Arginase is an enzyme that nephrologists can love
Friedrich C. Luft

In this issue of the journal, Demougeot et al. [1] report that arginase inhibition enhances endothelial function and attenuates high blood pressure development in spontaneously hypertensive rats (SHR). The word arginase conjured up ancient memories in my mind of dusty biochemistry classrooms and lectures from all too long ago. I recalled having learned that arginine is a semi-essential amino acid required during periods of maximal growth, severe stress, and injury. Arginine is not only a substrate for protein synthesis, but also modulates cellular biochemical functions via conversion to a number of biologically active compounds. Arginine is utilized by a vast variety of metabolic pathways that produce many regulatory compounds, such as nitric oxide, creatine phosphate, agmatine, polyamines, ornithine and citrulline. Arginine supplies are primarily regulated by two enzyme systems. The enzymes are arginase, which is part of the urea cycle, and nitric oxide synthase, which produces NO.

The urea cycle is the nephrologists’ daily bread and butter. The urea cycle was first proposed by Hans Krebs and Kurt Henseleit in 1932 (Fig. 1) and was the first cyclic metabolic pathway to be discovered. To synthesize urea (CON₂H₄), one of the nitrogen atoms in urea is transferred from the amino acid aspartate. The other comes directly from free NH₄⁺. The carbon atom comes from HCO₃⁻, derived from the hydration of CO₂. And there we have it! The urea cycle permits us to excrete nitrogen via urea instead of as the far more toxic NH₄⁺. This happy state-of-affairs separates us from the cyclostome fishes and other simple organisms. It also frees us from having to excrete nitrogen as uric acid, like the poor birds and reptiles do. Thus, we need not be bothered with a cloaca, but instead evolved a far more eloquent urethra to conduct our excretory affairs.

The urea cycle begins with the coupling of free NH₄⁺ with HCO₃⁻ to form carbamoyl phosphate. This complex triple step is catalysed by carbamoyl phosphate synthase. Ornithine and citrulline are amino acids, but they are not used for protein synthesis. Citrulline condenses with aspartate, the donor of the second amino group of urea. This synthesis of argininosuccinate, catalysed by argininosuccinate synthetase, is driven by the cleavage of ATP into AMP and pyrophosphate and by the subsequent hydrolysis of pyrophosphate. Argininosuccinase cleaves the argininosuccinate into arginine and fumarate. Finally, arginine is hydrolysed to generate urea and ornithine in a reaction catalysed by arginase. Ornithine is transported back into mitochondria to begin another cycle. The urea is excreted. Indeed, we humans excrete approximately 10 kg of urea per year! Nephrologists will quickly recognize the importance of the cycle not only for urea generation, but also for acid–base balance regulation. The urea cycle, the citric acid cycle, and the transamination of oxaloacetate are integrated by fumarate and aspartate.

Demougeot et al. [1] reasoned that arginase is also a critical factor in NO bioavailability. If arginase is inhibited, then more arginine would be available for nitric oxide synthase to produce more NO. To inhibit arginase, they used α-difluoromethylornithine (DMFO) in drinking water (0.02%) or 30 mg/kg per day. Hypertension development in treated SHR proceeded more slowly and in an attenuated fashion in rats over 5–10 weeks of age. Arginine activity in the kidney and liver was attenuated by treatment, and NO availability in the aortas was increased. Interestingly, Demougeot et al. [1] present evidence that aortic arginase activity is increased in untreated SHR, compared to Wistar–Kyoto rats and propose that a resultant vascular NO deficiency could account for some of the hypertension featured by SHR.

Demougeot et al. [1] discuss some earlier studies of their own, as well as those of other investigators, related to decreased arginase activity in hypertensive animal models. However, the authors did not have the opportunity to review a very recent paper by Johnson et al. [2] that is relevant to their study. Johnson et al. [2] investigated Dahl salt-sensitive and salt-resistant rats and found that arteriolar arginase expression was increased, and endothelium-dependent vasodilation was decreased, in high-salt-fed, salt-sensitive Dahl rats. Acute pretreatment in vitro with an arginase inhibitor or with L-arginine restored endothelium-dependent vasodilation and abolished the differences between high- and low-salt Dahl salt-sensitive groups. The results generated by Johnson et al. [2] suggest that enhanced vascular arginase activity

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contributes to endothelial dysfunction in Dahl salt-sensitive rats when they exhibit salt-induced hypertension. Similar to the study by Demougeot et al. [1], the data identify arginase as a potential therapeutic target to prevent endothelial dysfunction.

Demougeot et al. [1] note that earlier studies used DMFO to primarily assess the effects of polyamines on changes in vascular structure and subsequent blood pressure changes. In these studies, the intent was to inhibit ornithine decarboxylase and the effects on arginase were less appreciated. Higher doses (1–2% in drinking water) were used, compared to the 0.02% employed by Demougeot et al. [1]. The fact that DMFO is relatively non-toxic appears surprising. DMFO has been used as an experimental anti-cancer treatment in rodents at considerably higher doses [3] than employed here. The compound has also been used to inhibit growth of intracellular parasites [4]. Hepatic and renal arginase activities are pivotal to our metabolism. I could not find any reports indicating that DMFO administration resulted in reduced urea production, higher concentrations in toxic NH₄⁺ concentrations, or in defects related to HCO₃⁻ removal in the liver. Demougeot et al. [1] do not report on urea excretion in their animals, although arginase activities in the liver and kidney were decreased by treatment [1]. Whether or not specific vascular arginase inhibitors could be developed to increase vascular NO remains unknown. In the mean time, I guess we had just better eat more arginine!

References