Advanced chronic kidney disease populations have elevated trimethylamine N-oxide levels associated with increased cardiovascular events

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Cardiovascular disease is more common in patients with chronic kidney disease (CKD), and traditional risk factors do not adequately predict those at risk for cardiovascular (CV) events. Recent evidence suggests elevated trimethylamine N-oxide (TMAO), created by gut microflora from dietary L-carnitine and choline, is associated with CV events. We investigated the relationship of TMAO levels in patients with stages 3b and 4 CKD to ischemic CV events using the CanPREDDICT cohort, a Canada-wide observational study with prospective 3-year follow-up of adjudicated CV events. Baseline samples were obtained for 2529 CKD patients. TMAO, choline, and L-carnitine levels were measured using tandem mass spectrometry. Baseline median TMAO level was high for the whole cohort (20.41 *μM;* interquartile range [IQR]: 12.82–32.70 μM). TMAO was independently associated with CV events (hazard ratio 1.23; 95% confidence interval: 1.06–1.42 / 1 SD lnTMAO) after adjusting for all potential CV risk factors. Those in the highest TMAO quartile had significantly higher risk of CV events (adjusted hazard ratio 1.59; 95% confidence interval: 1.04–2.43; $P = 0.0351$) in the analysis of recurring ischemic events. Among those with stage 3b CKD (hazard ratio 1.45; 95% confidence interval: 1.12–1.87 / 1 SD lnTMAO), independent of kidney function, TMAO levels identified those at highest risk for events. Our results suggest that TMAO may represent a new potentially modifiable CV risk factor for CKD patients. Further studies are needed to determine sources of variability and if lowering of TMAO reduces CV risk in CKD.

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Patients with chronic kidney disease (CKD) are at an increased risk for adverse cardiovascular (CV) events, and traditional markers of CV disease do not appear to account for the high CV risk in this population.[1] Multiple interventions using conventional CV risk reduction strategies have had limited success in CKD populations.[2] Recently, a potential novel marker of CV disease, trimethylamine N-oxide (TMAO), was identified in subjects with normal renal function, where increased plasma concentrations of TMAO were associated with an increased risk of experiencing an adverse CV event.[3] Importantly, this association remained even after adjustment for traditional risk factors, suggesting plasma TMAO concentrations to be an independent biomarker of CV risk.[3]

TMAO is the primary metabolite of trimethylamine (TMA), and is formed in the liver via TMA conversion by flavin-containing monooxygenase isoform 3 (FMO3).[4,5] The metabolism and conversion of TMAO has been described in detail.[6] There is an important role of gut flora[7,8] in mediating the metabolism of the precursors, choline and L-carnitine, which are found abundantly in eggs and red meat. TMAO's role in the pathogenesis of CV disease is thought to be via enhanced accumulation of cholesterol in macrophages as well as accumulation of foam cells in artery walls.[7] TMAO is known to be renally excreted;[9] however, urinary excretion in healthy subjects varies nearly 700-fold, which suggests that variability from dietary sources[9] or other mechanisms may be important. It is known that TMAO plasma concentrations are increased in subjects with end-stage renal disease,[10] but TMAO levels have not been characterized in patients with different levels of reduced kidney function, notably those with estimated glomerular filtration rate (eGFR) <45 ml/min per 1.73 m^2.

The use of statins and the use of angiotensin-converting enzyme inhibitors or angiotensin receptor blockers are the predominant strategies currently proven to address CV risk in CKD,[11,12] but long-term mortality benefits are diminished when compared to non-CKD patients.[2] New strategies to mitigate or modify CV risk in CKD are needed to address the excess in CV disease in this high-risk population. Given the potential role of diet and drugs in reducing TMAO levels, we

were interested in assessing TMAO levels in CKD patients in the community. Importantly, we hypothesized that TMAO plasma concentrations would be significantly elevated in CKD patients and thereby increase the risk for CV events. Recently, a study by Tang *et al.* demonstrated that TMAO levels are elevated in CKD patients, and that those with higher TMAO levels were associated with higher mortality rate.[13] In the study by Tang *et al.*, higher TMAO levels contribute to renal fibrosis and dysfunction and thereby increase mortality risk from CKD.[13] However, the relationship between TMAO plasma concentrations and ischemic CV events has not been systematically assessed in a cohort of CKD patients, particularly with sufficient sample size to provide robust CV risk–associated outcomes in this group. If TMAO levels in CKD patients can be shown to be independently associated with higher CV risk in the CKD population, then non-pharmacologic interventions that focus on modulation of TMAO levels through diet or alteration of microbial gut flora may prove to be of particular benefit in this population in addition to current CV risk reduction strategies.

We thus evaluated the relationship between renal function, TMAO plasma concentrations, and ischemic CV risk, due to the proposed pathologic mechanism involving atherosclerotic CV disease, in a cohort of 2529 adult CKD patients who were enrolled across Canada in a multicenter prospective observational study (Supplementary Figure S1). The overall study focused on collection of clinical outcomes and biologic samples, with the goal of identifying more predictive biomarkers of CV disease in CKD subjects (Canadian Study of Prediction of Risk and Evolution to Dialysis, Death and Interim Cardiovascular Events Over Time [CanPREDDICT]).[14]

RESULTS

Baseline characteristics and TMAO levels

Table 1 describes the baseline characteristics for the overall CKD population, and in those patients who did and did not have a primary outcome, that is, an ischemic CV event over 3 years of follow-up. The mean age of the participants was 68 years; over 60% were men, and the prevalence of diabetes was high. One-third of study participants had a history of ischemic heart disease, while 46% of these patients also had a history of congestive heart failure. The mean (SD) eGFR at the baseline visit was 28.0 ml/min per 1.73 m^2 (9.0 ml/min per 1.73 m^2); 39% of patients had stage 3b CKD (eGFR 30–45 ml/min per 1.73 m^2) and 61% had stage 4 (eGFR <30 ml/min per 1.73 m^2).

Table 1 | Baseline characteristics, overall and according to ischemic cardiovascular events during 3-year follow-up

Characteristic	All participants $N = 2529$	Participants without event $N = 2265$	Participants with events $N = 264$	*P* value
Age	68.2 (12.7)	67.8 (12.9)	71.4 (10.2)	<0.0001
Male	1580 (62.5%)	1404 (62.0%)	176 (66.7%)	0.14
Caucasian	2243 (88.7%)	2007 (88.6%)	236 (89.4%)	0.70
Diabetes	1218 (48.2%)	1053 (46.5%)	165 (62.5%)	<0.0001
Ischemic heart disease	848 (33.5%)	701 (30.9%)	147 (55.9%)	<0.0001
Congestive heart failure	681 (26.9%)	584 (25.8%)	97 (36.7%)	0.0001
Systolic blood pressure, mm Hg	133.8 (20.0)	133.3 (19.6)	137.5 (22.8)	0.0054
Diastolic blood pressure, mm Hg	70.9 (11.9)	71.1 (12.0)	69.0 (10.8)	0.0034
Weight, kg	83.5 (19.7)	83.5 (19.8)	83.6 (19.6)	0.94
BMI, kg/m^2	29.5 (6.4)	29.5 (6.5)	29.5 (6.1)	0.98
TMAO, μM	20.41 [12.82–32.70]	19.71 [12.44–31.84]	26.21 [16.56–40.89]	<0.0001
Carnitine, μM	23.7 (6.3)	23.6 (6.3)	24.7 (6.4)	0.0084
Choline, μM	21.2 (7.3)	21.0 (7.3)	22.7 (6.9)	0.0003
Creatinine, mg/dl	2.31 (0.79)	2.3 (0.9)	2.52 (0.79)	<0.0001
eGFR, ml/min/1.73 m^2	28.0 (9.0)	28.2 (9.0)	25.9 (8.35)	<0.0001
eGFR ≥ 30 ml/min/1.73 m^2	989 (39.1%)	904 (39.9%)	85 (32.2%)	0.015
eGFR <30 ml/min/1.73 m^2	1540 (60.9%)	1361 (60.1%)	179 (67.8%)	
Urine ACR, mg/mmol	16.3 [3.0–86.6]	15.75 [2.90–80.30]	31.20 [4.40–159.30]	0.0003
Albumin, g/l	40.4 (4.3)	40.5 (4.2)	39.3 (4.7)	<0.0001
Hemoglobin, g/l	123.2 (15.6)	123.6 (15.6)	119.6 (15.5)	<0.0001
Calcium, mmol/l	2.31 (0.14)	2.31 (0.14)	2.28 (0.15)	0.0055
Phosphate, mmol/l	1.21 (0.25)	1.21 (0.24)	1.27 (0.27)	0.0006
1,84-Parathyroid hormone, pg/ml	44.2 [26.1–75.6]	15.75 [2.90–80.30]	31.20 [4.40–159.30]	0.0002
Bicarbonate, mmol/l	25. 5 (3.4)	25.5 (3.4)	25.5 (3.5)	0.96
Sodium, mmol/l	140.2 (4.9)	140.2 (5.0)	139.7 (3.3)	0.018
Total cholesterol, mmol/l	4.25 (1.17)	4.26 (1.12)	4.21 (1.58)	0.70
HDL, mmol/l	1.18 (0.44)	1.18 (0.44)	1.12 (0.48)	0.11
LDL, mmol/l	2.22 (0.87)	2.23 (0.87)	2.08 (0.80)	0.041
Triglycerides, mmol/l	1.94 (1.29)	1.91 (1.20)	2.21 (1.90)	0.057
Aspirin	1351 (53.4%)	1191 (52.6%)	160 (60.6%)	0.013
ACE inhibitors and/or ARBs	1823 (72.1%)	1639 (72.4%)	184 (69.7%)	0.36
β-Blockers	1155 (45.7%)	1003 (44.3%)	152 (57.6%)	<0.0001
Statins	1697 (67.1%)	1501 (66.3%)	196 (74.2%)	0.0091

ACE, angiotensin-converting enzyme; ACR, albumin-to-creatinine ratio; ARB, angiotensin receptor blocker; BMI, body mass index; eGFR, estimated glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; TMAO, trimethylamine N-oxide.