

Supplementary Materials: Elevation of Trimethylamine-N-Oxide in Chronic Kidney Disease: Contribution of Decreased Glomerular Filtration Rate

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Table S1. Multiple Reaction Monitoring (MRM) parameters used for LC-MS/MS analysis.

Compounds	MRM (<i>m/z</i>)	Cone Voltage (V)	Collision Energy (eV)
TMAO	75.9 → 58.9	20	11
² H ₉ -TMAO	85.0 → 68.0	20	11
Derivatized TMA	174.1 → 118.0	25	10
Derivatized [¹³ C ₃ , ¹⁵ N]-TMA	178.1 → 122.0	25	10
Betaine	118.1 → 58.1	40	22
¹³ C ₂ -betaine	120.1 → 58.1	40	22
Choline	104.1 → 60.1	40	15
² H ₉ -choline	113.2 → 69.1	40	15
Carnitine	162.1 → 103.0	25	14
² H ₉ -carnitine	171.1 → 69.1	25	14

Table S2. Baseline characteristics of Control subjects and CKD patients who performed TMAO clearance.

Variable	Controls	CKD Patients		<i>p</i> -Value
		Stage 1–2	Stage 3–5	
N	5	19	8	
Sex, male/female	2/3	12/7	3/5	0.387
Age, y	44 [34–50]	37 [23–57]	57 [23–69]	0.508
BMI, kg/m ²	22.4 [19.8–27.3]	23.7 [21.7–26.0]	23.5 [21.6–29.1]	0.852
Systolic Blood Pressure, mmHg	109 [96–146]	113 [105–130]	117 [101–135]	0.782
Diastolic Blood Pressure, mmHg	73 [61–89]	72 [64–81]	68 [59–77]	0.735
Heart Rate, bpm	74 [61–79]	62 [57–70]	66 [57–75]	0.434
Creatinine, μmol/L	72 [62–82] ^a	94 [84–109] ^{a,b}	123 [96–151] ^b	0.001
mGFR, mL/min/1.73m ²	98 [91–105] ^a	73 [67–79] ^b	51 [46–55] ^c	<0.0001
Urea, mmol/L	4.8 [3.7–5.5] ^a	5.5 [3.8–6.4] ^a	8.3 [6.3–10.7] ^b	0.005
UA/C, mg/mmol	0.4 [0.4–1.1]	0.9 [0.4–1.7]	3.5 [0.8–59.0]	0.101
Uric acid, mmol/L	252 [217–298]	319 [261–449]	390 [322–635]	0.063
Proteins intake, g/kg/day	1.02 [0.87–1.40]	0.82 [0.70–0.82]	0.97 [0.77–1.08]	0.218
Bicarbonates, mmol/L	24 [22–25]	25 [24–26]	26 [23–34]	0.277
Proteins, g/L	71 [70–74]	72 [70–75]	69.5 [65.3–72.8]	0.218
Triglycerides, mmol/L	0.94 [0.81–1.35] ^{a,b}	0.92 [0.83–1.88] ^a	2.12 [1.54–3.18] ^b	0.025
Total cholesterol, mmol/L	4.11 [3.92–4.79]	5.42 [4.19–5.90]	4.83 [3.76–5.42]	0.205
HDL-cholesterol, mmol/L	1.08 [0.98–1.97]	1.18 [1.07–1.68]	0.92 [0.79–1.31]	0.221
LDL-cholesterol, mmol/L	2.76 [2.24–2.90]	3.49 [2.40–4.08]	2.90 [1.99–3.50]	0.244
Lipid Lowering treatments, %	0.0	5.3	55.5	0.076

Data are expressed as medians [interquartile range] and compared with a Kruskal-Wallis test. Categorical data were compared using Fisher’s exact test. BMI: body mass index; CKD: chronic kidney disease; CRP: C-reactive protein; CV: cardiovascular; mGFR: measured glomerular filtration rate; RAA: renin angiotensin aldosterone; UA/C: urinary albumin/creatinine ratio. Different letters indicate a significant difference between groups ($p < 0.05$).

Table S3. mGFR and renal excretion of uric acid, choline, betaine and carnitine.

Variable	Parameter	mGFR, mL/min/ 1.73 m ²	FR _{Na} %	pUric acid μmol/L	Cl _{Uric acid}		pCholine μmol/L	Cl _{Choline}	
					mL/mi n/1.73m ²	FE _{Uric acid} %		mL/mi n/1.73 m ²	FE _{Choline} %
Controls <i>n</i> = 5	median	98	99.4	252	9	9	1.2	3	3
	IQR	[91–105]	[98.8–99.6]	[217–298]	[7–12]	[8–13]	[1.0–1.4]	[1–8]	[2–8]
CKD									
stages 1–2 <i>n</i> = 19	median	73	99.4	319	6	8	1.3	2	3
	IQR	[67–79]	[99.0–99.7]	[261–449]	[4–11]	[6–15]	[1.2–1.5]	[1–4]	[2–5]
CKD									
stages 3–5 <i>n</i> = 8	median	51	99.1	390	5	9	1.9	3	7
	IQR	[46–55]	[97.9–99.5]	[322–635]	[3–7]	[7–15]	[1.5–2.1]	[2–5]	[3–10]
<i>p</i> -value		<0.0001	0.401	0.063	0.117	0.262	0.005	0.796	0.172
Controls <i>n</i> = 5	median	98	99.4	29.9	2	2	40.2	2	1
	IQR	[91–105]	[98.8–99.6]	[14.8–46.3]	[2–4]	[2–4]	[30.8–47.3]	[1–3]	[1–3]
CKD									
stages 1–2 <i>n</i> = 19	median	73	99.4	32.8	1	2	40.8	2	3
	IQR	[67–79]	[99.0–99.7]	[27.3–38.4]	[1–6]	[1–8]	[35.7–44.3]	[1–3]	[1–4]
CKD									
stages 3–5 <i>n</i> = 8	median	51	99.1	29.0	4	8	60.3	2	4.0
	IQR	[46–55]	[97.9–99.5]	[15.4–36.3]	[1–12]	[3–23]	[36.7–67.8]	[1–3]	[2.3–5.0]
<i>p</i> -value		<0.001	0.401	0.365	0.543	0.079	0.174	0.983	0.145

Data are expressed as medians [interquartile range] and compared with a Kruskal-Wallis test. Abbreviations: CKD: chronic kidney disease; Cl: clearance; FE: fraction of excretion; FR: fraction of reabsorption; IQR: interquartile range; mGFR: measured glomerular filtration rate; p: plasma; TMAO: trimethylamine-N-oxide.

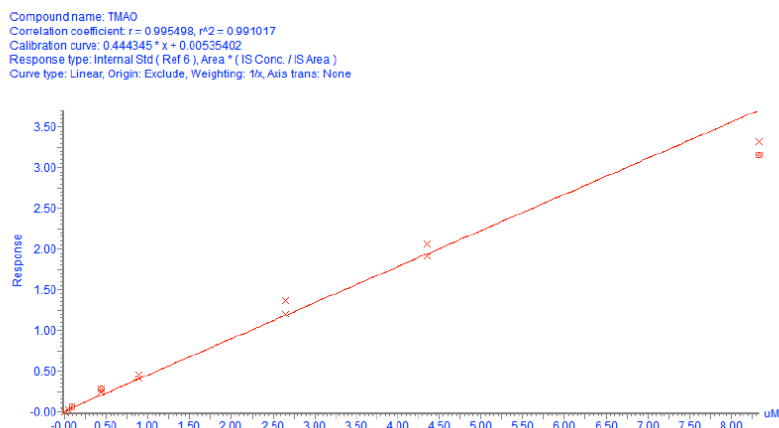


Figure S1. Typical calibration curve of TMAO in LC-MS/MS.

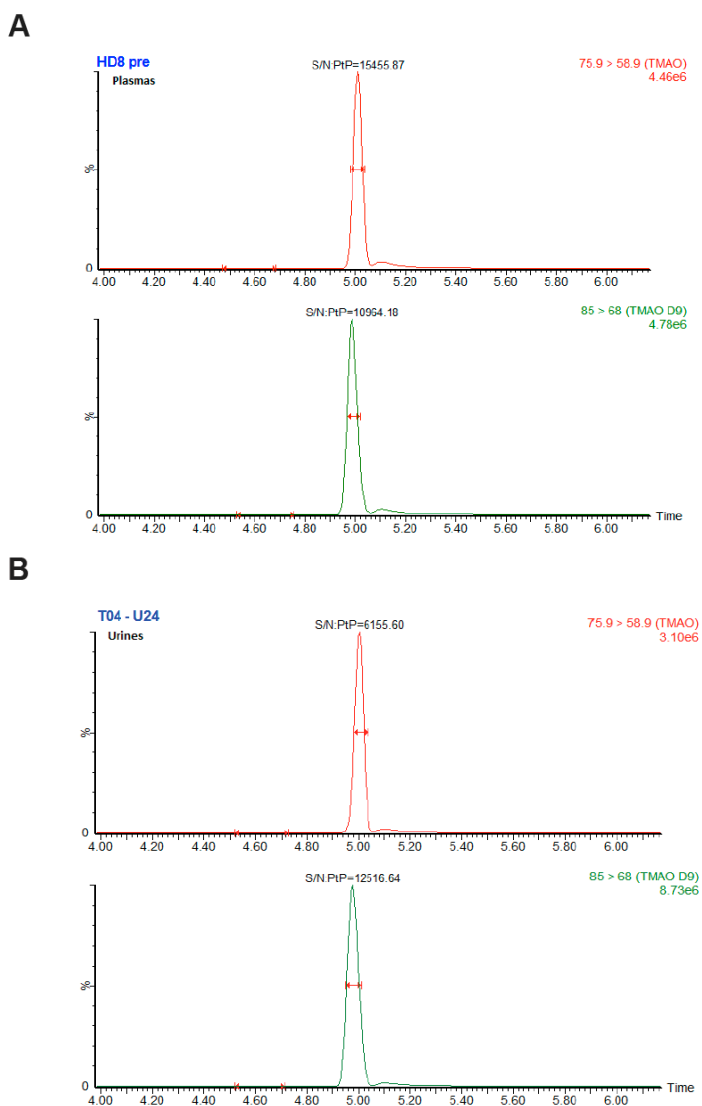


Figure S2. Representative LC-MS/MS chromatograms of TMAO in plasma (A) and urine (B). The deuterated standard (TMAO D9) is presented in green (lower panel).

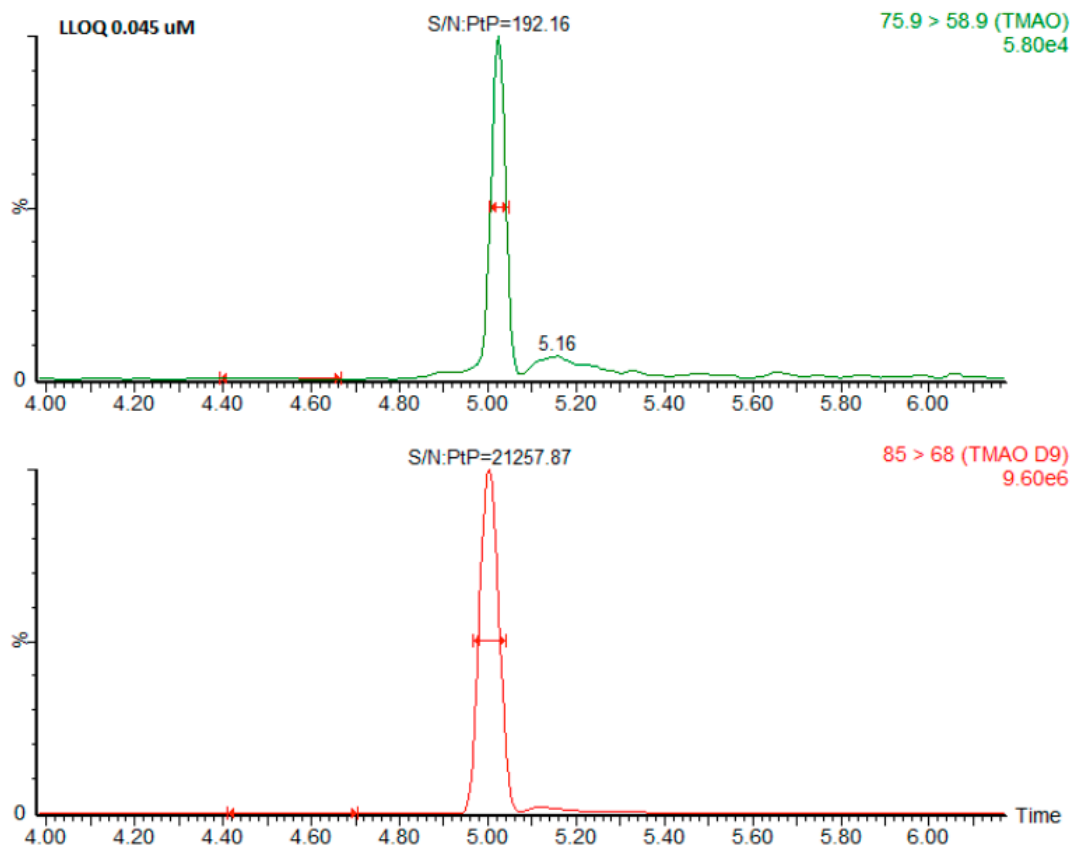


Figure S3. Representative LC-MS/MS chromatograms of TMAO at the lower limit of quantification (LLOQ). The deuterated standard (TMAO D9) is presented in red (lower panel).