. The relatively large

contribution of intestinal arginine synthesis compared with kidney synthesis in the neonate suggests that the intestine is an important source of arginine (Wu *et al.* 1997, 2004). A lack of arginine synthesis in the preterm intestine (Wu *et al.* 1995) further suggests a requirement for additional dietary arginine for premature infants.

ADMA is the most powerful endogenous competitive inhibitor of NOS. It competes with L-arginine for the active site of NOS and for transport-mediated uptake into cells (Leiper & Vallance, 2006). In sepsis, plasma ADMA is increased, and this is thought to contribute to sepsis-related pathology such as multiple organ system failure by interfering with physiological functions of NO (Nijveldt *et al.* 2003). Thus, ADMA could contribute to impaired blood flow (Cooke, 2000) by reducing NO. In NEC, a higher concentration of ADMA could be considered potentially dangerous because of consequent reduced microvascular blood flow mediated by lower NO levels.

The source of ADMA is via the catabolism of post-translationally modified proteins that contain methylated arginine residues. It is then metabolized and excreted in the urine. Thus, catabolism and renal dysfunction could contribute to elevated ADMA concentrations. In the article by Richir *et al.* (2007), the elevation in ADMA level was not associated with established NEC. Whether ADMA is elevated prior to the development of NEC is still unknown, and knowing this would be helpful in further understanding the pathogenesis of NEC.

Richir *et al.* (2007) also raise the notion that an increase in plasma arginine:ADMA ratio could increase NOS production. This is made possible by a phenomenon referred to as the 'arginine paradox', which refers to the phenomenon that the K_m for all three major isoforms of NOS is below the plasma concentration of arginine. Thus, all NOS isoforms should be saturated and additional arginine should not contribute to an increase in NO production. This is, however, not the case, and it has been hypothesized that this may be because inhibitors such as ADMA compete with the intracellular arginine. Thus, the addition of arginine will increase NOS activity and augment NO production (Tsikas *et al.* 2000).

So, are we getting close to being able to do a clinical trial of arginine supplementation in premature infants? The best answer is yes, based on the previous studies, but several factors should be considered in their design. Dosage, route of administration (enteral versus parenteral), mechanisms of action, other nutritional intake and careful monitoring of side effects will be critical. Dosages well above normal dietary intake may be harmful when taking into account the possibility of harmful peroxynitrite generation (Ford, 2006). The question of enteral versus parenteral administration should be carefully considered. One potential advantage of enteral administration is that the arginine could have significant beneficial effects on the intestinal mucosa itself, increasing proliferation and differentiation due to the production of other amino acids and polyamines. Careful attention to overall nutrition should also be given to prevent a catabolism-induced production of ADMA, which may decrease NOS activity and NO production, with a decreased arginine:ADMA ratio, thereby affecting the intestinal microvasculature and potentially predisposing to NEC.

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