Supplemental Experimental Procedures

Chemical synthesis of (2-Hydroxyethyl)(trimethyl)phosphonium chloride

The phosphorous analog of choline, (2-hydroxyethyl)(trimethyl)phosphonium chloride (P-choline), was prepared as described previously (Renshaw and Bishop, 1938) but with some minor modifications. In brief, trimethylphosphine (12g) was placed in a glass pressure vessel with an excess of 2-chloroethanol (15 g) dissolved in 20 ml of absolute ethanol. The material was then heated in a sealed tube for 7 h at 95 – 100 ^{o}C. The clear colorless reaction mixture was then cooled to room temperature and diluted with absolute ethanol and the phospho-choline chloride was crystalized out by the addition of anhydrous diethyl ether. The product was purified through three successive rounds of re-crystallization from ethanol and anhydrous diethyl ether. During the last re-crystallization, the addition of ether was performed slowly, and long (0.5 to 1 cm) white needle-like crystals were obtained. These crystals are very hygroscopic and the resulting pure material is extremely soluble in water and various alcohols. This material is essentially insoluble in ether, hydrocarbons (hexane, pentane) and acetone. TLC of the reaction mixture on Silica Gel 60 plates (EMD #5735-7) developed with 0.1% (v/v) formic acid in methanol and then visualized by iodine staining shows only one oblong spot at Rf = 0.35. High resolution mass spectrometry was performed on the triply recrystallized material (transparent white, long prismatic needles from absolute ethanol with ether) diluted to 10 μg/ml in 50% (v/v) water/methanol plus 0.1% (v/v) formic acid and infused at 10 μl/min into the ESI source of an AB Sciex 5600 triple TOF calibrated with CsI and ALILTLVS peptide in the positive ion and high resolution product ion modes. It showed a base peak molecular ion $[M]^+$ at m/z of 121.0779 amu, in agreement with the anticipated elemental composition (calculated m/z for C5H14OP = 121.0792 amu). A peak corresponding to $[M+1]^+$ at 5.4% the height of the base peak at m/z 122.0808 amu for the carbon 13 isotope was also observed. All other impurity peaks amounted to less than 1% of the base peak height. The high resolution product ion (positive mode, at 35 EV collision energy) of the precursor peak at 121.1 amu demonstrates a base peak fragment ion at 103.0676 amu, corresponding to $[M-H2O]^+$, along with other major product ions (>5% of base peak intensity) at m/z 62.0297 amu, 75.0371 amu, 88.0443 amu and 90.0588 amu (Figure S6).

Chemical synthesis of [d9-N,N,N-trimethyl]glycerophosphocholine

[d9-N,N,N-trimethyl]glycerophosphocholine (d9-GPC) was prepared from glycerophosphoethanolamine (Corden Pharma, Cambridge, MA, USA) in methanol with 3 equivalents of potassium hydrogen carbonate and 15 equivalents of d3-methyl iodide (all from Sigma-Aldrich, St. Louis, MO) essentially by the method of Morano et al (Morano et al., 2008). After 96 hours, the entire reaction mixture was quantitatively transferred onto a bed of silica gel (60Å pore 40-60 μm particle, Angela Technologies, Wilmington, DE) equilibrated in methanol in a coarse fritted Buchner pressure funnel. Non-polar material was removed by elution of the column with the 1.25 column volumes of methanol. The product d9-GPC was eluted in 2.5 column volumes of 30% (v/v) water in methanol. Rotary evaporation of this second fraction gave the crude product as a clear, colorless oil. Residual water was removed by rotary evaporation and final traces of moisture were removed azeotropically by sequential rotary evaporations of absolute ethanol under reduced pressure. The clear colorless oil was filtered from a solution in absolute ethanol to remove residual inorganic salts. The material was concentrated to dryness by rotary evaporation and placed under high vacuum (10 mtorr) overnight. This viscous, clear, colorless oil was stored at -20 ^{o}C. Concentrations of stock solutions of this material were

determined by stable isotope dilution LC/MS/MS analyses relative to a standard curve of authentic (natural abundance) GPC. High resolution mass spectrometry (m/z): $[M+H]^+$ calculated for C8H12 2H9NO6P, 267.1671; found, 267.1663; $[M+Na]^+$ calculated for NaC8H11 2H9NO6P, 289.1491; found, 289.1482; $[M+K]^+$ calculated for KC8H11 2H9NO6P, 305.1221; found, 305.1230; high resolution product ions (positive mode, at 30 ev collision energy),69.1463, 113.1759, 193.1504, corresponding to d9-TMA, d9-choline and d9-phosphocholine fragmented from $[M+H]^+$, respectively (Figure S7). d9-GPC is highly hygroscopic and the concentration of the aqueous solution was quantified by LC/MS/MS both using natural abundance GPC as above, as well as (with similar results) after 2 M NaOH hydrolysis at 60°C for 2 hours to release free d9-choline, which was similarly quantified by stable isotope dilution LC/MS/MS using non-labeled choline as standard.

Quantitation of choline uptake

Bacteria were grown in minimal medium with 1 mM amino acids (alanine, lysine, arginine, valine, leucine, isoleucine, tyrosine) and vitamin B1 added until an $OD_{600\ nm}$ of ~0.5. Cells were then harvested and re-suspended in the same volume of minimal medium. Intact cells were incubated with different concentrations of d9-choline in the presence or absence of DMB (2 mM) for 15 minutes and cell exterior d9-choline was removed by cold (0°C) PBS washing. Cellular uptake of d9-choline was quantified following freeze-thaw cycle induced lysis and release, with quantification by stable isotope dilution LC/MS/MS using [1,1,2,2-d4]choline as internal standard, which was monitored using multiple reaction monitoring of parent and characteristic daughter ion m/z 108→60.

Quantitation of plasma TMAO, TMA, choline and betaine

Stable isotope dilution liquid chromatography with on-line tandem mass spectrometry (LC/MS/MS) was used for quantification of plasma and tissue TMAO, choline and betaine as previously described (Wang et al., 2011; Wang et al., 2014). TMA in plasma or tissue was similarly quantified from plasma or tissue homogenate by LC/MS/MS using d9-TMA as internal standard. Samples used for TMA analyses were acidified (60 mM HCl final) immediately after collection/processing and prior to storage in gas tight vials at -80ºC until analysis. LC/MS/MS analyses were performed on an AB Sciex 4000 QTRAP mass spectrometer using electrospray ionization in positive-ion mode. TMAO, d9-TMAO, choline, d9-choline, betaine, d9-betaine, TMA and d9-TMA were monitored using multiple reaction monitoring of precursor and characteristic product ions: m/z 60→44 for TMA; m/z 69→49 for d9-TMA; m/z 76→58 for TMAO; m/z 85→66 for d9-TMAO; m/z 104→60 for choline and m/z 113→69 for d9-choline; m/z 118→59 for betaine and m/z 127→68 for d9-betaine.

Determination of TMA lyase activity

TMA lyase activity was quantified *in vitro* by incubating (typically 10-16h) the indicated enzyme activity source [cell lysate (typically 3 mg protein), isolated recombinant enzyme (typically 30 μg protein), cultured live microbe (typically $OD_{600\ nm}$ ~ 1.0), or cecal lysate (typically 3 mg protein)] with the indicated d9-labeled synthetic substrate (typically 100 μM), in PBS in the presence vs. absence of the indicated concentration of choline structural analogue or DMB (2 mM) at 37°C (unless otherwise stated). Except for reactions with cultured live microbes, reactions would also consist of co-factors including 1 mM S-adenosyl-L-methionine

and 2 mM $Na_2S_2O_4$ for purified CutC/D and 200 µM NADPH and 1 mM ammonium iron(II) sulfate hexahydrate for CntA/B and YeaW/X. Reactions (2 ml) were carried out in 13 x 100 mm threaded glass tubes with gas-tight mininert caps. TMA lyase activity was monitored by quantifying d9-TMA production by stable isotope dilution LC/MS/MS analysis. Briefly, reactions were quenched by addition of heavy isotope labeled [$^{13}C_3$,$^{15}N_1$]TMA (Sigma Chemicals, St. Louis, MO) in 0.2 N formic acid as an internal standard within the gas-tight reaction vials, followed by immediate extraction of TMA isotopologues into the organic layer produced following mixing with successive additions of 2 ml hexane, 1 ml butanol and 0.2 ml 1 N NaOH (to deprotonate the TMA isotopologues), vortexing and then centrifugation. The recovered upper hexane layer was transferred to a 12 x 75 mm PTFE capped threaded glass tube, acidified with the addition of 200 µl of 0.2 N formic acid, vortexed and the organic and aqueous layers were separated by centrifugation. The now protonated TMA isotopologues were recovered in the aqueous phase for quantitation by stable isotope dilution LC/MS/MS analysis. Sample aliquots were injected onto a reverse phase C18 HPLC column (2.0 × 150 mm, 5 µm, Phenomenex, Prodigy, Torrance, CA) eluted at a flow rate of 0.2 ml/min and resolved using a linear gradient between 0.2% formic acid in water and 0.2% formic acid in acetonitrile/methanol (95:5, v/v). HPLC column effluent was introduced into an AB Sciex 5500 QTRAP mass spectrometer using electrospray ionization in the positive-ion mode. Both d9-TMA and [$^{13}C_3$,$^{15}N_1$]TMA were monitored using multiple reaction monitoring of parent and characteristic daughter ions: m/z 69 → 49 for d9-TMA; and m/z 64 → 47 for [$^{13}C_3$,$^{15}N_1$]TMA. Reactions without microbial enzyme source (cells, cell lysates, or isolated proteins) were used as blank controls for each individual d9-TMA containing substrate and showed negligible (~0.25 nM) background d9-TMA production.

DMB analysis by gas chromatography mass spectrometry

Plasma or urine aliquots (50 µl) were acidified by adding 10% trichloroacetic acid (10 µl) to precipitate protein and DMB was extracted with hexane (50 µl). Hexane extract was injected onto a 6890/5973 gas chromatography mass spectrometry (GC/MS) instrument equipped with an automatic liquid sampler (Agilent Technologies, Santa Clara, CA) using the positive ion chemical ionization mode with methane as the reagent gas. The source temperature was set at 230°C. The electron energy was 240 eV and the emission current was 300 µA. The hexane extract was separated on a J and W Scientific (Folsom, CA) DB-5MSDG column (30 m, 0.25 mm inner diameter, 0.25 µm film thickness). The injector and the transfer line temperatures were maintained at 250°C. The initial GC oven temperature was set at 100°C and held for 2 minutes, and then increased 20°C/min to 200°C followed by increases of 100°C/min to 320°C. The selected ion mass spectra of DMB at m/z=41, 57, 69 and 86, were recorded. Spike and recovery experiments confirmed that under the experimental methods employed, the limit of detection (S/N 3:1) of DMB in biological matrices was 500 nM, and resolution from the structural isomer 2,3-dimethyl-1-butanol was confirmed with authentic standards. GC/MS analysis of DMB (Sigma-Aldrich, St. Louis, MO), demonstrated > 98% purity.

Cloning and Expression of *cutC/cutD, cntA/cntB* and *yeaW/yeaX*

P. mirabilis ATCC 29906 whole genome sequence, contig00092 (ACLE01000068.1) was used to acquire the nucleotide sequence of *cutC* (locus tag: HMPREF0693_2863), *cutD* (locus tag: HMPREF0693_2862) and the 104 bases of intergenic DNA between *cutC/D*. The open reading frames (ORFs) for *cutC/D* were codon optimized for expression in *E. coli*, synthesized

contiguously by GenScript (Piscataway, NJ) and cloned into pUC57 (Genescript). The synthesized *cutC*- intergenic region-*cutD* genes were inserted by ligation into the bacterial expression vector pBAD (Invitrogen, Carlsbad, CA) between the Nco I and Hind III sites and transformed into *E. coli* Top10 cells (Invitrogen). Sequence verified clones were used to express CutC and CutD as described below. For expression of amino-terminal 8x-His-tagged CutC and carboxy-terminal Strep-Tag II-tagged CutD, the codon optimized *cutC*-intergenic region-*cutD* from *P. mirabilis* described above was PCR amplified and cloned in-frame into the plasmid pGK::nucMCS that was isolated from *L. lactis* (ATCC® 8731™) behind an N-terminal 8x-His tag and in front of a Strep-tag II affinity tag. The plasmid pGK::nucMCS is an expression plasmid that drives constitutive expression of downstream genes from the strong *Staphlococcus* nuclease I promoter and selected with erythromycin (250 μg/ml) (Le Loir et al., 1996). Sequence verified clones were transformed into *E. coli* Stellar cells (Clontech, Moutainview, CA). Expressed 8x-His-tagged proteins were affinity purified using nickel immobilized affinity chromatography (IMAC), whereas Strep-Tag II- tagged proteins were affinity purified with Strep-Tactin resin (Qiagen GmbH, Hilden Germany).

The ORFs for *Desulfovibrio alaskensis* G20 *cutC/D* genes (Ddes_3282 and Ddes_1357 respectively) and their associated intergenic region were codon optimized for expression in *E. coli* and synthesized by Genscript for cloning into pUC57. To allow constitutive expression of the *D. alaskensis* G20 CutC/D proteins in *E. coli* Top10 cells (Invitrogen), we included the synthetic *em7* constitutive bacterial promoter with ribosome binding site (5'ATTAATCATCGGCATAGTATATCGGCATAGTATAATACGACAAGGT-GAGGAA-CTAAACCCAGGAGGCAGACC3') immediately 5' to the synthesized genes, while a Rho-independent terminator sequence (5' TAATCCCACAGCCGCCAGTTCCGCTGGCGGCATTTT-TTGGATCCAAGCTT3') was placed immediately 3' to the *cutC/D* genes.

The genes for y*eaW/yeaX* were cloned from *E. coli* K-12 DH10B genomic DNA by PCR, inserted into a modified pET22 vector backbone and expressed in *E. coli* BL21(DE3) (Invitrogen), as described previously (Zhu et al., 2014). For expression of 8x-His-tagged YeaW and 8x-His-tagged YeaX proteins, the relevant genes were PCR amplified and cloned in-frame behind an 8x-His-tag using Nde1 and Hind III restriction sites. All clones were sequence verified and transformed into *E. coli* BL21(DE3) for expression.

The genes for *cntA*/*cntB* from *Acinetobacter baumannii* ATCC 19606 as reported (Zhu et al., 2014) were codon optimized and synthesized independently by Genscript, cloned into pUC57 and sequence verified. The *cntA* and *cntB* encoding pUC57 plasmids were digested with NcoI and Hind III restriction enzymes, the gene fragments isolated and inserted separately into a modified pET22 vector backbone between the Nco I and Hind III restriction enzyme sites, and expressed as un-tagged proteins in *E. coli* BL21(DE3).

Growth and induction conditions for recombinant TMA lyases

Recombinant clones in *E. coli* Top10 and BL21(DE3), were grown in LB medium with 100 μg/ml ampicillin and 1.0% glucose at 37^{o}C overnight. The cultured bacteria were diluted to 1:20 with fresh medium under antibiotic selection but without glucose and grown to an $OD_{600\ nm}$ of 0.5. For recombinant protein expression, arabinose or isopropyl β-D-1-thiogalactopyranoside

(IPTG) was added at a final concentration of 0.2% or 0.4 mM for induction expression of TMA lyase subunits constructed in pBAD or pET, respectively, and cells were grown at 37^{o}C for an additional 5 hours. Bacterial cells were then harvested for TMA lyase activity assays (intact bacterial cell, bacterial lysate, or affinity purified protein) as described above. The *cutC/D* recombinant clones in the pGK or pUC57 vectors were grown in LB medium with erythromycin (250 μg/ml) or ampicillin (100 μg/ml), respectively, at 37^{o}C overnight to constitutively express the targeted TMA lyase subunit. Constructs in pUC57 constitutively expressing wild type or mutated *cutC/D* TMA lyase subunits were harbored in *E. Coli* Top10 cells and grown to an $OD_{600\ nm}$ of 0.5. Arabinose (0.2% final) was added to provide similar growth and induction conditions as *P. mirabilis* expressed *cutC/D*.

Construction of CutC, YeaW and CntA point mutants

The CutC proteins from *P. mirabilis* ATCC 29906 and *D. alaskensis* G20 share homology across 804 amino acids, with *P. mirabilis* having an additional amino-terminal 338 amino acids of non-homology. Mutants of *D. alaskensis* CutC (Ddes_3282) reported to lack catalytic activity include those with alanine replacement of either of the conserved residues C489 or G821, residues which play a part in catalysis through formation of a thiyl- or glycyl-radical, respectively (Craciun and Balskus, 2012). The homologous residues in CutC from *P. mirabilis* ATCC 29906 are C781 and G1117. Using pBAD *cutC*-intergenic region-*cutD* cloned from *P. mirabilis* ATCC 29906 as template DNA, the residues C781 and G1117 were individually mutated to an alanine using the Quickchange lightning mutagenesis kit (Agilent, San Diego, CA) as per the manufacturer's instructions. Mutants *cutC*(C781A)/*cutD* and *cutC*(G1117A)/*cutD* were confirmed by DNA sequencing and transformed into *E. coli* Top10 cells for expression. *D. alaskensis* G20 *cutC/D* in pUC57, described above, was also singly mutated at CutC residues C489 and G821 (each to an alanine), as previously described (Craciun and Balskus, 2012), using the Quickchange lightning mutagenesis kit. Mutants *cutC*(C489A)/*cutD* and *cutC*(G821A)/*cutD* were confirmed by DNA sequencing and transformed into *E. coli* Top10 cells for expression. The *Acinetobacter baumannii* (ATCC 19606) E205D catalytically-inactive *cntA* mutant (Zhu et al., 2014) was made by site-directed mutagenesis using the pET22-*cntA* construct described above as template DNA. Resulting mutants were DNA sequence verified and transformed into *E. coli* BL21(DE3) for expression as described above. YeaW is a Rieske-type oxygenase that shows sequence homology at the amino acid level to CntA from *A. baumannii*. It contains a conserved glutamic acid immediately in front of the mononuclear iron center at amino acid position 208. YeaW(E208D) was constructed by site-directed mutagenesis using pET22-*yeaW*- (*E. coli* K-12 DH10B) described above as the template DNA. Mutants were DNA sequence verified and transformed into *E. coli* BL21(DE3) for expression. Recombinant proteins containing an 8x-His tag were nickel IMAC purified as described above.

Statistical analyses

Student's t test and ANOVA with post hoc t test with Holm-Bonferroni correction were used to compare difference in mean value between different groups for most studies, as indicated. Pearson's correlation was used to calculate the association between two variables after demonstration of normal distribution of data. For all statistical tests $p < 0.05$ was considered significant. For animal atherosclerosis studies, we performed a power analysis using R Project Package 'pwr' v1.1-3 (https://github.com/heliosdrm/pwr), indicating that using a group size of

N=15, we have 80% power to detect a 27% difference in lesion area, assuming a coefficient of variation of 25%, using a two tailed Students' t test at a=0.05. In other mouse studies we used an a=0.05 and a 79% probability to detect a difference of 50% with a group size of N=5, assuming a coefficient of variation of 25% employing a 2-tailed Student's t test analysis. Biological replicates were used in all experiments. Results from all animals in a given experiment were included in the analyses. Investigators performing quantitative analyses of endpoints (e.g. atherosclerotic plaque quantification, TMAO levels) were blinded to group allocation with samples labeled by code only. Investigators were not blinded to mouse group allocation during the performance of animal husbandry requirements for experiments.

Table S1 | Effects of DMB on lipid profiles, glucose, renal and liver function in C57BL/6J *Apoe*$^{-/-}$ mice, Related to Figure 3

Sex		Chow	Chow + DMB	Choline	Choline + DMB
Male	N=	21	22	21	20
	Weight (g)	33.7 ± 1.6	32.7 ± 2.0 (p=0.09)	31.7 ± 1.7 (p=0.0005)	29.4 ± 1.9 (p<0.0001, 0.0003*)
	Cholesterol (mg/dL)	340 ± 93	335 ± 108 (p=0.87)	383 ± 95 (p=0.14)	383 ± 49 (p=0.53, 0.98*)
	Triglyceride (mg/dL)	105 ± 49	119 ± 50 (p=0.38)	116 ± 44 (p=0.44)	136 ± 39 (p=0.28, 0.14*)
	Creatinine (μM)	7.8 ± 1.1	8.5 ± 1.6 (p=0.14)	8.5 ± 2.6 (p=0.30)	7.9 ± 0.9 (p=0.93, 0.34*)
	ALT	56.6 ± 26.9	41.9 ± 21.6 (p=0.08)	38.9 ± 24.6 (p=0.04)	32.6 ± 11.1 (p=0.002, 0.30*)
	AST	137 ± 56	162 ± 201 (p=0.60)	120 ± 119 (p=0.57)	119 ± 198 (p=0.48, 0.97*)
	Glucose (mg/dL)	277 ± 64	300 ± 111 (p=0.41)	259 ± 91 (p=0.46)	250 ± 55 (p=0.10, 0.71*)
	Insulin (μg/L)	0.19 ± 0.23	0.10 ± 0.10 (p=0.09)	0.12 ± 0.10 (p=0.18)	0.13 ± 0.07 (p=0.47, 0.95*)
	BUN (mg/dL)	20.6 ± 2.5	20.9 ± 3.0 (p=0.68)	22.8 ± 2.9 (p=0.01)	21.9 ± 2.2 (p=0.09, 0.26*)
	Total bilirubin (mg/dL)	0.029 ± 0.046	0.024 ± 0.043 (p=0.69)	0.019 ± 0.040 (p=0.62)	0.0067 ± 0.0258 (p=0.06, 0.21*)
	Choline (μM)	26.4±9.1	22.6±5.1 (p=0.10)	31.0±8.2 (p=0.09)	27.8±6.5 (p=0.57, 0.18*)
Female	N=	13	17	17	19
	Weight (g)	24.7 ± 1.2	24.9± 2.5 (p=0.74)	24.3± 2.6 (p=0.67)	22.8± 2.0 (p=0.008, 0.10*)
	Cholesterol (mg/dL)	335 ± 46	265 ± 38 (p=0.0002)	330 ± 46 (p=0.83)	313 ± 51 (p=0.22, 0.31*)
	Triglyceride (mg/dL)	100 ± 27	76 ± 18 (p=0.01)	95 ± 19 (p=0.73)	72 ± 17 (p=0.004, 0.0007*)
	Creatinine (μM)	8.8 ± 1.4	8.6 ± 2.0 (p=0.75)	8.6 ± 1.0 (p=0.65)	9.3 ± 1.7 (p=0.34, 0.13*)
	ALT	38.2 ± 21.0	34.0 ± 8.3 (p=0.55)	36.0 ± 11.7 (p=0.84)	39.2 ± 15.9 (p=0.90, 0.80*)
	AST	138 ± 150	139 ± 71 (p=0.98)	139 ± 125 (p=0.96)	153 ± 130 (p=0.78, 0.74*)
	Glucose (mg/dL)	263 ± 67	221 ± 67 (p=0.10)	233 ± 60 (p=0.28)	196 ± 42 (p=0.005, 0.04*)
	BUN (mg/dL)	20.8 ± 3.7	18.3 ± 2.8 (p=0.02)	20.7 ± 2.6 (p=0.95)	22.3 ± 3.4 (p=0.24, 0.17*)
	Total bilirubin (mg/dL)	0.094 ± 0.023	0.010 ± 0.031 (p<0.0001)	0.074 ± 0.045 (p=0.08)	0.057 ± 0.051 (p=0.02, 0.35*)
	Choline (μM)	14.5±2.1	14.9±2.8 (p=0.69)	20.8±5.3 (p=0.0002)	18.5±3.3 (p=0.0002, 0.13*)

Blood, collected from C57BL/6J *Apoe*$^{-/-}$ mice was snap-frozen and stored at -80 ^{o}C until use. Data are presented as mean ± SD from the indicated number of mice in each group. All p values (Student's t-test with 2 tails) were compared with chow diet except *, which were compared with choline diet.

Table S2. DMB effects on Choline, betaine, TMAO and TMA in liver, kidney, plasma and urine, Related to Figure 3

Tissues	Diet	Chow (n=12)	Chow + DMB (n=12)	Choline (n=12)	Choline + DMB (n=12)
Liver	choline (nmol/mg fresh weight)	0.69 ± 0.22	0.51 ± 0.14 (p=0.09)	0.39 ± 0.13 (p=0.003)	0.48 ± 0.13 (p=0.12, 0.08*)
	betaine (nmol/mg fresh weight)	0.80 ± 0.20	2.55 ± 1.42 (p=0.002)	2.95 ± 1.02 (p<0.001)	2.49 ± 0.93 (p<0.001, 0.33*)
	TMAO (nmol/mg fresh weight)	0.00 ± 0.00	0.00 ± 0.00	0.040 ± 0.015 (p<0.001)	0.014 ± 0.009 (p<0.001, <0.001*)
	TMA (nmol/mg fresh weight)	0.00 ± 0.00	0.00 ± 0.00	0.15 ± 0.13 (p<0.001)	0.072 ± 0.062 (p=0.002, 0.06*)
Kidney	choline (nmol/mg fresh weight)	4.93 ± 0.85	5.43 ± 1.91 (p=0.16)	4.32 ± 0.74 (p=0.21)	4.14 ± 1.06 (p=0.41, 0.73*)
	betaine (nmol/mg fresh weight)	1.07 ± 0.21	2.05 ± 0.72 (p<0.001)	1.70 ± 0.47 (p=0.002)	2.08 ± 0.33 (p<0.001, 0.048*)
	TMAO (nmol/mg fresh weight)	0.008 ± 0.002	0.005 ± 0.003 (p=0.04)	0.094 ± 0.013 (p<0.001)	0.052 ± 0.007 (p<0.001, <0.001*)
	TMA (nmol/mg fresh weight)	0.00 ± 0.00	0.00 ± 0.00	0.50 ± 0.27 (p<0.001)	0.19 ± 0.08 (p<0.001, 0.003*)
Plasma	choline (uM)	7.24 ± 1.03	8.04 ± 1.35 (p=0.12)	11.4 ± 3.7 (p=0.002)	11.3 ± 2.6 (p<0.001, 0.94*)
	betaine (uM)	44.2 ± 7.4	69.3 ± 12.9 (p<0.001)	74.8 ± 17.9 (p<0.001)	98.1 ± 25.0 (p<0.001, 0.02*)
	TMAO (uM)	1.03 ± 0.32	0.69 ± 0.22 (p=0.007)	23.2 ± 7.5 (p<0.001)	16.8 ± 3.4 (p<0.001, 0.02*)
	TMA (uM)	42.5 ± 4.5	41.4 ± 3.4 (p=0.53)	307.5 ± 146.2 (p<0.001)	225.6 ± 77.8 (p<0.001, 0.12*)
Urine	choline (mol/mol creatinine)	0.021 ± 0.006	0.036 ± 0.019 (p=0.04)	0.18 ± 0.36 (p=0.19)	0.11 ± 0.04 (p<0.001, 0.52*)
	betaine (mol/mol creatinine)	0.13 ± 0.05	0.30 ± 0.18 (p=0.02)	0.43 ± 0.83 (p=0.28)	0.53 ± 0.36 (p=0.01, 0.74*)
	TMAO (mol/mol creatinine)	0.128 ± 0.026	0.085 ± 0.024 (p=0.003)	2.46 ± 0.39 (p<0.001)	2.03 ± 0.41 (p<0.001, 0.03*)
	TMA (mol/mol creatinine)	0.91 ± 0.38	0.64 ± 0.17 (p=0.09)	25.0 ± 10.4 (p<0.001)	21.3 ± 6.9 (p<0.001, 0.38*)

Male C57BL/6J *Apoe*$^{-/-}$ mice at 10 weeks of age were fed a chow diet or a 1.0% choline supplemented chow diet with or without DMB provided in the drinking water. Two weeks later, mice were sacrificed and tissues were collected for measurement of choline, betaine, TMAO and TMA by LC/MS/MS. Data were presented as mean ± SD from the indicated number of mice. All p values (Student's t-test with 2 tails) were compared with chow diet except *, which were compared with choline diet.

Table S3. Proportion (%) of gut microbiota taxa showing significant correlation to plasma TMAO, TMA or aortic lesion in male C57BL/6J *Apoe*$^{-/-}$ mice, Related to Figure 5

Taxa	Chow (n=10)	chow+DMB (n=10)	Choline (n=9)	choline+DMB (n=9)	Aortic lesion		TMAO		TMA	
					r	p	r	p	r	p
p__Actinobacteria	0.15±0.14	0.42±0.18*	0.81±0.55	0.86±0.65	0.25	0.27	0.41	0.01	0.34	0.04
c__Coriobacteriia	0.05±0.03	0.14±0.12	0.23±0.12	0.47±0.30*	-0.04	0.85	0.58	0.000	0.52	0.001
o__Coriobacteriales	0.05±0.03	0.14±0.12	0.23±0.12	0.47±0.30*	-0.04	0.85	0.58	0.000	0.52	0.001
f__Coriobacteriaceae	0.05±0.03	0.14±0.12	0.23±0.12	0.47±0.30*	-0.04	0.85	0.58	0.000	0.52	0.001
g__	0.03±0.03	0.10±0.13	0.12±0.15	0.45±0.29**	-0.13	0.56	0.52	0.001	0.48	0.002
f__Bacteroidaceae	0.01±0.01	0.00±0.00	0.01±0.01	0.00±0.00	0.49	0.02	0.17	0.30	0.15	0.37
g__Bacteroides	0.01±0.01	0.00±0.00	0.01±0.01	0.00±0.00	0.49	0.02	0.17	0.30	0.15	0.37
f__Rikenellaceae	5.63±2.34	2.43±1.95**	0.01±0.01	0.01±0.01	-0.31	0.18	-0.66	0.0000	-0.64	0.000
g__	5.63±2.34	2.43±1.95**	0.01±0.01	0.01±0.01	-0.31	0.18	-0.66	0.0000	-0.64	0.000
c__Bacilli	0.63±0.69	0.99±1.88	1.86±1.00	1.79±0.72	0.37	0.10	0.34	0.04	0.30	0.07
o__Bacillales	0.00±0.00	0.00±0.00	0.01±0.01	0.13±0.24	-0.24	0.30	0.32	0.05	0.44	0.01
f__Staphylococcaceae	0.00±0.00	0.00±0.00	0.01±0.01	0.13±0.24	-0.24	0.30	0.32	0.05	0.44	0.01
g__Staphylococcus	0.00±0.00	0.00±0.00	0.01±0.01	0.13±0.24	-0.24	0.30	0.32	0.05	0.44	0.01
f__Clostridiaceae	0.03±0.02	0.03±0.04	0.35±0.32	0.13±0.06	0.44	0.04	0.58	0.000	0.49	0.002
g__	0.02±0.02	0.02±0.04	0.34±0.32	0.12±0.06	0.44	0.04	0.58	0.000	0.50	0.002
f__Dehalobacteriaceae	0.02±0.01	0.03±0.02	0.04±0.02	0.04±0.01	0.15	0.50	0.39	0.02	0.41	0.01
g__Dehalobacterium	0.02±0.01	0.03±0.02	0.04±0.02	0.04±0.01	0.15	0.50	0.39	0.02	0.41	0.01
f__Lachnospiraceae	6.23±7.76	5.60±1.80	9.37±3.96	8.28±2.24	0.19	0.42	0.32	0.05	0.25	0.13
g__Coprococcus	0.14±0.17	0.14±0.10	0.26±0.20	0.19±0.08	0.20	0.40	0.34	0.04	0.25	0.13
g__Dorea	0.02±0.02	0.01±0.01	0.07±0.07	0.06±0.04	0.20	0.39	0.41	0.01	0.39	0.01
f__Ruminococcaceae	5.18±2.75	4.52±0.99	6.45±2.10	8.04±1.76	0.12	0.62	0.52	0.001	0.52	0.001
g__	1.01±0.59	0.89±0.52	1.14±0.64	2.86±0.97	-0.10	0.65	0.51	0.001	0.58	0.000
c__Erysipelotrichi	0.38±0.58	0.21±0.21	0.82±0.83	1.11±0.60	0.33	0.14	0.44	0.01	0.47	0.003
o__Erysipelotrichales	0.38±0.58	0.21±0.21	0.82±0.83	1.11±0.60	0.33	0.14	0.44	0.01	0.47	0.003
f__Erysipelotrichaceae	0.38±0.58	0.21±0.21	0.82±0.83	1.11±0.60	0.33	0.14	0.44	0.01	0.47	0.003
g__Allobaculum	0.23±0.60	0.04±0.03	0.71±0.88	1.07±0.61	0.32	0.16	0.48	0.00	0.50	0.001
o__A eroplasmatales	0.96±1.20	1.19±1.48	0.65±0.43	0.00±0.01***	-0.21	0.36	-0.36	0.03	-0.36	0.02
f__A eroplasmataceae	0.96±1.20	1.19±1.48	0.65±0.43	0.00±0.01***	-0.21	0.36	-0.36	0.03	-0.36	0.02
g__A eroplasma	0.96±1.20	1.19±1.48	0.65±0.43	0.00±0.01***	-0.21	0.36	-0.36	0.03	-0.36	0.02

Male C57BL/6J *Apoe*$^{-/-}$ mice (after weaning at 4 weeks of age) were fed a chow, or 1% choline supplemented chow diet with or without DMB supplementation in the drinking water for 16 weeks. At 20 weeks of age, mice were sacrificed to collect blood, heart and cecum. The heart was fixed in formalin and used for aortic lesion quantitation. Plasma TMAO and TMA were measured. Bacterial 16s rRNA was sequenced from each individual mouse cecum and each taxon expressed as a percentage to the total bacterium population. Bacterium % in the same diet fed mice was grouped as mean ± SD. Pearson's correlation was used to calculate the correlation coefficient and p values. Different taxonomic levels were p_, phylum, c_, class; o_, order, f_, family; g_, genus. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ vs. similar diet without DMB.

Table S4. Proportion (%) of gut microbiota taxa showing significant correlation to plasma TMAO, TMA or aortic lesion in female C57BL/6J *Apoe*$^{-/-}$ mice, Related to Figure 5

Taxa	Chow (n=11)	Chow+DMB (n=11)	Choline (n=12)	Choline+DMB (n=10)	Aortic lesion		TMAO		TMA	
					r	p	r	p	r	p
g_Adlercreutzia	0.04±0.08	0.02±0.04	0.07±0.12	0.11±0.16	-0.25	0.208	0.18	0.242	0.33	0.042
p_Bacteroidetes	40.9±9.2	53.2±7.3**	37.8±6.1	51.0±5.4***	-0.40	0.037	-0.29	0.057	-0.34	0.032
c_Bacteroidia	40.9±9.1	53.0±7.1**	37.0±6.1	51.0±5.4***	-0.41	0.035	-0.29	0.058	-0.34	0.032
o_Bacteroidales	40.9±9.1	53.0±7.1**	37.0±6.1	51.0±5.4***	-0.41	0.035	-0.29	0.058	-0.34	0.032
f_S24-7	38.9±7.8	44.2±5.8	35.8±6.4	45.6±7.1**	-0.42	0.029	-0.20	0.192	-0.31	0.053
f_S24-7;g_	38.9±7.8	44.2±5.8	35.8±6.4	45.6±7.0**	-0.42	0.029	-0.20	0.192	-0.31	0.053
p_Firmicutes	56.1±8.9	44.0±7.2**	59.6±5.8	45.6±5.8***	0.40	0.036	0.29	0.060	0.34	0.032
c_Bacilli	1.9±0.4	2.4±5.8	2.7±0.5	1.3±0.2	0.42	0.031	-0.03	0.832	-0.11	0.491
o_Turicibacterales	0.6±0.8	1.1±1.1	0.7±1.1	0.05±0.08	0.56	0.002	-0.12	0.435	-0.18	0.261
f_Turicibacteraceae	0.6±0.8	1.1±1.1	0.7±1.1	0.05±0.08	0.56	0.002	-0.12	0.435	-0.18	0.261
g_Turicibacter	0.6±0.8	1.1±1.1	0.7±1.1	0.05±0.08	0.56	0.002	-0.12	0.435	-0.18	0.261
c_Clostridia	53.8±8.8	39.8±7.1***	52.8±6.5	43.1±5.8***	0.31	0.114	0.23	0.126	0.35	0.027
o_Clostridiales	53.7±8.8	39.7±7.1***	52.8±6.5	43.0±5.8***	0.31	0.113	0.23	0.126	0.35	0.027
o_Clostridiales;f_	14.1±3.5	10.8±5.3	22.0±5.8	13. 9±5.2**	0.42	0.031	0.52	0.0003	0.38	0.018
o_Clostridiales;f_;g_	14.1±3.5	10.8±5.3	22.0±5.8	13.7±4.9**	0.42	0.031	0.52	0.0003	0.38	0.018
f_Lachnospiraceae	8.1±2.8	6.3±2.5	7.2±2.4	4.3±1.1**	0.41	0.033	-0.07	0.648	-0.07	0.672
f_Lachnospiraceae;g_	4.0±1.8	2.3±0.8*	2.9±1.7	1.8±0.6*	0.32	0.104	0.56	0.0001	0.49	0.002
f_Ruminococcaceae	7.2±2.6	7.4±1.5	7.2±3.3	6.1±2.0	0.45	0.019	0.00	0.994	0.10	0.556
g_Ruminococcus	1.1±1.1	0.8±0.4	1.9±0.9	1.4±0.6	0.43	0.024	0.47	0.001	0.45	0.004
f_Ruminococcaceae;g_	0.6±0.6	0.6±0.2	0.8±0.8	0.6±0.5	0.47	0.013	-0.31	0.044	-0.22	0.175
c_Erysipelotrichi	0.2±0.2	0.8±0.7*	1.8±1.8	0.2±0.4*	0.49	0.009	0.36	0.015	0.19	0.250
o_Erysipelotrichales	0.2±0.2	0.8±0.7*	1. 8±1.8	0.2±0.4*	0.49	0.009	0.36	0.015	0.19	0.250
f_Erysipelotrichaceae	0.2±0.2	0.8±0.7*	1. 8±1.8	0.2±0.4*	0.49	0.009	0.36	0.015	0.19	0.250
g_Allobaculum	0.1±0.2	0.7±0.7*	1.7±1.8	0.2±0.3*	0.54	0.004	0.36	0.016	0.18	0.281
p_Tenericutes	0.6±0.6	0.6±0.8	0.1±0.3	0.2±0.3	0.16	0.436	-0.34	0.024	-0.33	0.040
c_Mollicutes	0.6±0.6	0.6±0.8	0.1±0.3	0.2±0.3	0.16	0.436	-0.34	0.024	-0.33	0.040

Female C57BL/6J *Apoe*$^{-/-}$ mice (after weaning at 4 weeks of age) were fed a chow, or 1% choline supplemented chow diet with or without DMB supplementation in the drinking water for 16 weeks. At 20 weeks of age, mice were sacrificed to collect blood, heart and cecum. The heart was fixed in formalin and used for aortic lesion quantitation. Plasma TMAO and TMA were measured. Bacterial 16s rRNA was sequenced from each individual mouse cecum and each taxon expressed as a percentage to the total bacterium population. Bacterium % in the same diet fed mice was grouped as mean ± SD. Pearson's correlation was used to calculate the correlation coefficient and p values. Different taxonomic levels were p_, phylum, c_, class; o_, order, f_, family; g_, genus. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$ vs. similar diet without DMB.

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