SUPPLEMENTAL MATERIAL

Table S1. Baseline characteristics of US men and women in the Cardiovascular Health Study by quintiles of trimethylamine N-oxide*

	Trimethylamine N-oxide					p-value
	Q1 (n=826)	Q2 (n=827)	Q3 (n=825)	Q4 (n=827)	Q5 (n=826)	
Median TMAO (IQR), µmol/L	2.29 (1.84, 2.61)	3.49 (3.19, 3.77)	4.72 (4.40, 5.14)	6.77 (6.16, 7.69)	13.2 (10.4, 19.9)	
Demographics						
Age, (SD) years	71.7 (5.0)	71.7 (4.9)	72.3 (5.5)	72.4 (5.3)	72.8 (5.6)	<0.001
Female, n (%)	584 (70.7)	541 (65.4)	511 (61.9)	492 (59.5)	495 (59.9)	<0.001
Race, n (%)						0.001
White	661 (80.0)	686 (83.0)	698 (84.6)	704 (85.1)	721 (87.3)	
Education, n (%)						
<High school	223 (27.0)	227 (27.4)	229 (27.8)	235 (28.4)	207 (25.1)	
High school	245 (29.7)	240 (29.0)	237 (28.7)	233 (28.2)	234 (28.3)	0.884
Some college	188 (22.8)	202 (24.4)	180 (21.8)	186 (22.5)	203 (24.6)	
College graduate	170 (20.6)	158 (19.1)	179 (21.7)	173 (20.9)	182 (22.0)	
Income group, n (%)						
<$11,999	224 (27.1)	193 (23.3)	190 (23.0)	198 (23.9)	191 (23.1)	
$12,000-$24,999	275 (33.3)	315 (38.1)	295 (35.8)	307 (37.1)	294 (35.6)	0.032
$25,000-$49,999	226 (27.4)	221 (26.7)	244 (29.6)	212 (25.6)	203 (24.6)	
>$50,000	101 (12.2)	98 (11.9)	96 (11.6)	110 (13.3)	138 (16.7)	
Lifestyle and risk factors						
Self-reported health status, n (%)						
Excellent/ Very good	356 (43.1)	355 (42.9)	361 (43.8)	345 (41.7)	334 (40.4)	0.121
Good	303 (36.7)	317 (38.3)	326 (39.5)	317 (38.3)	299 (36.2)	
Fair/poor	167 (20.2)	155 (18.7)	138 (16.7)	165 (20.0)	193 (23.4)	
Smoking, n (%)						
Never smoked	447 (54.1)	387 (46.8)	396 (48.0)	362 (43.7)	377 (45.6)	
Former smoker	282 (34.1)	326 (39.4)	336 (40.7)	356 (43.0)	349 (42.3)	0.002
Current smoker	97 (11.7)	114 (13.8)	93 (11.3)	109 (13.2)	100 (12.1)	
Physical activity, (SD) kcal	1,181 (1,599)	1,136 (1,480)	1,162 (1,555)	1,122 (1,537)	1,147 (1,493)	0.947
Alcohol, (SD) drinks/wk	2.2 (6.2)	2.8 (7.3)	2.2 (5.6)	2.9 (7.0)	2.9 (9.7)	0.091
BMI, (SD) kg/m^2	26.2 (4.8)	26.7 (4.9)	26.9 (4.6)	27.2 (4.7)	26.6 (5.0)	<0.001

	Trimethylamine N-oxide					p-value
	Q1 (n=826)	Q2 (n=827)	Q3 (n=825)	Q4 (n=827)	Q5 (n=826)	
Waist circumference, (SD) cm	92.4 (13.7)	93.4 (13.3)	94.6 (13.2)	95.7 (13.4)	94.4 (13.8)	<0.001
Systolic blood pressure, (SD) mmHg	137 (21)	136 (22)	135 (21)	137 (22)	135 (21)	0.070
Diastolic blood pressure, (SD) mmHg	72 (11)	72 (11)	71 (11)	72 (11)	70 (11)	0.004
Biochemical, (SD)						
HDL cholesterol, mg/dL	57.8 (15.9)	56.7 (16.0)	55.0 (15.1)	54.5 (15.7)	55.0 (15.8)	<0.001
LDL cholesterol, mg/dL	131 (36)	130 (34)	130 (36)	128 (33)	128 (36)	0.333
Triglycerides, mg/dL	130 (63)	134 (66)	136 (70)	145 (85)	139 (75)	<0.001
C-reactive protein, mg/L	4.3 (7.2)	4.6 (8.4)	4.5 (6.7)	4.8 (8.7)	4.6 (7.2)	0.690
Estimated glomerular filtration rate, mL/min/1.73m^2	77.3 (14.0)	73.5 (14.1)	70.0 (15.1)	65.6 (16.1)	62.4 (18.4)	<0.001
Medical history, n (%)						
Prevalent diabetes	84 (10.2)	85 (10.3)	101 (12.2)	95 (11.5)	85 (10.3)	0.570
Lipid lowering medication	35 (4.2)	36 (4.4)	39 (4.7)	52 (6.3)	43 (5.2)	0.303
Hypertension medication	282 (34.1)	307 (37.1)	331 (40.1)	376 (45.5)	386 (46.7)	<0.001
Antibiotics in past 2 weeks	22 (2.7)	26 (3.1)	21 (2.5)	22 (2.7)	25 (3.0)	0.935
Dietary habits, (SD)						
Fruits, servings/d	2.2 (1.0)	2.1 (1.0)	2.2 (1.0)	2.1 (1.1)	2.2 (1.0)	0.623
Vegetables, servings/d	2.6 (1.3)	2.5 (1.3)	2.6 (1.4)	2.5 (1.4)	2.6 (1.4)	0.475
Fiber, g/d	29.6 (11.8)	30.0 (11.8)	29.4 (11.8)	28.6 (11.6)	28.7 (11.9)	0.074
Total animal product, servings/d	1.65 (0.92)	1.67 (0.96)	1.71 (1.02)	1.79 (0.96)	1.77 (1.00)	0.012

* Values reported as mean (SD) for continuous variables, and frequency, percentage (%) for categorical variables, unless otherwise stated. 3,877 participants entered the study at baseline (1989-90 or 1992-93) and 254 participants at 1996-97. IQR= interquartile range (25th, 75th)

Table S2. Spearman correlation coefficient for repeated within-individual measures of trimethylamine-N-oxide over two time points*

	TMAO measured at baseline (1989-90 or 1992-93)
TMAO measured in 1996-97	0.254

*2,470 participants who had two TMAO measures at baseline (1989-90 or 1992-93) and 1996-97.

Table S3. Trimethylamine N-oxide and the risk of atherosclerotic cardiovascular disease in the Cardiovascular Health Study: analysis of potential interaction by age, sex, body mass index, and estimated glomerular filtration rate with respective stratified analyses with Bonferroni correction (significance<0.01)*

(Cases/Total population)	TMAO	
	HR (95% CI)	***P*-value**
Age		
≤71 years (912/2,237)	1.08 (0.98, 1.18)	0.03
>71 years (854/1,894)	1.04 (0.97, 1.12)	
Sex		
Female (1,108/2,623)	1.06 (0.99, 1.14)	0.74
Male (658/1,508)	1.04 (0.95, 1.14)	
BMI		
<25kg/m^2 (645/1,597)	1.06 (0.98, 1.15)	0.50
25-29.9 kg/m^2 (754/1,708)	1.06 (0.97, 1.16)	
≥30 kg/m^2 (367/826)	1.04 (0.88, 1.22)	
eGFR		
≤60 mL/min/1.73m^2 (471/1,058)	1.09 (1.02, 1.17)	<0.001
>60 mL/min/1.73m^2 (1,295/3,073)	0.97 (0.88, 1.06)	

Multivariable adjustments include age (65-74y, 75-84y, 85y+), sex (male, female), race (white, non-white), study site (Bowman Gray, Davis, Hopkins, Pittsburgh), education (<high school, high school, some college, college graduate), income (<$11,999, $12,000-$24,999, $25,000-$49,999, >$50,000/y), self-reported health status (excellent, very good, good/fair/poor), smoking status (never, former, current), alcohol intake (0, <1, 1-2.49, 2.5-7.49, 7.5-14.49, >14.5 drinks/wk), physical activity (<500, 500-1000, 1000-1500, >1500 kcal/wk), BMI (<18.5, 18.5-25, 25.1-30, >30 kg/m^2), waist circumference (cm), lipid lowering medication (yes/no), anti-hypertensive medication (yes/no), antibiotics (yes/no), prevalent diabetes (yes/no), high-density lipoprotein cholesterol (mg/dL), low-density lipoprotein cholesterol (mg/dL), triglycerides (mg/dL), C-reactive protein (mg/L), systolic blood pressure (mmHg), diastolic blood pressure (mmHg)

*ptext*P-value obtained from continuous interaction term for age, BMI, and eGFR, and categorical interaction term for sex (females as reference).

Table S4. Relative risk of atherosclerotic cardiovascular disease (n=1,739) associated with long-term levels of plasma trimethylamine among 4,066 older men and women in the Cardiovascular Health Study, excluding participants who had taken antibiotics in the 2 weeks prior to study entry (n=65).

	Trimethylamine N-oxide quintiles					p-trend
	Q1	Q2	Q3	Q4	Q5	
Median TMAO, µmol/L	2.3	3.5	4.7	6.8	13.1	
Cases/Total	246/814	323/812	397/814	423/812	350/814	
Person-years	9,560	11,123	11,249	11,846	9,846	
Age, sex adjusted	1.00 (Reference)	1.05 (0.89-1.24)	1.24 (1.05-1.45)	1.21 (1.04-1.42)	1.24 (1.06-1.47)	0.014
Multivariable[†]	1.00 (Reference)	1.04 (0.88-1.23)	1.24 (1.06-1.46)	1.17 (1.00-1.38)	1.23 (1.04-1.45)	0.028
Multivariable and diet[‡]	1.00 (Reference)	1.05 (0.89-1.24)	1.24 (1.05-1.45)	1.17 (0.99-1.37)	1.22 (1.04-1.45)	0.035
Multivariable, diet, and renal function[§]	1.00 (Reference)	1.01 (0.86-1.20)	1.16 (0.99-1.37)	1.07 (0.91-1.26)	1.08 (0.91-1.29)	0.579

[†]Multivariable adjustments include age (65-74y, 75-84y, 85y+), sex (male, female), race (white, non-white), study site (Bowman Gray, Davis, Hopkins, Pittsburgh), education (<high school, high school, some college, college graduate), income (<$11,999, $12,000-$24,999, $25,000-$49,999, >$50,000/y), self-reported health status (excellent, very good, good/fair/poor), smoking status (never, former, current), alcohol intake (0, <1, 1-2.49, 2.5-7.49, 7.5-14.49, >14.5 drinks/wk), physical activity (<500, 500-1000, 1000-1500, >1500 kcal/wk), BMI (<18.5, 18.5-25, 25.1-30, >30 kg/m^2), waist circumference (cm), lipid lowering medication (yes/no), anti-hypertensive medication (yes/no), antibiotics (yes/no), prevalent diabetes (yes/no), high-density lipoprotein cholesterol (mg/dL), low-density lipoprotein cholesterol (mg/dL), triglycerides (mg/dL), C-reactive protein (mg/L), systolic blood pressure (mmHg), diastolic blood pressure (mmHg)

[‡]Diet adjustments include fruits (servings/d), vegetables (servings/d), fiber (g/d), and total animal source food (servings/week).

[§]Estimated glomerular filtration rate (mL/min/1.73m^2) is calculated based on the CKD-EPI creatinine-cystatin C equation.[39]

Table S5. Sensitivity analyses for the associations between long-term levels of plasma trimethylamine among 4,031 older men and women in the Cardiovascular Health Study excluding events within the first 2 years of follow-up*

	Trimethylamine N-oxide quintiles					p-trend
	Q1	**Q2**	**Q3**	**Q4**	**Q5**	
Median TMAO, μmol/L	2.3	3.5	4.7	6.7	13.2	
Cases/Total	238/807	307/805	389/806	395/807	337/806	
Person-years	9,443	11,157	11,613	12,192	9,942	
Age, sex adjusted	1.00 (Reference)	1.00 (0.84-1.18)	1.16 (0.99-1.37)	1.10 (0.94-1.29)	1.19 (1.01-1.41)	0.032
Multivariable[†]	1.00 (Reference)	0.98 (0.83-1.16)	1.15 (0.98-1.36)	1.05 (0.89-1.24)	1.17 (0.99-1.39)	0.053
Multivariable and diet[‡]	1.00 (Reference)	0.98 (0.82-1.16)	1.15 (0.98-1.36)	1.04 (0.89-1.23)	1.17 (0.99-1.39)	0.049
Multivariable, diet, and renal function[§]	1.00 (Reference)	0.95 (0.80-1.12)	1.08 (0.92-1.28)	0.96 (0.81-1.13)	1.04 (0.87-1.25)	0.576

*100 ASCVD events occurred within the first 2 years of follow-up to minimize reverse causation (pre-existing subclinical disease leading to changes in levels of TMAO).

[†]Multivariable adjustments include age (65-74y, 75-84y, 85y+), sex (male, female), race (white, non-white), study site (Bowman Gray, Davis, Hopkins, Pittsburgh), education (<high school, high school, some college, college graduate), income (<$11,999, $12,000-$24,999, $25,000-$49,999, >$50,000/y), self-reported health status (excellent, very good, good/fair/poor), smoking status (never, former, current), alcohol intake (0, <1, 1-2.49, 2.5-7.49, 7.5-14.49, >14.5 drinks/wk), physical activity (<500, 500-1000, 1000-1500, >1500 kcal/wk), BMI (<18.5, 18.5-25, 25.1-30, >30 kg/m^2), waist circumference (cm), lipid lowering medication (yes/no), anti-hypertensive medication (yes/no), antibiotics (yes/no), prevalent diabetes (yes/no), high-density lipoprotein cholesterol (mg/dL), low-density lipoprotein cholesterol (mg/dL), triglycerides (mg/dL), C-reactive protein (mg/L), systolic blood pressure (mmHg), diastolic blood pressure (mmHg)

[‡]Diet adjustments include fruits (servings/d), vegetables (servings/d), fiber (g/d), and total animal source food (servings/week).

[§]Estimated glomerular filtration rate (mL/min/1.73m^2) is calculated based on the CKD-EPI creatinine-cystatin C equation.[39]

Table S6. Sensitivity analyses for the associations between long-term levels of plasma trimethylamine among 4,055 older men and women in the Cardiovascular Health Study excluding those who reported poor self-reported health at study entry*

	Trimethylamine N-oxide quintiles					p-trend
	Q1	**Q2**	**Q3**	**Q4**	**Q5**	
Median TMAO, μmol/L	2.3	3.5	4.7	6.8	13.2	
Cases/Total	249/811	309/810	404/811	418/812	351/811	
Person-years	9,483	11,140	11,406	11,903	9,937	
Age, sex adjusted	1.00 (Reference)	0.99 (0.84-1.17)	1.21 (1.03-1.42)	1.17 (1.00-1.37)	1.21 (1.03-1.42)	0.016
Multivariable[†]	1.00 (Reference)	0.97 (0.82-1.15)	1.22 (1.04-1.43)	1.14 (0.97-1.33)	1.19 (1.01-1.41)	0.032
Multivariable and diet[‡]	1.00 (Reference)	0.97 (0.82-1.15)	1.21 (1.04-1.43)	1.13 (0.96-1.33)	1.19 (1.01-1.41)	0.031
Multivariable, diet, and renal function[§]	1.00 (Reference)	0.94 (0.79-1.11)	1.15 (0.98-1.35)	1.04 (0.88-1.22)	1.06 (0.89-1.26)	0.510

* Excluded 61 individuals who self-reported poor health at baseline (1989-90 or 1992-93) and 15 individuals who entered in 1996-97 and who self-reported poor health.

[†] Multivariable adjustments include age (65-74y, 75-84y, 85y+), sex (male, female), race (white, non-white), study site (Bowman Gray, Davis, Hopkins, Pittsburgh), education (<high school, high school, some college, college graduate), income (<$11,999, $12,000-$24,999, $25,000-$49,999, >$50,000/y), self-reported health status (excellent, very good, good/fair/poor), smoking status (never, former, current), alcohol intake (0, <1, 1-2.49, 2.5-7.49, 7.5-14.49, >14.5 drinks/wk), physical activity (<500, 500-1000, 1000-1500, >1500 kcal/wk), BMI (<18.5, 18.5-25, 25.1-30, >30 kg/m^2), waist circumference (cm), lipid lowering medication (yes/no), anti-hypertensive medication (yes/no), antibiotics (yes/no), prevalent diabetes (yes/no), high-density lipoprotein cholesterol (mg/dL), low-density lipoprotein cholesterol (mg/dL), triglycerides (mg/dL), C-reactive protein (mg/L), systolic blood pressure (mmHg), and diastolic blood pressure (mmHg)

[‡] Diet adjustments include fruits (servings/d), vegetables (servings/d), fiber (g/d), and total animal source food (servings/week).

[§] Estimated glomerular filtration rate (mL/min/1.73m^2) is calculated based on the CKD-EPI creatinine-cystatin C equation.[39]

Table S7. Relative risk of atherosclerotic cardiovascular disease associated with levels of plasma trimethylamine among 4,131 older men and women in the Cardiovascular Health Study (simple updating approach)*

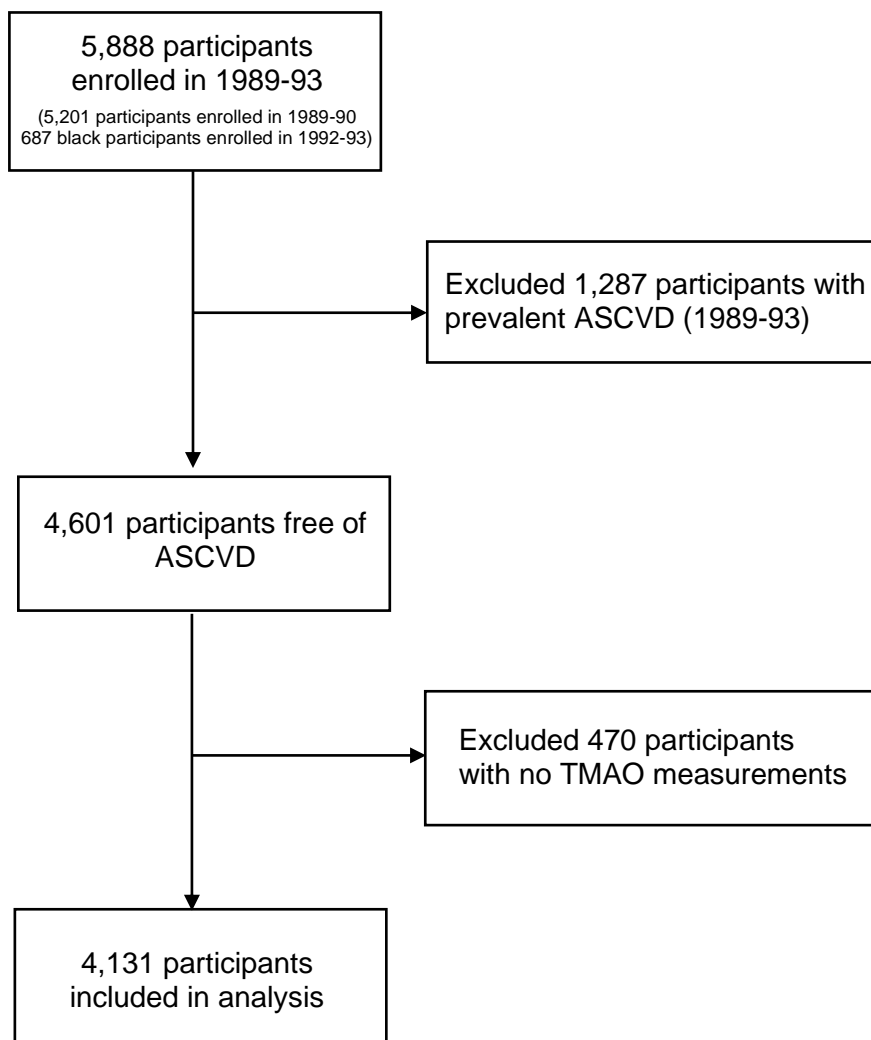|	Q1	Q2	Q3	Q4	Q5	p-trend
			Trimethylamine N-oxide quintiles			
Overall individuals (n=4,131)						
Median TMAO, µmol/L	2.3	3.5	4.7	6.8	13.1	
Cases/Total	280/827	280/826	309/826	370/826	527/826	
Person-years	10,306	10,141	10,402	10,412	13,186	
Age, sex adjusted	1.00 (Reference)	1.00 (0.85-1.18)	1.03 (0.87-1.21)	1.22 (1.04-1.42)	1.21 (1.05-1.41)	0.001
Multivariable†	1.00 (Reference)	1.01 (0.85-1.19)	1.04 (0.88-1.23)	1.17 (1.00-1.37)	1.17 (1.01-1.36)	0.013
Multivariable and diet‡	1.00 (Reference)	1.01 (0.85-1.19)	1.03 (0.88-1.22)	1.16 (0.99-1.36)	1.16 (1.00-1.35)	0.023
Multivariable, diet, and renal function§	1.00 (Reference)	0.98 (0.82-1.15)	0.97 (0.82-1.14)	1.05 (0.89-1.24)	1.01 (0.87-1.19)	0.653

* Plasma TMAO levels at baseline (1989-90 or 1992-93) were related to risk of ASCVD from 1989-90 or 1992-93 to 1996-97, and the plasma TMAO levels in 1996-97 were related to risk of ASCVD from 1996-97 to 2015.

†Multivariable adjustments include age (65-74y, 75-84y, 85y+), sex (male, female), race (white, non-white), study site (Bowman Gray, Davis, Hopkins, Pittsburgh), education (<high school, high school, some college, college graduate), income (<$11,999, $12,000-$24,999, $25,000-$49,999, >$50,000/y), self-reported health status (excellent, very good, good/fair/poor), smoking status (never, former, current), alcohol intake (0, <1, 1-2.49, 2.5-7.49, 7.5-14.49, >14.5 drinks/wk), physical activity (<500, 500-1000, 1000-1500, >1500 kcal/wk), BMI (<18.5, 18.5-25, 25.1-30, >30 kg/m^2), waist circumference (cm), lipid lowering medication (yes/no), anti-hypertensive medication (yes/no), antibiotics (yes/no), prevalent diabetes (yes/no), high-density lipoprotein cholesterol (mg/dL), low-density lipoprotein cholesterol (mg/dL), triglycerides (mg/dL), C-reactive protein (mg/L), systolic blood pressure (mmHg), diastolic blood pressure (mmHg)

‡Diet adjustments include fruits (servings/d), vegetables (servings/d), fiber (g/d), and total animal source food (servings/week).

§ Estimated glomerular filtration rate (mL/min/1.73m^2) is calculated based on the CKD-EPI creatinine-cystatin C equation.[39]

Figure S1. **Flow chart of this study.**


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        ┌─────────────────────────────────────────┐
        │          5,888 participants              │
        │          enrolled in 1989-93             │
        │ (5,201 participants enrolled in 1989-90  │
        │  687 black participants enrolled in 1992-93) │
        └─────────────────────────────────────────┘
                         │
                         │          ┌──────────────────────────────────┐
                         ├─────────▶│ Excluded 1,287 participants with  │
                         │          │ prevalent ASCVD (1989-93)         │
                         │          └──────────────────────────────────┘
                         ▼
        ┌─────────────────────────────────────────┐
        │       4,601 participants free of         │
        │               ASCVD                      │
        └─────────────────────────────────────────┘
                         │
                         │          ┌──────────────────────────────────┐
                         ├─────────▶│ Excluded 470 participants         │
                         │          │ with no TMAO measurements         │
                         │          └──────────────────────────────────┘
                         ▼
        ┌─────────────────────────────────────────┐
        │          4,131 participants              │
        │          included in analysis            │
        └─────────────────────────────────────────┘
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ASCVD, atherosclerotic cardiovascular disease; TMAO, trimethylamine-N-oxide