TMAO Is Both a Biomarker and a Renal Toxin

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In 2011, Hazen and colleagues¹ made the seminal discovery that 3 metabolites of dietary phosphatidylcholine (choline, trimethylamine N-oxide [TMAO] and betaine) predicted risk for cardiovascular disease in an independent large clinical cohort. They also demonstrated that supplementing the diet with choline or TMAO promoted atherosclerosis in a mouse model.¹ The gut flora was shown to be required for the production of TMAO, and when the gut flora was suppressed, dietary choline–enhanced atherosclerosis was inhibited.¹

The robustness of TMAO derived from choline in dietary phosphatidylcholine as a predictor of cardiovascular risk was subsequently confirmed in a larger cohort.²,³ It was also demonstrated that l-carnitine, a constituent of red meat, was another excellent substrate for gut flora to produce TMA, which was then converted to TMAO.² More recently, it was shown that after l-carnitine ingestion, γ-butyrobetaine is produced as an intermediary metabolite by gut bacteria at a rate 1000-fold higher than the formation of TMA and in mice is converted into TMA and TMAO and accelerates atherosclerosis.⁴

Other studies demonstrated that TMAO predicted risk in patients with heart failure.⁴ Patients with heart failure with high TMAO levels had increased long-term mortality independent of traditional risk factors and independent of cardiorenal indexes.⁶ In these studies⁶ it was noted that there was an inverse correlation between TMAO levels and the estimated glomerular filtration rate (r=−0.55; P<0.001). An accompanying editorial⁷ stated that “…the strong correlation between TMAO concentration and kidney function raises the following question: given the importance of the kidney in eliminating TMAO, is higher TMAO level just a marker of renal impairment (7)?” The reference⁷ cited in the editorial⁷ reported that TMAO (which is produced in the intestine by gut bacteria and transported to the liver where it is acted on by flavin monoxygenase family members⁸ to form TMAO) and TMAO itself were both elevated in the plasma from 10 patients with end-stage renal disease undergoing hemodialysis when compared with 10 healthy adults.⁹ Moreover, the authors observed that the elevated levels in the patients with end-stage renal disease were efficiently reduced during a single hemodialysis treatment.⁹

It has been recognized that chronic kidney disease (CKD) alters the intestinal microbial flora.¹⁰-¹² In a recent publication from the Framingham Heart Study, liquid chromatography/mass spectrometry–based metabolite profiling on plasma from 1434 participants demonstrated that 9 metabolites predicted the development of CKD.¹³ Interestingly, choline was 1 of 3 markers that remained significant after adjustment for estimated glomerular filtration rate, age, sex, diabetes mellitus, hypertension, and proteinuria at baseline.¹³

In this issue of Circulation Research, Tang et al¹⁴ provide evidence that the TMAO pathway not only is a biomarker for renal disease but likely contributes to the progression of renal disease and contributes to the risk of mortality in CKD.

The authors used samples collected from adults who underwent elective diagnostic coronary angiography for cardiac evaluation from 2001 to 2007 at the Cleveland Clinic.² They defined CKD as estimated glomerular filtration rate <60 mL/min per 1.73 m², and they ascertained all-cause mortality at 5 years by telephone contact and chart review plus interrogation of the Social Security Death Index to the year 2011. Of the 3687 subjects considered, 521 met the criteria for CKD leaving 3166 subjects classified as not having CKD. The median TMAO level in the CKD group was 7.9 μmol/L and the median value in the non-CKD group was 3.4 μmol/L (P<0.001). Comparing those CKD subjects with TMAO levels in the highest quartile with those subjects with TMAO levels in the lowest quartile revealed a 2.8-fold increase in all-cause mortality at 5 years (P<0.001). After adjusting for traditional cardiovascular risk factors, those CKD subjects in the highest quartile still had a 1.9-fold poorer 5-year survival (P<0.05).

Stratifying the subjects according to median TMAO levels (7.9 μmol/L) showed that higher TMAO levels conferred a 1.7-fold increase in risk for all-cause mortality (P<0.001) and remained significant even after adjusting for high-sensitivity C-reactive protein and cystatin C levels. Elevated TMAO levels were associated with a higher 5-year mortality risk among subjects with either normal or elevated cystatin C levels.

In mouse studies, 6 weeks after adding either 1.0% choline or 0.12% TMAO to a chemically defined diet comparable with normal mouse chow (which contains 0.08 g% choline) there was a significant increase in TMAO to levels seen in the human CKD subjects. The elevated TMAO levels were associated with significant increases in tubulointerstitial fibrosis and collagen deposition. The phosphorylation of Smad3 which regulates the profibrotic transforming growth factor-β/Smad3 signaling pathway was also significantly increased. Moreover, the mice-fed diets to raise TMAO levels showed significant increases in kidney injury marker-1 levels. TMAO levels were significantly correlated with collagen in the kidney, the ratio of phosphorylated Smad3 to total Smad3, and the expression of kidney injury marker-1 suggesting causality. Extending the feeding of choline or TMAO to 16 weeks resulted in a significant increase in serum cystatin C levels.
Gut Bacteria act on dietary phosphatidylcholine, choline or L-carnitine to produce TMA.

TMA is transported to the liver where FMO converts TMA to TMAO.

TMAO is transported to the kidneys where it promotes renal injury and phosphorylation of Smad3 leading to renal fibrosis and renal dysfunction.

**Figure.** A schematic diagram of the formation of trimethylamine (TMA) in the gut, its conversion in the liver to trimethylamine N-oxide (TMAO) by members of the flavin monooxygenase (FMO) family, which results in TMAO-mediated renal injury, fibrosis, and dysfunction.

The sequence of events leading from the gut to the kidney is depicted schematically in the Figure. The discovery of this novel connection between the gut, the liver, and the kidneys opens the possibility for new therapeutic approaches at each level. The diet may be targeted or the gut flora may be manipulated. The metabolism of TMA in the liver may be altered, or the action of TMAO on the kidneys may be ameliorated. These discoveries by Hazen and colleagues present exciting new research opportunities for slowing or reversing the growing CKD epidemic.

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Dr Fogelman is a principal and an officer in Bruin Pharma.

**References**


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