Editorial Commentary

Asymmetric dimethylarginine, erythropoietin resistance, and anemia in CKD

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In a recent issue of the *Journal of American Society of Nephrology*, Yokoro *et al.* (1) examined the contribution of asymmetric dimethylarginine (ADMA) to anemia of chronic kidney disease (CKD). Based on human and animal studies, the Authors concluded that accumulation of ADMA in erythrocytes worsens anemia. The Authors first measured levels of ADMA in erythrocytes and in plasma from 54 patients with advanced CKD [mean estimated glomerular filtration rate (eGFR) 13.7 mL/min/1.73 m^2]. They found that lower hemoglobin concentrations were associated with higher levels of ADMA in erythrocytes but not in plasma. Erythrocyte ADMA levels directly correlated with the erythropoiesis stimulating agent (ESA) resistance index, calculated as the ESA dose per unit body weight divided by the hemoglobin concentration (in 14 ESA-treated patients) and the ESA demand index, calculated as the plasma erythropoietin concentration divided by the hemoglobin concentration (in 28 patients not treated with ESA). To assess the effect of reducing erythrocyte ADMA levels on hemoglobin, the authors studied CKD in mice with increased expression of the ADMA-breakdown enzyme dimethylarginine dimethylaminohydrolase (DDAH-1). CKD mice with increased DDAH-1 had lower erythrocyte and plasma ADMA levels, higher hemoglobin concentrations, and greater gene expression of erythropoietin-related receptors and hormones than control CKD mice. Overall, the authors concluded that ADMA accumulation in erythrocytes may contribute to anemia by impairing erythropoiesis. A major barrier faced by all investigations of solute toxicity, however, is the potential confounding effects of the numerous solutes that accumulate when kidney function declines. Additionally, the unique characteristics of ADMA, such as its different elimination pathways and its distribution between body compartments, make it particularly difficult to prove its toxicity.

The report by Yokoro *et al.* adds to the growing body of evidence for ADMA toxicity (2,3). ADMA is one of numerous small solutes referred to as "uremic solutes" or "uremic toxins" which accumulate in the plasma when kidney function declines (4). ADMA has been associated with cardiovascular disease and mortality in patients with CKD as well as persons with normal or near normal kidney function (3). Its adverse effects have been attributed to the inhibition of nitrous oxide production. Nitrous oxide may promote erythrocyte production as well as vasodilation (5,6). Because ADMA levels are elevated with impaired kidney function, it is logical to examine its contribution to anemia in CKD.

The report of Yokoro *et al.* (1) presents a diligent effort to elucidate the effects of ADMA on anemia. The authors faced the problem common to all investigations of solute toxicity in kidney disease—numerous solutes exist (4,7-9). Because hundreds of uremic solutes have been

identified, it is difficult to determine the contribution of individual solutes to clinical outcomes. There are three potential approaches to the problem. The first approach is to associate plasma or serum solute concentrations or some other metric of solute burden, with outcomes, as the Authors had done by examining the correlation of erythrocyte ADMA levels with hemoglobin in CKD. Two other approaches are (I) to test the effects of increasing solute burden; and (II) to test the effects of reducing solute burden. These arguably stronger approaches to elucidate solute toxicity are most often employed in animal models and cultured cells, as increasing solute concentrations is ethically questionable in humans and consistently reducing solute concentrations has so far proven difficult. Yokoro *et al.* (1) therefore took the approach of using genetic mouse models to show that by reducing levels of erythrocyte ADMA, adverse effects on erythropoiesis were attenuated.

Another difficulty that the Authors faced in assessing toxic effects of ADMA is the unusual pattern of its accumulation in CKD. Plasma ADMA concentrations tend to rise in early stages of CKD, but do not continue to rise in proportion to further declines in kidney function (10-12). This pattern occurs because, unlike creatinine, the most commonly employed marker solute reflecting kidney function, ADMA is not cleared exclusively by the kidneys. The kidney contributes to only about 20% of ADMA clearance (2). The rest is accounted for by the breakdown by two intracellular enzymes, DDAH and alanine glyoxylate aminotransferase. Additionally, the production of ADMA may be variable. It is known to be produced from the metabolism of post-transcriptionally modified intracellular proteins by protein-arginine methyltransferase (2). However, control of ADMA production has not been fully characterized.

A characteristic of ADMA particularly relevant to the findings of Yokoro *et al.* (1) is the presence of ADMA in both the cell and plasma compartments. ADMA moves in and out of cells through cationic amino acid transporters (3,13). The stimuli contributing to the bidirectional transport, however, are not known. The key findings of Yokoro *et al.* (1) that erythrocyte ADMA levels were inversely correlated with hemoglobin concentrations suggest that erythrocyte levels are the more clinically relevant measure. This emphasizes an important point when studying solute toxicity—in which body compartment does the solute exert its toxicity? Previous studies have measured ADMA levels in erythrocytes and plasma, but all studies of toxicity in humans have assessed outcomes to levels in plasma (14-16). It is not known what regulates levels of ADMA between erythrocyte and plasma. A previous study by Billecke *et al.* (14) compared ADMA levels in erythrocyte and plasma in control subjects and in patients receiving hemodialysis. In control subjects, they found that erythrocyte ADMA levels were roughly 1.7-fold higher than those in plasma. In patients receiving hemodialysis, however, erythrocyte ADMA levels were similar to those in plasma. In contrast, Yokoro *et al.* (1) found erythrocyte ADMA levels to be higher than those in plasma for control subjects and for patients with advanced CKD. Of note, the Authors reported the erythrocyte ADMA level in nanomols per gram of cellular protein. The erythrocyte level could be converted to the same units as the plasma concentration (micromoles per liter) by assuming hemoglobin to be the major protein component of plasma and adjusting for the fraction of water content of erythrocytes. After the unit conversions, erythrocyte ADMA levels appeared to be 3.4-fold higher than plasma concentrations in control subjects and 2.3-fold higher in patients with advanced CKD.

Given ADMA's distribution between cell and plasma along with its complex removal and production, there are limited ways to lower levels. In other studies, more intensive hemodialysis did not significantly reduce plasma concentrations due largely to ADMA's substantial non-kidney clearance (17,18). Dietary maneuvers have reduced plasma concentrations of putative uremic solutes derived from protein breakdown or colon microbial metabolism, but such therapies have not yet been tested for ADMA (19-21). Enhancing the enzymatic breakdown and suppressing enzymatic production of ADMA are options, but specific therapies have not yet been developed. If targeted reduction were advisable, we would not yet know whether to target reduction in erythrocytes or plasma or both.

Although these complexities of ADMA are difficult to overcome, further analysis could strengthen the Authors' observation that erythrocyte ADMA was related to anemia. First, the Authors would need to confirm that the estimated effect of ADMA was independent of kidney function; other factors (e.g., hepcidin and/or other inflammatory markers) could confound the ADMA—anemia relation. Second, the association of erythrocyte ADMA with the erythropoietin resistance index would need to be replicated in a larger population.

Other limitations of this study should be considered. First, as with all cross-sectional studies, association does not prove causation. Second, findings from the mice studies cannot be directly linked to the findings from the

human studies. The Authors performed careful studies in mice, showing that CKD mice with increased DDAH-1 expression had lower ADMA levels and higher hemoglobin concentrations than control CKD mice. However, both erythrocyte and plasma ADMA were lower in the DDAH-1 CKD mice. Therefore, the Authors cannot distinguish whether higher hemoglobin concentrations were due to lower ADMA levels in erythrocytes versus plasma. Another notable finding was that of decreased expression of erythropoietin-related receptors and hormones in DDAH-1 CKD mice with lower ADMA levels. However, gene expression was performed only in the spleen, and as the Authors appropriately noted, they could not describe the fraction of erythropoiesis that occurs in the spleen versus other organs.

Overall, this study adds to the growing evidence base highlighting potential toxicities of ADMA. More studies are required to prove causation. Much remains unknown regarding the regulation of ADMA production and elimination from the body as well as its distribution into different body compartments. If ADMA is indeed toxic, then developing therapies to reduce its levels has the potential to improve lives of patients with CKD.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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