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See related article, "IgG Donor-Specific Anti-Human HLA Antibody Subclasses and Kidney Allograft Antibody-Mediated Injury," on pages 293–304.

Trimethylamine *N*-Oxide as a Novel Therapeutic Target in CKD

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Patients with CKD are at an increased risk for the development of cardiovascular disease even beyond traditional risk factors. Although uremic toxins have been proposed as important contributors in the pathogenesis of cardiovascular and renal disease progression in CKD, the sources and the processes of generating these toxins are diverse. Perturbations of the intestinal microbiota composition in both human and experimental CKD have demonstrated significant elevations of gut-derived uremic toxins.[1] Such alterations have been linked to the increased burden of systemic inflammation, as well as the pathogenesis of cardiovascular and renal disease progression in CKD.[2]

Our greatest environmental exposure likely comes from dietary intake, which in turn exerts important influences on the metabolism and composition of intestinal microbiota. There is increasing appreciation that intestinal microbiota play an important role in the pathophysiology of various diseases in part *via* complex interactions with the host. Our group recently discovered that certain dietary nutrients possessing a trimethylamine (TMA) moiety, namely choline/phosphatidylcholine and L-carnitine, participate in the development of atherosclerotic heart disease *via* a meta-organismal pathway involving intestinal microbiota and host hepatic enzymes

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(especially flavin monooxygenase 3[3,4]). Specifically, gut microbes were shown to play an obligatory role in trimethylamine *N*-oxide (TMAO) formation upon processing of common dietary components (*e.g.*, choline in egg yolk or L-carnitine in meat) both in mice[4,5] and in humans.[6–9] These studies also demonstrated that TMAO was not merely a biomarker, but it was also a mediator of alterations in cholesterol and sterol metabolism and can directly enhance atherogenesis.[4,5] Pathophysiologically, TMAO may also interact with other host neurohormonal systems, as observed in a rat model whereby angiotensin II infusion in the setting of elevated TMAO levels led to sustained augmentation of BP.[10] Specific microbial genre potentially responsible for enhanced TMA/TMAO generation have been identified,[4,11] and microbial transplantation studies have confirmed the obligatory role of dietary-induced TMAO-producing intestinal microflora in atherogenesis.[11] In a subsequent study of >4000 patients undergoing elective coronary angiography, elevated TMAO levels predicted death, myocardial infarction, and stroke over a 3-year period of stable patients who electively underwent coronary angiography.[6] Although elevated circulating L-carnitine and choline/betaine levels (all substrates of TMAO production) were also associated with future risk, their prognostic values were primarily restricted to those with concomitant elevated TMAO levels.[4,7]

Over the past decades, there have been reports from small cohorts ($n<20$) that have observed that circulating TMAO levels are elevated in patients with ESRD and CKD.[12,13] In our cohort, TMAO levels remained elevated in CKD (eGFR<60 ml/min per 1.73 m^2, calculated using the Modification of Diet in Renal Disease equation; $n=583$) compared with patients without CKD, and the prognostic value for TMAO in predicting future mortality risk remained robust in the CKD cohort even after adjustment for traditional risk factors.[6,9] Interestingly, within the non-CKD cohort, higher levels of TMAO were observed in the highest tertile of cystatin C,[9] implying that a rise in TMAO levels may precede overt renal insufficiency. Indeed, animal studies have corroborated this concept, with direct mechanistic links between dietary choline intake, TMAO accumulation, and progressive renal tubulointerstitial fibrosis and dysfunction.[9] A metabolomics report from the Framingham Heart Study further observed that elevated choline and TMAO levels among individuals with normal renal function predicted increased risk for incident development of CKD.[14] Taken together, the pathophysiologic role of TMAO may extend not only to augmentation of cardiovascular risk but also to progression of renal dysfunction. However, a recent report by Kaysen *et al.* did not observe a significant increase in both all-cause and cardiovascular mortality risk in a national cohort of patients new to dialysis, despite markedly elevated TMAO levels.[15] These findings have challenged the extension of TMAO-associated cardiorenal risk to more advanced CKD stages.

In this issue of *JASN*, Stubbs *et al.* measured circulating TMAO levels in stored samples from five separate, single-center cohorts of patients across the spectrum of CKD.[16] They confirmed the graded rise in circulating TMAO levels with progressive renal dysfunction and added evidence to show the resolution of high TMAO after kidney transplantation. Importantly, they also provided independent validation of the prognostic role of TMAO in an independent cohort of patients with CKD and confirmed the direct correlation between atherosclerotic burden and TMAO levels in CKD. The claim that TMAO clearance from the circulation being largely dependent on urinary excretion was based on a modest ($r^2=-0.31$) correlation between serum TMAO and eGFR, which was consistent with previously reported associations ($r=-0.48$ with eGFR; $r=0.46$ with cystatin C).[9] TMAO, with a molecular mass of 75.1 Da, is readily filtered and removed by hemodialysis.[12] Interestingly, higher rather than lower TMAO urinary levels have been related to rejection of the transplanted kidneys, yet the degree and pattern of rise in TMAO levels were not uniformly concordant with the degree of reduction in GFR.[17–19] Meanwhile, in the proximal tubules of the chicken nephron, circulating TMAO is passively excreted, whereas *N*-oxidation of TMA occurs *via* a quinidine-sensitive transporter at the basal side and is excreted *via* probenecid-sensitive transporter at the luminal side.[20] Clearly, the handling of TMAO by the kidneys may be more complicated than previously thought, and it remains to be determined whether TMAO transporters are present in the human nephron. Whether an increase in TMAO levels in patients with CKD is simply a product of increased production, reduced clearance, or a combination of both is still debated and needs to be systematically examined. We previously demonstrated that TMAO production is largely induced by dietary nutrients[4]; therefore, dietary sources of TMAO generation, such as some species of deep-sea fish, eggs, and meat, should be reviewed and possibly reduced, because they may pose further unwanted consequences in the setting of CKD. The resilience and adaptive capacity of microbiota may preclude the effectiveness of short-term antibiotic administration, whereas chronic antibiotics may likely induce colonization of antibiotic-resistant microbes.[21] At the same time, there is much excitement over the prospects of modulating intestinal microbiota as a therapeutic strategy in CKD, as promised by several notable proof-of-concept animal studies using oral biosorbants to prebiotics/probiotics in the setting of acute cardiorenal syndromes.[22] Further studies are warranted to determine whether dietary modification with the target to reduce TMAO levels may delay the progression of CKD and cardiovascular disease in the setting of CKD. It is also conceivable that TMAO can someday serve as a therapeutic target to modify dietary exposures, a strategy that has great potential to reduce heart and kidney disease susceptibility in those at risk and needs to be further tested. There is much to learn in this complex relationship between ourselves and the microbes living within us.

DISCLOSURES

None.

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See related article, "Serum Trimethylamine-*N*-Oxide is Elevated in CKD and Correlates with Coronary Atherosclerosis Burden," on pages 305–313.