Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Tang WHW, Wang Z, Levison BS, et al. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N Engl J Med 2013;368:1575-84. DOI: 10.1056/NEJMoa1109400

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Intestinal Microbial Metabolism of Phosphatidylcholine and Cardiovascular Risk

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Figure S1

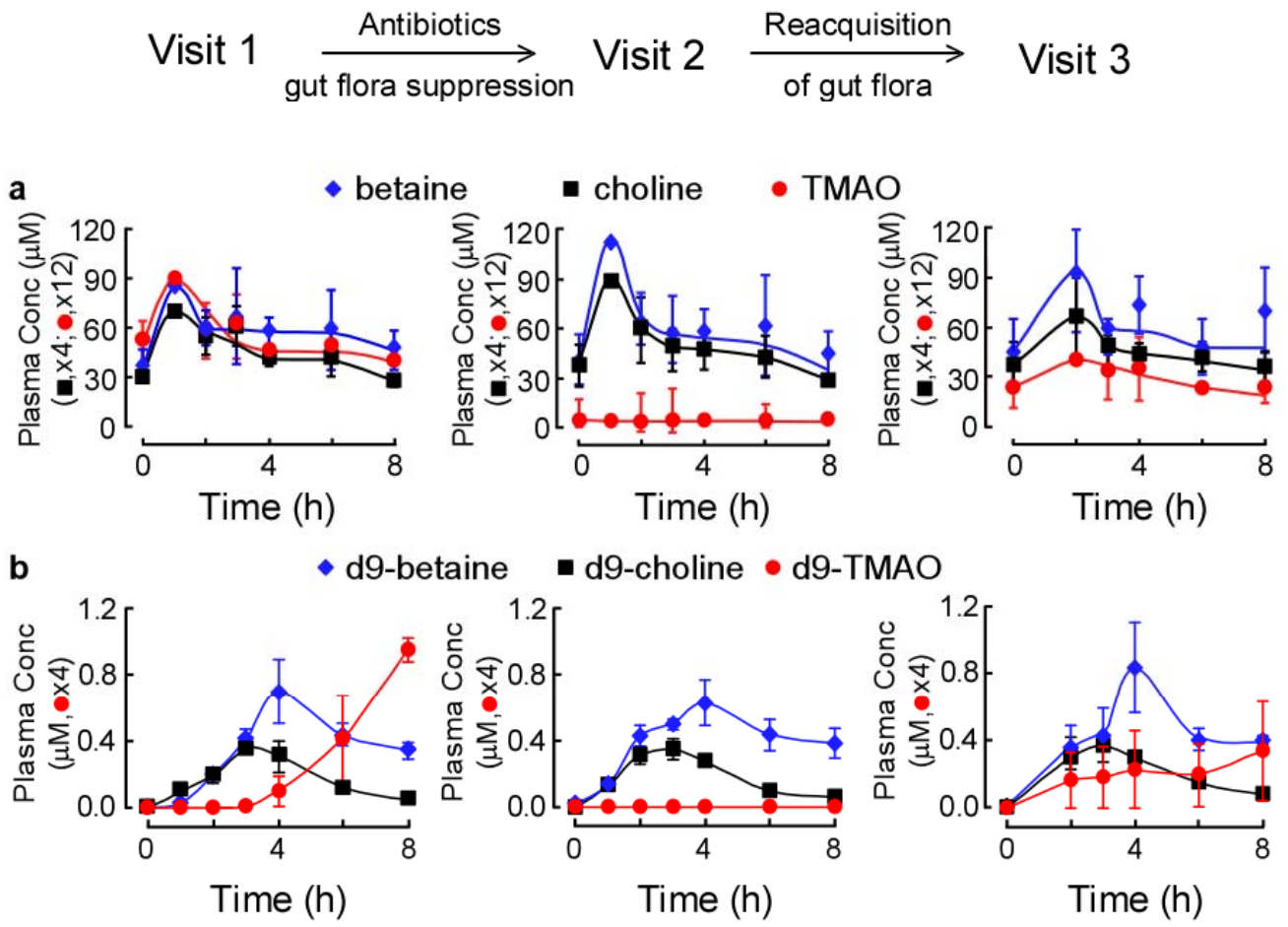

Figure S1 Human Plasma Levels of Phosphatidylcholine Metabolites (Trimethylamine-*N* oxide, Choline, Betaine) after Oral Ingestion of Two Hard-Boiled Eggs and d9-Phosphatidylcholine Before and After Antibiotics. At the top of the figure, the visit sequence is shown. All 40 study participants (healthy volunteers) participated in the first dietary phosphatidylcholine challenge (Visit 1). Six participants were then administered broad-spectrum antibiotics for one week, followed by a second phosphatidylcholine challenge (Visit 2). These same participants returned again at least one month after discontinuing antibiotics for a third challenge (Visit 3). Panels a and b show the time course of plasma concentrations of betaine, choline and trimethylamine-*N*-oxide (Panel a) and of their d9 isotopologues (Panel b). Note that,

in Panel a, the concentrations of choline are multiplied by 4, and the concentration of trimethylamine-*N*-oxide are multiplied by 12; in Panel b, the concentrations of d9-trimethylamine-*N*-oxide are multiplied by 4. All left panels show data from Visit 1; center panels, from Visit 2; and right panels, from Visit 3.

Figure S2

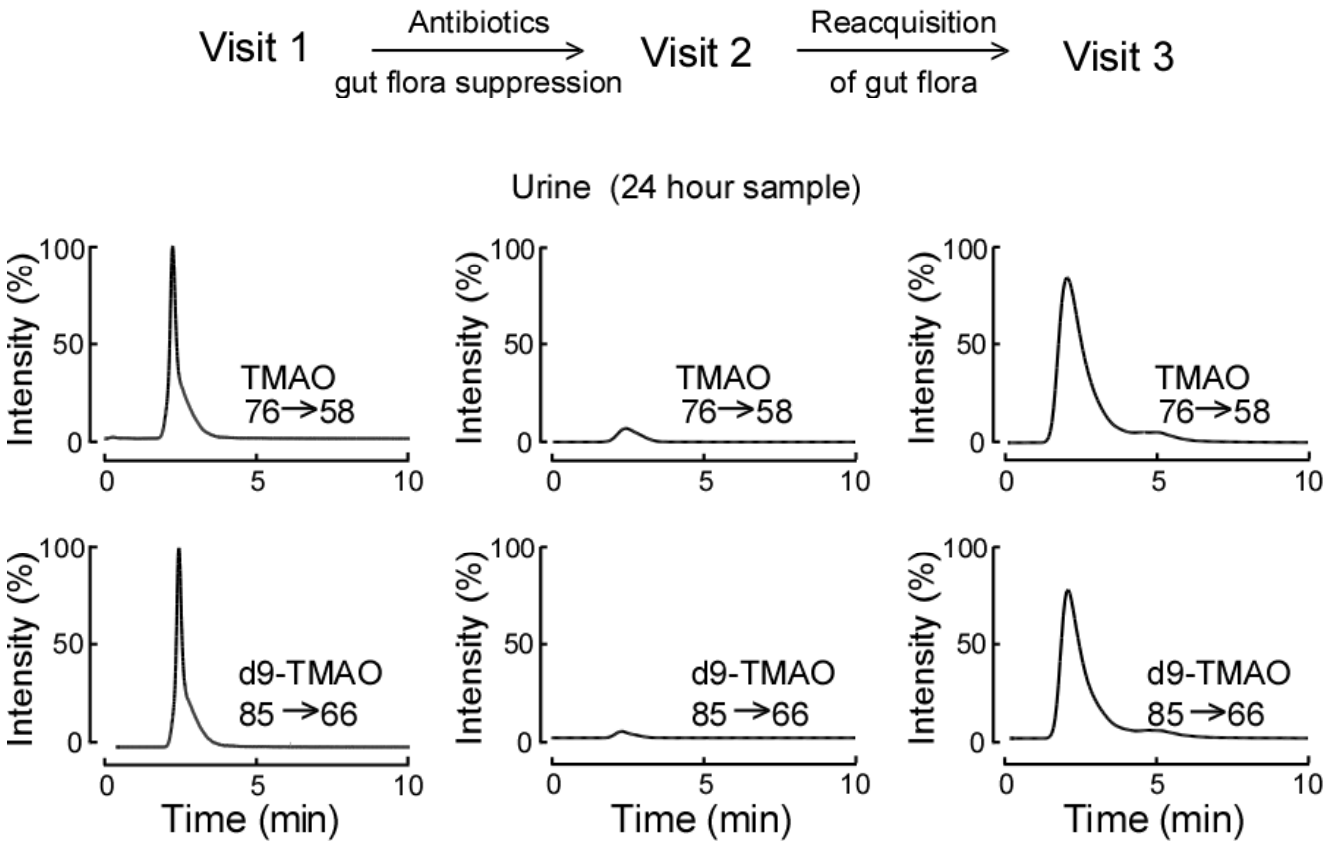

Figure S2. Human 24-hour Urine Levels of Trimethylamine *N*-Oxide (TMAO) after Oral Ingestion of Two Hard-Boiled Eggs and d9-Phosphatidylcholine Before and After Antibiotics. Numbers in label represent the mass-to-charge ratios for the precursor → product ion transitions monitored for TMAO and d9-TMAO.

Figure S3

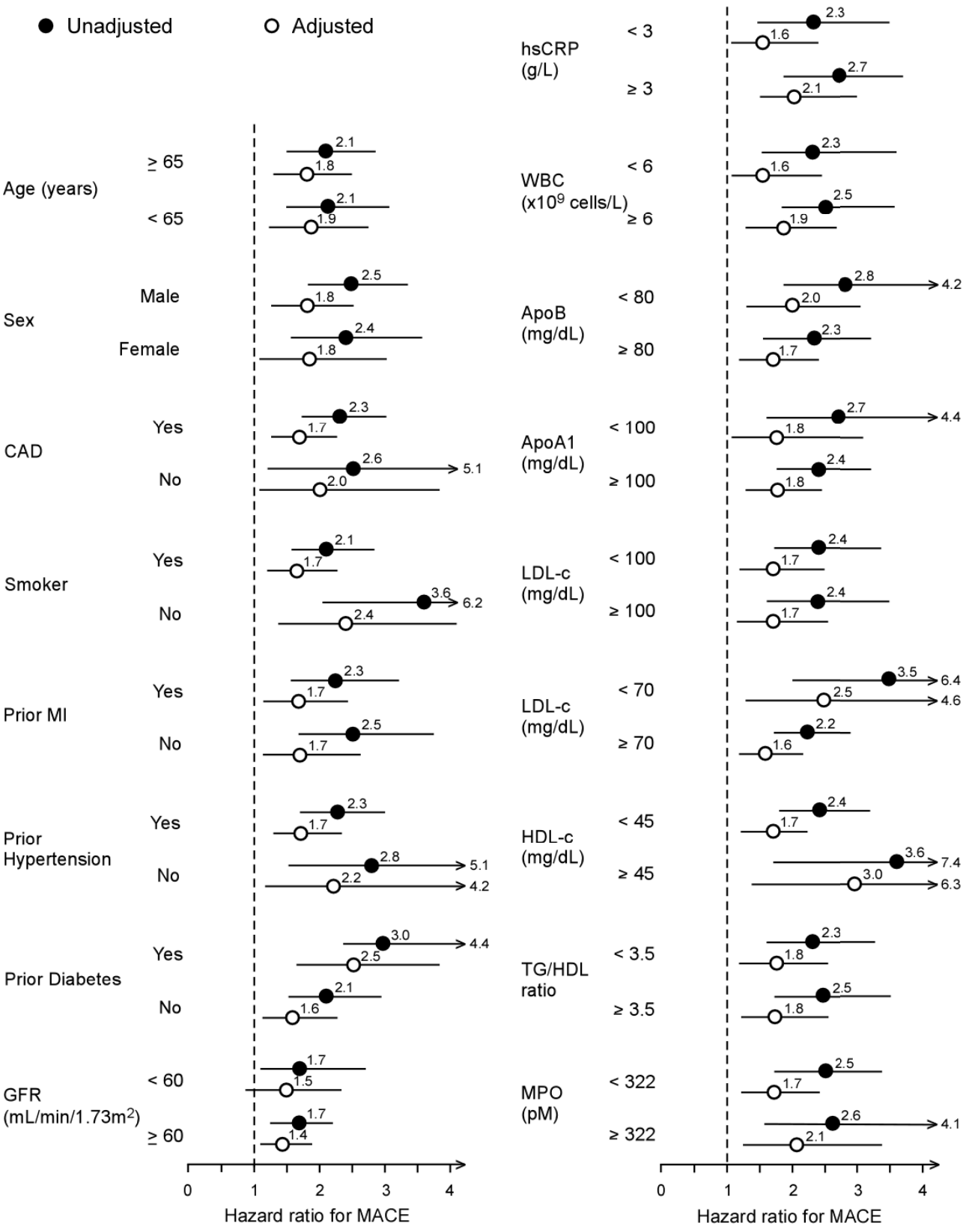
Unadjusted
Adjusted
Age (years)
≥ 65
< 65
Sex
Male
Female
CAD
Yes
No
Smoker
Yes
No
Prior MI
Yes
No
Prior Hypertension
Yes
No
Prior Diabetes
Yes
No
GFR (mL/min/1.73m2)
< 60
≥ 60
hsCRP (g/L)
< 3
≥ 3
WBC (x10^9 cells/L)
< 6
≥ 6
ApoB (mg/dL)
< 80
≥ 80
ApoA1 (mg/dL)
< 100
≥ 100
LDL-c (mg/dL)
< 100
≥ 100
LDL-c (mg/dL)
< 70
≥ 70
HDL-c (mg/dL)
< 45
≥ 45
TG/HDL ratio
< 3.5
≥ 3.5
MPO (pM)
< 322
≥ 322
0
1
2
3
4
Hazard ratio for MACE
Hazard ratio for MACE

Figure S3. Risks of Major Adverse Cardiovascular Events (MACE) among Patient Subgroups, According to Baseline Trimethylamine *N*-Oxide Levels. Hazard ratios compare top to bottom quartiles. Significant interactions were observed between plasma trimethylamine-*N*-oxide and cigarette smoking (p=0.027) as well as plasma trimethylamine-*N*-oxide and plasma myeloperoxidase (p=0.012).

Table S1: Baseline Characteristics of Cohort According to TMAO Quartiles

Characteristic	**Quartile 1** (n=1001)	**Quartile 2** (n=998)	**Quartile 3** (n=1003)	**Quartile 4** (n=1005)	**p-value**
(TMAO, µM)	(<2.4)	(2.4-3.6)	(3.7-6.2)	(>6.2)	
Age (years)	59±11	62±11	65±10	66±10	<0.001
Male Gender (%)	67	67	63	61	0.008
Body mass index	28.4 (25.4-32)	28.7 (25.6-32.8)	28.7 (25.9-32.6)	28.4 (25.7-33.1)	0.138
Diabetes mellitus (%)	24	28	31	42	<0.001
Hypertension (%)	68	69	70	79	<0.001
History of MI (%)	41	39	42	43	0.317
# of diseased vessels					
0	30	26	27	21	<0.001
1	22	22	18	18	0.017
2	21	21	19	18	0.233
3	27	30	36	42	<0.001
Past or current Smoking (%)	63	65	67	65	0.314
LDL-c (mg/dL)	97 (79-117)	98 (81-120)	95 (78-116)	92 (74-114)	<0.001
HDL-c (mg/dL)	34 (29-42)	35 (29-42)	34 (29-41)	33 (27-40)	<0.001
Triglycerides (mg/dL)	115 (84-166)	115 (84-163)	121 (86-178)	123 (86-180)	0.089
ApoB (mg/dL)	82 (70-97)	83 (69-98)	82 (69-95)	80 (68-94)	0.05
ApoA1 (mg/dL)	116 (103-134)	117 (104-132)	117 (104-133)	115 (101-132)	0.121

Fasting glucose	100 (91-114)	101 (92-116)	102 (93-119)	107 (95-134)	<0.001
hsCRP (ng/L)	2.3 (0.9-6.3)	2.2 (1-5.6)	2.3 (1.1-5)	3.1 (1.2-6.8)	<0.001
MPO (pM)	127.3 (78.7-264.6)	115.3 (76.9-242.4)	112.9 (75.8-235.2)	110.8 (74.2-226.8)	0.092
eGFR(ml/min/1.73m^2)	92 (81-103)	86 (75-96)	79 (67-91)	69 (52-83)	<0.001
Total leukocyte count (WBC, x10^9)	6.2 (5.1-7.6)	6.1 (5.1-7.5)	6.1 (5-7.5)	6.1 (5-7.5)	0.524
Baseline drugs (%):					
Aspirin	76	76	73	70	0.007
ACE inhibitor/ARB	44	47	53	57	<0.001
Statin	64	61	58	57	0.005
Beta blockers	62	64	63	64	0.823

Table S1. Values expressed in mean ± standard deviation or median (interquartile range).

Abbreviations: MI, myocardial infarction; LDL-c, low-density lipoprotein cholesterol; HDL-c, high-density lipoprotein cholesterol; ApoB, apolipoprotein B; ApoA1, apolipoprotein A1; hsCRP, high sensitivity C-reactive protein; MPO, myeloperoxidase; WBC, white blood cell; ACE, angiotensin converting enzyme; ARB, angiotensin receptor blocker; TMAO, trimethylamine *N*-oxide