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Reviews

Balancing the Equation: A Natural History of Trimethylamine and Trimethylamine-*N*-oxide

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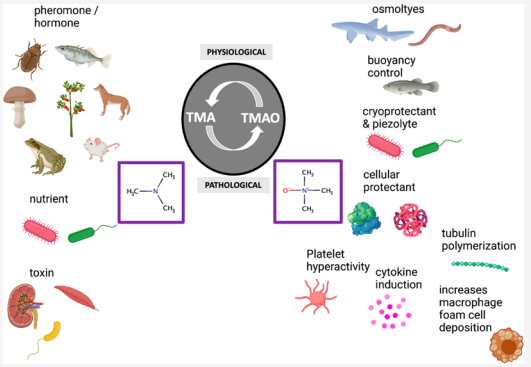
ABSTRACT: Trimethylamine (TMA) and its *N*-oxide (TMAO) are ubiquitous in prokaryote and eukaryote organisms as well as in the environment, reflecting their fundamental importance in evolutionary biology, and their diverse biochemical functions. Both metabolites have multiple biological roles including cell-signaling. Much attention has focused on the significance of serum and urinary TMAO in cardiovascular disease risk, yet this is only one of the many facets of a deeper TMA−TMAO partnership that reflects the significance of these metabolites in multiple biological processes spanning animals, plants, bacteria, and fungi. We report on analytical methods for measuring TMA and TMAO and attempt to critically synthesize and map the global functions of TMA and TMAO in a systems biology framework.



KEYWORDS: *metabolic profiling, symbiotic, host−microbial cometabolism, osmolyte, trimethylamine, trimethylamine N-oxide, cardiovascular disease, FMO3*

1. METHYLAMINES IN BIOLOGY

Methylamines are found in organisms spanning all five major kingdoms (Animalia, Plantae, Fungi, Protista, and Monera) and can be synthesized in shared and unique metabolic pathways in both eukaryotic and prokaryotic organisms or even abiotically (Figure 1). The ubiquitous presence of methylamines is consistent with them having fundamental roles in evolutionary biology. This Review summarizes the multiple biological functions of trimethylamine (TMA) and its oxide, trimethylamine-*N*-oxide (TMAO), with a focus on their role in humans and their influence on physiological and pathophysiological processes. Microbes capable of *de novo* TMA production are widely distributed across multiple ecosystems including marine sediments, algal communities in fresh and salt water, and the mammalian gut, where the predominant source of TMA generation is from the microbial breakdown of choline or betaine or via bacterial reduction of dietary TMAO.

Whereas there has been much recent interest in the role of TMA and TMAO with respect to the development of atherosclerosis, its other biological functions are rarely acknowledged, and much of the older literature on TMA has been largely forgotten or ignored. A review by Chhibber-Goel et al. provides a concise summary of some of the lesser known history of TMA research.[1] Although chemically related, TMA and TMAO have very different physicochemical properties and biological functions, with TMA frequently acting as a pheromone or chemical signaling agent and TMAO most often serving as a cellular protector or disruptant depending on the chemical context. TMA is characterized by its strong fish-like odor, and because of its odorant properties, it can act as both a chemical attractant and repellent in various species.[2] TMA is oxidized to its nonodorous form, TMAO, in the liver via the hepatic flavin-monooxygenase 3 (FMO3) gene of most higher vertebrates. TMAO can act as an osmolyte, shielding cellular proteins from the denaturizing effects of urea and other perturbing osmolytes in some fish (e.g., elasmobranchs and teleosts) and other marine organisms, including invertebrates, where it serves to protect organisms from osmotic stress,[3] low temperatures,[4] and high hydrostatic pressure.[5,6] However, the mechanism by which TMAO stabilizes proteins remains controversial. On the basis of infrared studies and molecular dynamics simulations, TMAO has been proposed to weaken peptide−water interactions by strengthening the water hydrogen-bonding network.[7] An expanded summary of the biological functions of TMA and TMAO is provided in Figure 2.

2. ANALYTICAL MEASUREMENT OF METHYLAMINES

TMA was first isolated and formally identified by Hofmann in early 1852.[8] TMA, like most amines, has a strong smell. The organic/water partition coefficients for TMA are low (chloro-

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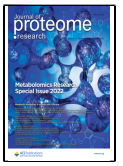

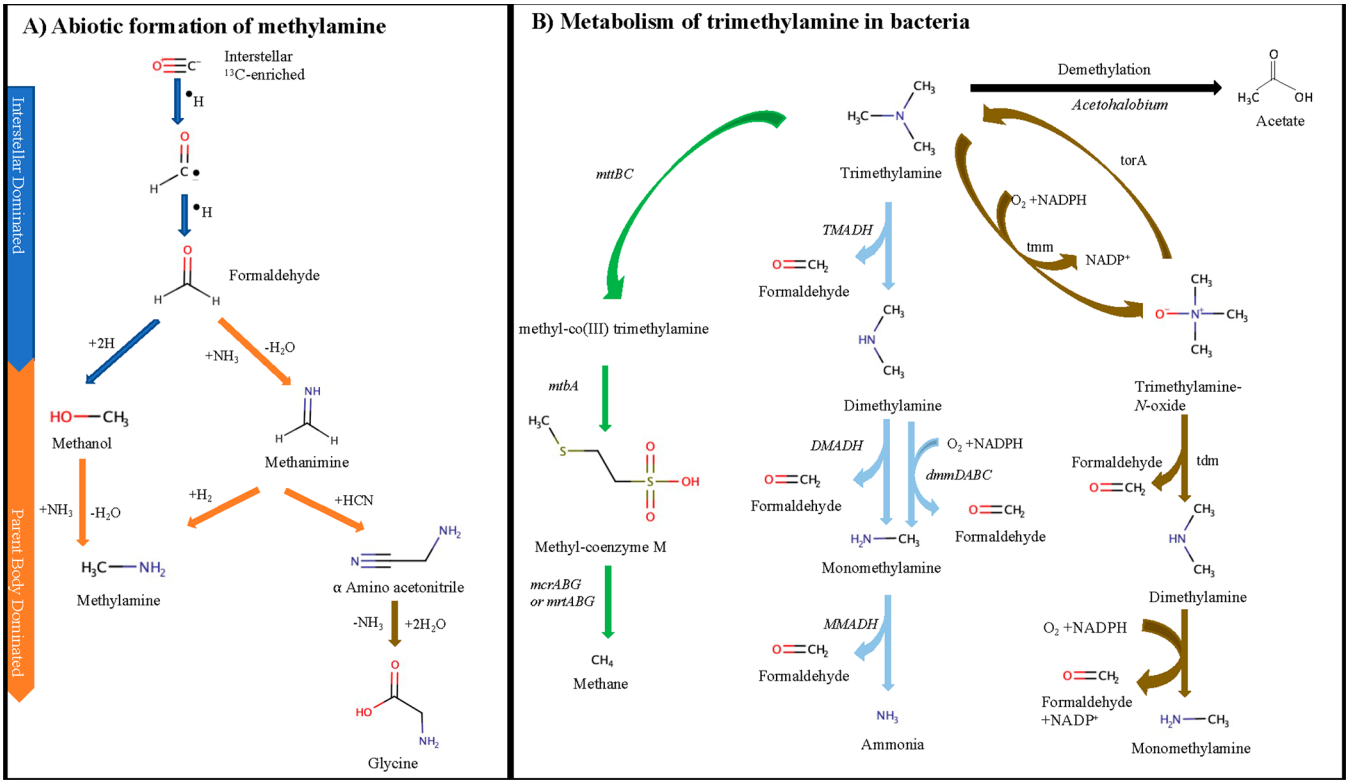

Figure 1. (A) Overview of an abiotic formation of methylamine and (B) the four main microbial pathways of TMA metabolism including methanogenesis (green), anaerobic TMA dehydrogenase (pale blue), and aerobic TMA oxidation (brown) and acetogenesis (black). Key: mttBC, TMA methyltransferance; mtbA, CoM methyltransferase; mcrABC or mrtABC, methyl-CoM reductase; TMADH, trimethylamine dehydrogenase; DMADH, dimethylamine dehydrogenase; MMADH, monomethylamine dehydrogenase; NADPB, nicotinamide adenine dinucleotide phosphate; tmm, TMA monooxygenase; torA, TMAO reductase; tdm, TMAO demethylase; dmmDABC, DMA monooxygenase.

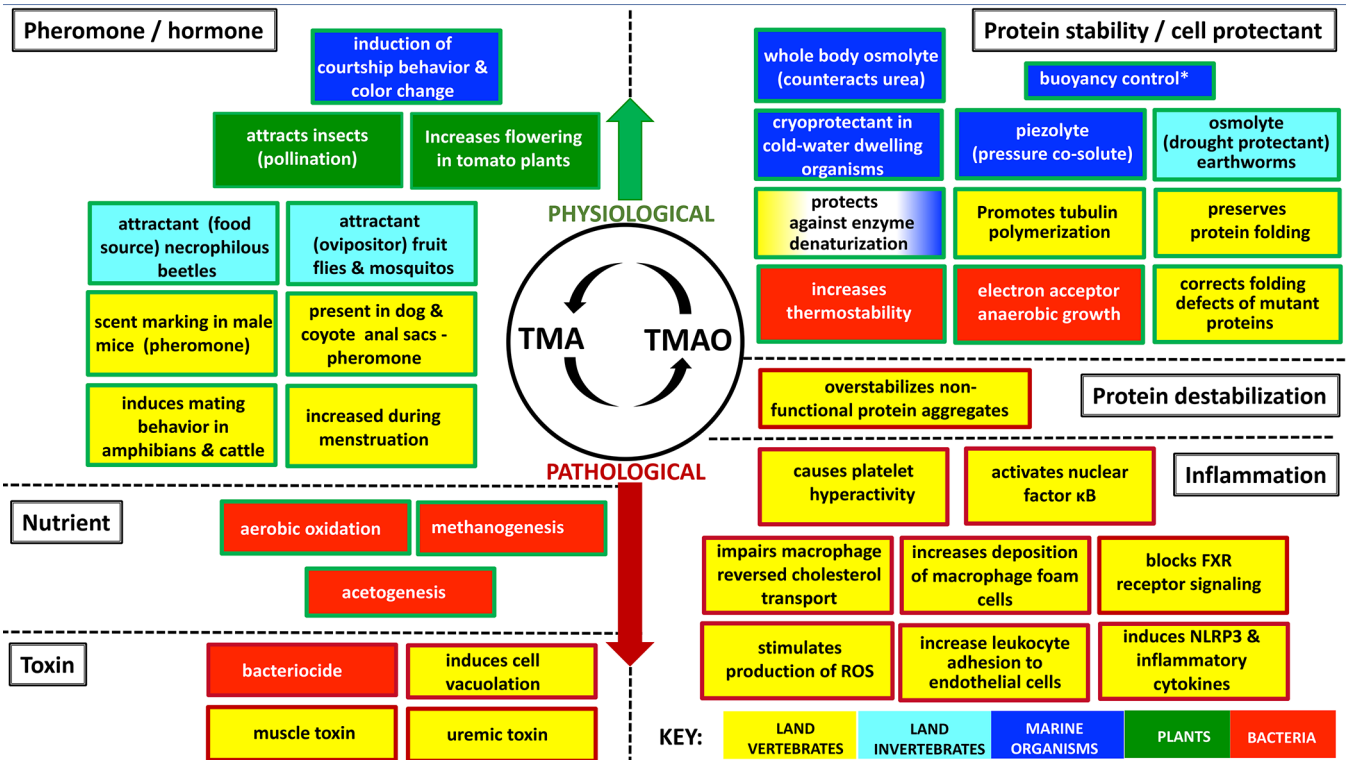

Figure 2. Summary of exemplar biological functions of TMA and TMAO on various prokaryotic and eukaryotic organisms. Key: * it is dependent on physiocohhemical properties of body fluids rather than a protein stability; green edge indicates beneficial effects; red edge indicates adverse biological effects.

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Table 1. Summary of Different Analytical Methods Used to Analyze TMAO and TMA Together with Their Intra- and Interday Precision[c]

method	specimen type	analytes	intraday precision CV (%)[a]	interday precision CV (%)[a]	run time/sample (min)	method applied to measure concentrations in	TMAO range[b]	TMA range[b]	stability[a]
^{1}H NMR[27]	serum and plasma	TMAO (plus 20 other endogenous and exogenous compounds)	4.3−10.3	9.8−14.5	5.5	153 healthy fasting donors (validated against MS platforms)	3.3−21.1 μM (2.5−97.5 percentile); 3.3−72.2 μM (full range)	NA	stable up to 15 days at room temperature, refrigerated (2−8 °C), frozen (at −25 °C to −10 °C and −80 °C), and up to three freeze−thaw cycles.
^{1}H NMR[28]	urine	TMAO (plus 14 other compounds)	NA	NA	NA	NA	54−73 μmol/mmol creatinine		stable up to 40 days at −80 °C
^{1}H NMR[29]	urine	TMAO and TMA	NA	NA	NA	five healthy volunteers and a patient with TMAU and his family (N = 8)	118.66 ± 83.18 μmol/mmol creatinine; 15.72 μmol/mmol creatinine (TMAU patient)	7.7 ± 7.4 μmol/mmol creatinine; 21.94 μmol/mmol creatinine (TMAU patient)	NA
^{1}H NMR[30]	urine	TMAO, glycine betaine, proline betaine, trigonelline, dimethylglycine, TMA, creatinine	2.5−4.3	2.0−8.5	<5	two suspected cases of TMAU and a control patient (after 600 g of fish intake)	236.05 μM; 603.8−1102.27 μM (suspected TMAU)	2.09 μM; 30.01−56.22 μM (suspected TMAU)	NA
direct infusion ESI Q-Tof-MS[31]	urine	TMAO and TMA	NA	NA	2	40 individuals	NA	NA	NA
FAB-MS[32]	urine	TMAO and TMA	NA	NA	NA	20 healthy volunteers (10 Caucasian and 10 Asian from North America) and 7 patients with homozygous/heterozygous for FMO3 shown to cause TMAU	53 ± 57 μmol/mmol creatinine; 36 ± 21 μmol/mmol creatinine (TMAU)	0.7 ± 0.43 μmol/mmol creatinine; 45 ± 13 μmol/mmol creatinine (TMAU)	NA
capillary GC-MS[22]	blood, urine, liver, kidney muscle	TMAO, TMA, and dimethylamine	1.26	1.78	NA	NA	NA	NA	once derivatives were formed, they were stable at room temperature or at −20 °C for 2 weeks
flow injection ESI-MS/MS[33]	urine	TMAO and TMA	2.8−4.6	4.6−5.7	2	27 control and 5 TMAU patients	13.5−181 μmol/mmol creatinine; 0.36−607 μmol/mmol creatinine (TMAU)	0.11−1.19 μmol/mmol creatinine; 5.3−230 μmol/mmol creatinine (TMAU)	prepared samples stable for 1 week at room temperature
headspace GC-MS[34]	urine	nine aliphatic amines including TMA and TMAO	5.0 ± 0.8	7.2	7	10 individuals	10.7−60.6 mg/day; 8.3−54.8 mg/day (TMAU)	0.2−2.7 mg/day; 8.7−56.1 mg/day (TMAU)	stable at −20 °C and −70 °C for 3 months
HS-SPME-GC-MS[35]	plasma	TMAO and TMA	NA	NA	NA	10 healthy individuals	22.5−79.4 μM	0.271−0.651 μM	NA
HS-SPME-GC-MS[36]	urine	TMAO and TMA	6.1−12.2 (based on TMA)	NA	30	10 healthy adults	208−2768 μmol/L	1.4−11.0 μmol/L	NA
HS-SPME-GC-MS[37]	fecal	TMA, TMAO, short-chain fatty acids (acetic, propionic, butyric, and valeric acid)	6.55	9.94	NA	two healthy volunteers (N = 5 samples)	0.015−0.058 μmol/g	0.08−0.146 μmol/g	NA

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Table 1. continued

method	specimen type	analytes	intraday precision CV (%)[a]	interday precision CV (%)[a]	run time/sample (min)	method applied to measure concentrations in	TMAO range[b]	TMA range[b]	stability[a]
MALDI-TOF-MS[38]	urine	TMAO, TMA	0.29−2.59	0.31−2.68	NA	three healthy individuals and two patients with TMAU	22.24−28.08 μmol/mmol creatinine; 28.20−53.81 μmol/mmol creatinine (TMAU)	0.53−0.87 μmol/mmol; creatinine; 34.52−37.41 μmol/mmol creatinine (TMAU)	NA
LC-ESI-MS/MS[39]	urine	TMAO (and nine other Krebs-cycle-, purine-, and oxidative-stress-related metabolites)	1.8−3.4	5.2−6.9	5.5	120 healthy adults and 36 healthy pediatric	0.008−0.147 μmol/L/mOsm (adults); 0.096−0.575 μmol/L/mOsm (children)	NA	post-preparative samples stable at 4 °C for 48 h; samples stable at −20 °C and −80 °C for up to 6 months
LC-TQ-ESI-MS/MS[26]	urine	TMAO, TMA	1.2−1.5	2.0−2.9	4	118 healthy individuals	6.8−181.2 μmol/mmol Cr	0.209−0.977 μmol/mmol Cr	samples stable at 4 and 20 °C for 14 days, at −20 °C for 30 days, and up to five freeze−thaw cycles; post-preparative stable for 42 h at 4 °C
LC-TQ-ESI-MS/MS[40]	serum	TMAO, leucine, isoleucine, and valine)	1.1−8.2	3.9−12.0	4	10 individuals	NA	NA	NA
LC-QTRAP-ESI-MS/MS[21]	serum, plasma	TMAO	1.3−6.4	5.2−9.9	11	349 healthy volunteers	2.25−5.79 μM (IQ range)	NA	stable up to five freeze−thaw cycles
UPLC-QTRAP ESI-MS/MS[41]	serum, plasma, urine	TMAO, choline, betaine, carnitine, and acetyl-carnitine	1.9−3.6	1.8−6.8	11	13 healthy individuals	0.7−5.0 μmol/L	NA	NA
UPLC-TQ-ESI-MS/MS[25]	urine	TMAO and three other aliphatic nitrogenous analytes	2.1−5.8	4.7−11.0	5	spiked in analytes in samples	NA	NA	stable at room temperature, 4 °C, and −20 °C for 7 days; stable at −70 °C for 9 months and for up to seven freeze−thaw cycles
UPLC-Q-Exactive quadrupole-Orbitrap-MS[42]	serum	TMAO, TMA, choline, betaine, carnitine	3.0−5.0	NA	14	67 healthy individuals	0.16−17.52 μmol/L	0.29−1.66 μmol/L	TMAO stable for three freeze−thaw cycles, refrigerated for 7 days and at room temperature for 7 days
UPLC-TOF-ESI-MS/MS[43]	plasma	TMAO	1.0−11.4	1.4−20.8	2.5	43 systolic heart failure patients and 42 healthy individuals	3.1−5.11 μmol/L; 4.2−14.1 μmol/L (SHF)	NA	NA
UPLC-TQ-ESI-MS/MS[24]	plasma	TMAO, carnitine, γ-butyrobetaine	3.41−6.72	2.27−10.62	6	applied to analyze six plasma samples from healthy volunteers	1.26−3.88 μmol/L	NA	stable after three freeze−thaw cyclers, at room temperature for 4 h and at 10 °C for 20 h
UPLC-TQ-ESI-MS/MS[44]	plasma	TMAO, betaine, and choline	3.5−6.1	5.1−11.2	3	222 stroke patients	103.9−2784.5 ng/mL (IQ range)	NA	stable after three freeze−thaw cyclers, at room temperature and 4 °C for 4 h and

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Table 1. continued

method	specimen type	analytes	intraday precision CV (%)[a]	interday precision CV (%)[a]	run time/sample (min)	method applied to measure concentrations in	TMAO range[b]	TMA range[b]	stability[a]
									at −80 °C for 30 days
UPLC-TQ-ESI-MS/MS[45]	plasma, urine	TMAO, TMA, choline, betaine, creatinine	1.8−5.2	4.1−5.1	5	six volunteers fed with 0−6 egg yolks in a dietary intervention study	NA	NA	stable for up to eight freeze−thaw cycles
UPLC-TQ-ESI-MS/MS[46]	plasma, urine	TMAO, choline, and betaine	plasma: 4.7−6.7; urine: 3.8−5.8	plasma: 4.6−5.2; urine: 6.1−7.5	5	pilot study for investigating atherosclerosis in patients with kidney disease	NA	NA	post-preparative samples stable at room temperature for 4 h, and 10 °C for 24 h; samples stable up to three freeze−thaw cycles; stock solution stable for 264 days at −80 °C
UPLC-TQ-ESI-MS/MS[47]	plasma, urine	TMAO, TMA, and taurine	1.6−2.4	5.2−6.2	6	20 normal renal function and 19 renal diseases	plasma: 4.04 (SD 3.8) μmol/L, 89.0 (SD 78.0) μmol/L for renal patients; urine: 577 (SD 276) μmol/L, 475 (SD 389) μmol/L for renal patients	plasma: 0.86 (SD 30.41) μmol/L, 0.57 (SD 0.17) μmol/L for renal patients; urine: 3.4 (SD 3.4) μmol/L, 19.9 (SD 78.2) μmol/L for renal patients	plasma: stable at 4 °C for 7 days or at −70 °C for 14 months and up to three freeze−thaw cycles
UPLC-MIS ESI-MS/MS[48]	plasma, DBS	TMAO, choline, carnitine, and acetyl-carnitine	low range: 2.1−3.4; high range: 1.4−2.6	low range: 1.0−4.0; high range: 0.5−5.7	6	56 DBS only	plasma: 2.7 (IQ range 2.2−3.7) μmol/L; DBS: 1.4 (IQ range 1.0−2.3) μmol/L	NA	DBS: stable at room temperature, 4 °C, and −20 °C for up to 30 days

[a]For TMAO only. [b]For healthy volunteers unless otherwise specified. [c]DBS, dried blood spot; ESI, electrospray ionization; FAB, fast atom bombardment; FMO3, flavin-monooxygenase 3; GC, gas chromatography; ^{1}H NMR, proton nuclear magnetic resonance; HS-SPME, headspace−solid-phase microextraction; IQ, interquartile; LC, liquid chromatography; MIS, matrix-induced suppression; MS, mass spectrometry; NA, not available; Q-Tof, quadrupole time-of-flight; QTRAP, quadrupole ion trap; SHF, systolic heart failure; TOF, time-of-flight; TMA, trimethylamine; TMAO, trimethylamine *N*-oxide; TMAU, trimethylaminuria; TQ, triple quadrupole; UPLC, ultraperformance liquid chromatography.

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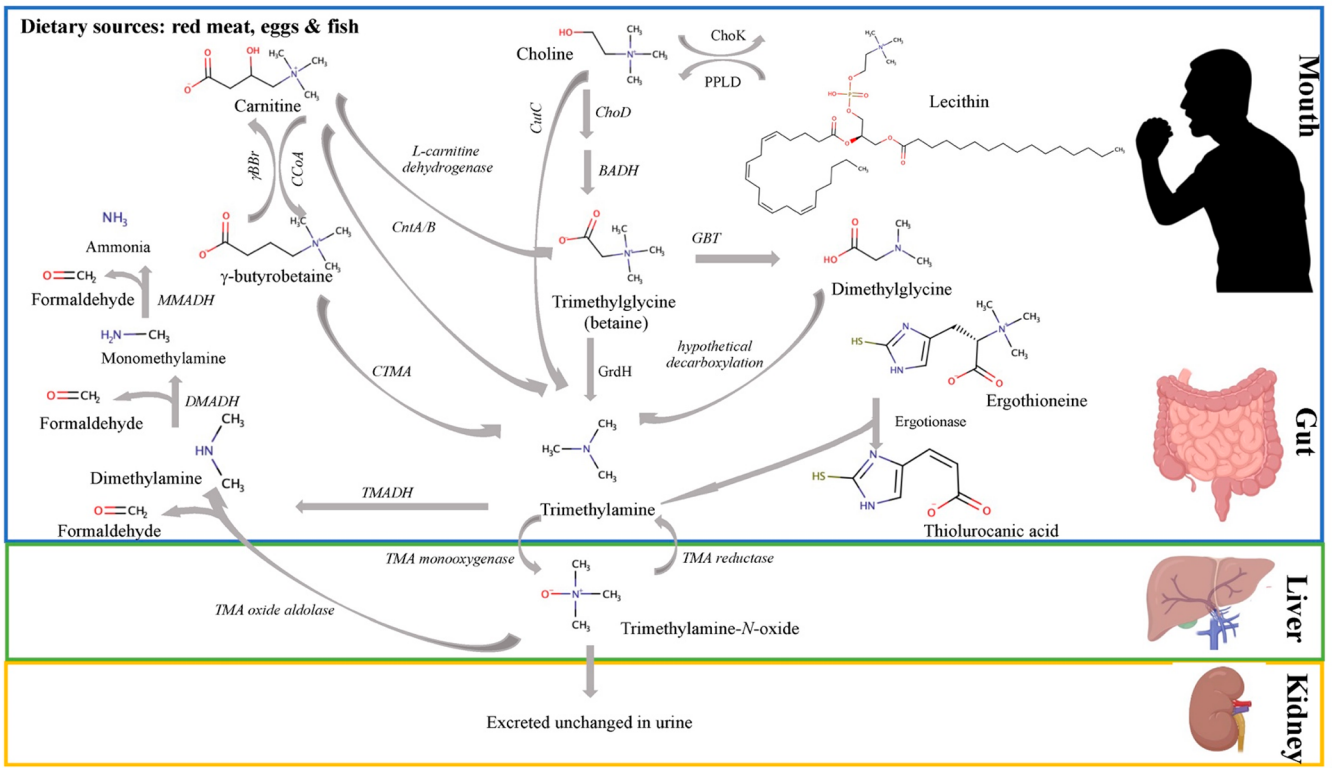

Figure 3. Major routes of metabolism of TMA by human microbiota (adapted from Fennema et al.[58]). Key: γ-butyrobetainyl CoA carnitine, CoA transferase; γBBr, γ-butyrobetaine hydroxylase; CutC, choline TMA lyase; CntA/B, carnitine monooxygenase; CTMA, carnitine TMA lyase; ChoD, choline dehydrogenase; BADH, betaine aldehyde dehydrogenase; GrdH, betaine reductase; GBT, glycine betaine transmethylase; PPLD, phospholipase D; ChoK, choline kinase; TMADH, trimethylamine dehydrogenase; MMADH, methylamine dehydrogenase; DMADH, dimethylamine dehydrogenase; CTMA, carnitine TMA lyase. The images of the gut, liver, and kidney were prepared in BioRender.com, and the image of the silhouette of a man was purchased from Getty Images.

form logP 0.54),[9] with a pK_a of 9.8, and are twice the density of air, which accounts for the lasting effect of the odor around organisms that generate TMA. TMA has a low boiling point (3 °C) and a high vapor pressure (1610 mmHg at 25 °C).[10] It is widespread in the environment because it is generated from the general decomposition of organic matter, sewage, and food waste, in particular, from the food-fishery industry.[11] The presence of TMA in human tissues and body fluids such as urine, blood, feces, bile, and semen was recognized by the early 1900s,[9] and numerous methods for measuring methylamine and its related compounds such as choline and betaine in biological specimens including urine, blood (plasma and serum), liver,[12] fecal water, brain, muscle,[13] and both raw and processed fish-meat[14,15] have been developed. Similarly, numerous methods have been devised for the measurement of TMA and other methylamines in environmental samples such as seawater,[16,17] air particulates,[18] marine sediments, and marine plankton.[19] Many assays utilize spectroscopic methods, such as nuclear magnetic resonance (NMR) spectroscopy and liquid or gas chromatography coupled to mass spectrometry (LC-MS and GC-MS respectively; Table 1 and Table S1). Recent studies have largely followed the U.S. Food and Drug Administration (FDA) guidelines on bioanalytical method validation[20] and have provided information on the limit of detection (LOD) or limit of quantification (LOQ, usually three times the LOD), linearity, and intra- and interday coefficients of variation to assess the reproducibility of the analytical measurements.

Many LC-MS methods have adopted a multiple reaction monitoring (MRM) approach to selectively focus on specific types of methylamines using a variety of chromatographic columns to optimize molecular separation, including silica,[21−23] amide,[23,24] C18,[25] and pentafluorophenyl (PFP) columns.[26] The analytical conditions for these methods, including the mobile phases and sample preparation steps, are summarized in Table S1. Several application-focused publications have required the reduction of TMAO to TMA, preventing the differentiation of these two compounds, and thus it is recommended that methodology sections are carefully read to ascertain the limitations of the method. Typically, these compounds can be measured in high-throughput mode because sample preparation is relatively simple and the analytical run times for these methods are short (<10 min), with the exception of GC-MS, which requires appropriate chemical derivatization steps. In terms of the biochemical stability, both urinary and plasma/serum TMAO concentrations have been shown to be robust to storage and to multiple freeze−thaw cycles (Table 1).

3. BIOLOGICAL ROLES OF TMA AND TMAO IN BACTERIA

TMA and TMAO serve as nutrients for numerous bacteria in marine, freshwater, and mammalian gut environments. The relationship of these methylamines and bacteria can be studied using genomic technologies to identify bacterial genes that are capable of synthesizing or metabolizing TMA/TMAO or by

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correlating changes in the concentrations of TMA/TMAO with bacterial classes or species.

3.1. TMA Metabolism in Marine and Freshwater Bacteria

In marine habitats, heterotrophic marine bacteria, in particular, those from the SAR11 and *Roseobacter* clades, are capable of generating TMA and of subsequent oxidation of TMA.[49] The SAR11 Alphaproteobacteria are the most abundant of the heterotrophs colonizing marine environments and have a relatively small genome (1 308 759 base pairs),[50] which encodes pathways for the oxidation of 1-carbon compounds and compounds with methyl groups, including methanol, formaldehyde, methylamine, betaine, TMA, TMAO, and dimethyl-sulfoniopropionate.[51] Thus TMA and TMAO are important components of marine nitrogen and carbon cycles, with both compounds being found in seawater itself.[52]

TMAO can act as a substrate for anaerobic respiration in bacteria, serving as a terminal electron acceptor of a respiratory transport chain[52] in the bacterial conversion of TMAO to TMA. The reduction of TMAO to TMA is a general feature of bacterial metabolism. It has been observed in marine bacteria but also is known to occur in freshwater (*Rhodobacter* sp.) and in most enterobacteria in the mammalian gut.[53,54] The marine bacteria *Shewanella*, which are a Gamma Proteobacteria class that have been found in West Pacific deep sea sediment, have been reported to use TMAO, nitrate, and dimethylsulfate as electron acceptors during anaerobic growth.[51]

Metagenomic analysis of marine sediments has found genes encoding three main enzymes involved in the production of TMA from TMAO, including choline-TMA lyase (CutC; prevalent in anaerobic marine sediments), TMAO reductase (TorA; abundant in marine sediments and open oceans), and glycine betaine reductase (GrdH; present in low abundance).[51] The four pathways for the microbial metabolism of TMA include acetogenesis, methanogenesis, dehydrogenation, and aerobic oxidation, and thus in marine environments, the main products of TMA metabolism are acetate, methane, ammonia, and formaldeyde (Figure 1B), examples of both marine and mammalian bacteria associated with the metabolism of TMA and TMAO are summarized in Table S2.

Bacterial enzymes are adaptive to their environment. For example, increased hydrostatic pressure causes an increase in TMAO reductase activity in marine bacteria, which may be consistent with a strategy for adaptation to deep sea environments that have relatively higher concentrations of TMAO.[52] Lidbury et al. showed that in some marine bacteria, TMAO-specific transporters were necessary for uptake into cells and identified a unique adenosine triphosphate (ATP)-binding cassette transporter (TmoXWV).[52]

3.2. TMA and TMAO Metabolism in Mammals

In mammals, the dietary conversion of choline and carnitine to TMA is dependent on the gut bacteria. The bacterial genes and species associated with TMA production in the mammalian gut have been extensively reviewed by Falony et al.[55] and share many similarities with the TMA-producing bacteria in marine and other aquatic systems (Figure 1). In mammals, gut bacterial genes that are capable of generating TMA include CutC, carnitine monooxygenase (CntA/B), and GrdH (Figure 3; Table S2). Similar to marine environments, the TMAO reductase pathway is the most prevalent in the human gut, with Proteobacteria (mainly from the *Klebsiella* and *Escherichia* genera) contributing to the majority of the TMAO reductase sequences.[56] In contrast, CutC and GrdH demonstrate a stronger correlation with Firmicutes.[57]

Certain members of the *Enterobacteriaceae* family (phylum Proteobacteria) are also capable of retroconversion of TMAO to TMA, which precedes reuptake and transformation to TMAO and has also been shown to be a major contributor to circulating TMAO.[59] In vitro fermentation studies from fecal batches demonstrated a correlation between TMA and bifidobacteria, Clostridia (sensu stricto), and coriobacteria, whereas the addition of TMAO to the system caused an increased growth of *Enterobacteriaceae*.[59]

In humans, the capacity for TMA and TMAO generation has been shown to be higher in carnivores and omnivores than in vegetarians, which is consistent with the observation that bacterial enzymes required for TMA production are absent (CntA/B) or present in reduced (CutC) levels in herbivores together with lower circulating levels of TMAO compared with omnivores.[57] Supplementation of the diet with carnitine results in increased plasma concentrations of TMAO, but this effect can be reversed by antibiotic administration, further evidence of the critical role of the microbiome in the metabolism of carnitine to TMA and TMAO.[60] This association is further supported by the extensive work published in animal models. Tang et al. have shown that the capacity to generate TMAO is absent from both germ-free and antibiotic-suppressed rodents.[61,62] On exposure to the laboratory environment, urinary concentrations of TMAO increase, reaching a steady state at around 21 days in germ free rats.[63] In conventional mice, carnitine supplementation increases circulating TMA and TMAO and has been shown to result in increased fecal abundance of *Anaeroplasma*, *Prevotella*, and *Mucispirillum*,[64] whereas supplementation with γ-butyrobetaine, a precursor of TMA, is associated with an increased abundance of *Staphylococcus* spp., *Akkermansia*, *Parasutterella*, *Prevotella*, and *Bacteroides*.[55,65] The relationship of the microbiome and TMA and, consequently, TMAO production are further explored in the following sections, contrasting the function of TMAO as a cellular protectant versus its potential role in relation to the development of cardiometabolic and other chronic diseases.

4. TMAO AS AN OSMOLYTE AND CELLULAR PROTECTANT IN MARINE AND TERRESTRIAL ENVIRONMENTS

All organisms have developed strategies for coping with osmotic stress resulting from changes in the solute concentration surrounding cells. Fluctuations in the salinity (marine environments), the accumulation of cellular waste products (mammalian kidneys), high temperatures (evaporation and desiccation), and extracellular freezing all result in a net efflux of water from the cell.[66] Osmolytes are molecules, which accumulate in cells that counteract the effects of osmotic stress. The accumulation of inorganic ions such potassium or destabilizing solutes such as urea can regulate the aqueous environment but also potentially damage proteins within the cellular environment. Examples of destabilizing osmolytes include urea, ethanol, and arginine,[67] which interact with the protein surface to lower the free energy and favor the unfolded state. Urea can directly interact with polarized groups such as peptides on the protein backbone, which weakens intermolecular bonds on the protein surface and disrupts the secondary and tertiary structures. It also acts indirectly with the solvent environment and can alter the structure of hydrophobic

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molecular groups and the dynamics of water to increase solvation.[68] Most organisms regulate osmotic pressure by the accumulation of "compatible" osmolytes, which are small organic molecules, typically uncharged or zwitterionic, that predominantly interact with the tertiary folding structure of proteins to stabilize them, usually by hydrogen bonding.[69]

Examples of stabilizing or protective osmolytes include sugars, polyols (sorbitol and inositol), amino acids (e.g., glycine and proline), and methylamines (e.g., TMAO and betaine), all of which act as cellular protectants.[70] Stabilizing osmolytes favor the folded state of cellular proteins by raising the free energy of the unfolded state. They are widely used in organisms to counteract the effects of destabilizing osmolytes such as urea and to protect against dehydration, high salinity, and extreme temperatures.[71,72] For example, one of the main adaptive strategies of marine molluscs, crustaceans, and fish is the utilization of relatively nontoxic organic osmolytes such as TMAO (derived from direct assimilation from marine sediments or degradation of dietary choline)[73] to regulate the cell volume under the high osmotic pressure of salt water.[66,74] Yancey et al. demonstrated that TMAO was a better protein stabilizer than other osmolytes in marine organisms, including glycine betaine and glycerol, and the optimal biological ratio of urea to nonperturbing osmolytes such as TMAO has been empirically shown to be 2:1.[5] In some marine organisms, such as crustaceans and certain elasmobranchs, TMAO is the main osmolyte and can be present in concentrations of up to 0.2 M.[75] In mammals and other organisms, methylamines serve a similar purpose as a nondeleterious osmolyte and cellular protectants and are accumulated in the renal cortex and medulla to prevent dehydration and to counteract the effects of urea and other toxins that are accumulated as urine passes through the loops of Henle in the renal nephrons.[76,77] Whereas TMAO does not appear to be accumulated in significant proportions in the renal medulla, it has been proposed as a renal cellular protectant because it has been shown to reverse the accumulation of ninhydrin-positive substances caused by hyperosmotic NaCl and urea,[78] and the administration of renal toxins such as bromoethanamine and propyleneimine to rodent models results in an accumulation of TMAO in the renal cortex and a corresponding decrease in the urinary excretion of TMAO.[76]

The mechanisms by which TMAO protects against the destabilizing effects of urea have been studied by a range of technologies such as infrared spectrometry, neutron scattering measurements, X-ray Raman scattering and molecular dynamics simulations.[79−81] To an extent, the mechanisms by which TMAO stabilizes the protein structure remain under debate, but the main mechanisms proposed involve:

(i) Reduction of the strength of protein−water hydrogen bonds: At physiological pH, TMAO is extremely hydrophilic due to the presence of the strong dipole moment (N^+-O^- group) and preferentially hydrogen bonds with water, with each TMAO oxygen atom interacting with two/three water molecules. This increases the strength of the water hydrogen-bonding network and can attenuate the strength of the hydrogen bonds that form between the water and the polar protein groups, resulting in the preferential exclusion of the TMAO complex from the protein surface.[82,83] This increased hydrogen bonding water structure, termed "iceberg water", dehydrates the carbonyl functional groups of the protein backbone, making the unfolded protein structure less favorable.[84] In this sense, TMAO acts as a cosolvent, a molecule that interacts with water by increasing the miscibility between water and cellular proteins without having to interact directly with the protein. Additionally, molecular simulation and solubility experiments at various pressures showed that TMAO indirectly contributes to stabilizing internal hydrogen bonds in proteins via weakening water hydrogen bonds with the protein backbone.[85]

(ii) Molecular crowding: TMAO is repelled from the protein surface, as evidenced by the identification of molecular crowding of the water molecules around the protein surface, and the contribution to protein stabilization is assumed to be achieved via its effect on the water structure surrounding the protein.[86] A recent molecular dynamics study has shown that TMAO increases the fluid/gel transition temperature in lipid membranes and enhances the orientational order of lipid molecules, resulting in greater packing density.[87]

(iii) Modification of peptide−urea solvation properties: The preferential interaction of urea with the protein backbone causes the unfolding of the protein. TMAO has been shown to directly interact with the amine groups in urea, thereby reducing the capacity for urea to interact with cellular proteins.[88] Experimentally, TMAO has been shown to increase the melting temperature and enhance the thermal stability[70] and unfolding free energy of proteins. Thus TMAO can stabilize the folded state of proteins or reverse misfolded or denatured proteins such as lactate dehydrogenase.[70]

In contrast with these proposed mechanisms of stabilization, using X-ray Raman scattering spectroscopy, Liao et al. showed that other osmolytes had a stronger influence on the water structure than TMAO.[7] Thus they proposed that the stabilization mechanism could result from TMAO acting as a surfactant at the interface of polar and nonpolar regions of folded protein surfaces rather than molecular crowding or hydrogen bonding.

In its role as a cellular protectant, TMAO contributes in multiple ways. In marine environments, TMAO also protects proteins against denaturation from temperature and pressure, acting as a piezolyte.[89,90] For example, TMAO concentrations in the muscles and plasma of teleost fish have been shown to increase linearly with the habitat depth,[74] suggesting accumulation in response to increasing pressure. Functionally, this increase in TMAO concentration can serve to reduce the hydrostatic-pressure-induced loss of activity of certain enzymes such as lactate dehydrogenase and pyruvate kinase, as supported by evidence from in vitro experiments showing that 250 mmol/L TMAO can reduce the impact of hydrostatic pressure on lactate dehydrogenase.[91] The behavior of osmolytes in the mammalian kidney are analogous to that found in marine organisms with TMAO functioning as a protein stabilizer. When the kidney is under osmotic stress due to dehydration and high levels of urea and other toxicants, an accumulation of medullary TMAO occurs[77] and can slow down the dissociation of enzymes such as lactate dehydrogenase under high osmotic pressure and prevent urea-induced inactivation.[92] In contrast, Rani et al. have shown that TMAO can act as a cellular disruptant. In scenarios where TMAO concentrations are high or there are fewer available water molecules, TMAO can interact directly with the exposed protein surface or even enter the hydrophobic pockets, which decreases the free energy of the unfolded protein state, shifting the balance toward denaturization.[93]

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Another example of TMAO functioning as a cellular protectant is its accumulation in renal tissue in animals exposed to renal toxins, implying that it may accumulate in tissues as a response to the toxin-induced cellular stress.[94] In the plant kingdom, TMAO operates as a protective osmolyte in plants and as an activator of abiotic-stress-induced gene expression,[95] resulting in increased tolerance of drought, high-salinity, and low-temperature conditions. TMAO has also been shown to promote the polymerization of microtubules, which are necessary structures for mitosis and intracellular transport in eukaryotes and are composed of helical polymers consisting of tubulin bound to additional proteins. TMAO, but not other methylamines, can also protect against the depolymerization of microtubules caused by urea and cold stress.[96] As discussed, TMAO does not directly interact with the protein but causes an increase in hydrogen bonding, resulting in preferential hydration of the protein that minimizes the exposed protein surface area.[84] However, given the high concentrations of TMAO in the kidney, the counteracting effect against the urea-induced destabilization of tubulin is not fully accounted for by TMAO alone and is likely to be a composite effect involving other nonperturbing osmolytes.

Although the primary role of TMAO appears to be as a cellular protectant, several species appear to have developed more specific uses (Figure 2). For example, apart from osmotic control, some marine organisms are thought to use TMAO as a mechanism of buoyancy control. In terms of the contribution of TMAO and other methylamines to buoyancy control, Withers et al. calculated the solute composition of plasma and muscles using molecular weights and partial molar volumes. TMAO contributes a positive lift of 1.7 g/L and is considerably less dense than equimolar solutions of most other solutes (e.g., Na^+ and K^+).[97] Another particularly niche function is found in the hagfish, which, when threatened, expels a large volume of slime, formed from interwoven and colorful skeins of mucus, that clogs the gills of its predators. These mucus skeins are prevented from unravelling via an "adhesive" that contains high levels of methylamines including TMAO, dimethylglycine, and betaine, which, as discussed, can prevent the destabilization of proteins.[98]

5. TMAO AS A CELLULAR DISRUPTANT

Although protein stabilization by osmolytes such TMAO generally acts to protect cells and tissues, as Yancey has demonstrated, protein stabilization in the absence of a cellular disruptant may confer disadvantages;[99] therefore, the term "chemical chaperone" or "counteracting solute" is a more accurate description than "compatible solute". Whereas the TMAO-enhanced stabilization of proteins and ligand binding has been shown to counteract the effect of urea in multiple cell and tissue systems across the animal kingdom, it can overstabilize nonfunctional protein aggregates by promoting their formation, for example, β-amyloid.[100] Other research groups have shown that TMAO is capable of both enzyme activation and inhibition and that it does not always counteract the effect of urea.[101] For example, whereas urea inhibits alcohol dehydrogenase, lactate dehydrogenase, xanthine oxidase, and catalase, TMAO activates alcohol dehydrogenase and lactate dehydrogenase, weakly inhibits xanthine oxidase, and increases the thermal stability of catalase. This strongly suggests that TMAO does not always counteract the effects of urea. Mashino et al. suggested that TMAO inhibits enzymes in cases where it selectively stabilizes the ground state of the catalytic cycle and activates enzymes in cases where it selectively stabilizes the transition state of the catalytic cycle.[101] Whether TMAO acts to stabilize or destabilize proteins is partially dependent on the pH of the chemical environment. For some proteins such as prions, lysozyme, ribonuclease A, and apo-α-lactalbumin, TMAO improves the thermal stability above its pK_a but acts as a destabilizer at pH values below its pK_a.[102] Another factor that may determine whether TMAO serves to stabilize proteins is the kinetics of protein folding. Kumari et al. suggested that restoration of folding of mutant proteins occurs in fast-folding proteins such as the prion protein[103] but that for slow-folding proteins such as carbonic anhydrase, horseradish peroxidase, and choline acetyltransferase, where proline residues are prominent, TMAO can act to stabilize the intermediate forms and decrease the rate of formation of the fully folded protein. Further experimental work showed that the slow refolding stages of carbonic anhydrase were due to TMAO inhibition of cis−trans isomerization of the proline residues and that this effect was specific to TMAO and was not observed for other osmolytes such as sorbitol or betaine. Treatment of HeLa cells with TMAO resulted in a 2−7% decline in viable cells and in an increase in the population of cells in the S phase of the cell cycle (from 11.25 to 20.01%), suggesting that TMAO can arrest the cell cycle at the synthesis phase.[103]

Thus the interaction of TMAO with proteins has been variously reported to have both positive and negative effects on physiological functions. When assessing the literature, it must be borne in mind that much of the in vitro experimental work does not establish an experimental system within the normal physiological range for concentration, pH, or temperature and should be interpreted with caution.

6. SIGNALING FUNCTIONS OF TMA

The broad function of TMAO can be broadly classified as protein stabilization, whereas the role of TMA can be predominantly considered as chemical signaling. TMA has been found to serve as either a chemical attractant or repellent in multiple animal and plant species including slugs, cockroaches, mice, flies, water fleas, ducks, fish, and snakes.[9,104] However, different species have evolved different functionalities such as the detection of pheromones, attraction to rotting food sources, or avoidance of predators. For example, although trace amine receptors, which are part of the olfactory system and induce an innate animal behavioral response, are conserved across mammals. TMA is a repellent in humans and rats but is a chemical attractant in mice[105] and in various flies, mosquitoes,[106] and fish species,[107] whereas some plants use TMA as a deterrent to herbivory.[108] TMA is also accumulated in the reproductive structures of various plants, animals, and even certain fungi[2,109] and has a profound influence on hormonal and behavioral patterns in animals, inducing courtship behavior in some amphibians and fish.[109,110] Indeed sexually dimorphic urinary TMA excretion in mice has evolved as part of their behavioral signaling pattern,[111] which may partially explain the higher concentration of TMA found in mouse urine in comparison with rats.[112] TMA also serves as a chemical attractant in other mammalian species such as cattle, where its presence in the vaginal fluid of cows during estrus has been shown to induce mating behavior in bulls.[113] In contrast, mice and some aquatic organisms such as the fathead minnow also secrete TMA as a territorial signaling molecule.[110] TMA

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Table 2. Summary of the Effects of TMAO and TMA in Normal Physiology and Different Pathologies

physiological or pathological condition TMAO	number of studies	biological matrix	summary for TMAO and TMA	refs
healthy volunteers	9	urine	healthy individuals metabolized TMA to TMAO, and saturation of metabolism of TMA was observed; contradictory results on gender differences for excretion of TMAO	26, 28, 33, 122, 124−128
	1	feces	fecal content of short-chain fatty acid, TMA, and TMAO was established for two healthy volunteers	37
	1	vaginal secretion	TMA in vaginal fluid is relatively stable for normal women, primiparae, secundiparre, and those in parturition; at climaterium or postmenopausal, TMA is either reduced or absent	129
trimethylaminuria (TMAU, fish odor syndrome)	6	urine	in TMAU, urinary excretion of TMAO is reduced and TMA is enhanced under normal dietary conditions; heterozygotes are similar to control, but ingestion of TMA at a dose of 600 mg results in an increase in the urinary TMA/TMAO ratio	26, 33, 127, 128, 130, 131
cardiovascular disease (CVD)	13	plasma, serum, and urine	high TMAO concentrations are associated with major adverse cardiovascular events, and all cause mortality; circulating TMAO is directly associated with inflammation; contradictory results observed in carotid intima-media thickness, with one study showing no association and another study showing positive association	126, 132−143
bariatric surgery	6	plasma, serum, and feces	increased serum/plasma TMAO concentrations post-Roux-en-Y gastric bypass surgery but not for other type of bariatric surgical procedures; fecal TMAO was reduced 4 years postsurgery in one study	144−149
adiposity and obesity	5	plasma, serum, and urine	contradictory results on the TMAO relationship with obesity/body mass index (BMI): one study showed a positive association of TMAO with visceral fat; positive association between TMA and BMI reported in two studies	150−154
diabetes mellitus (DM)	11	plasma, serum, and urine	increased serum/plasma TMAO in DM and insulin resistance and levels associated with increase all cause mortality, including cardiovascular events	155−163
gestational diabetes mellitus (GDM)	3	plasma and serum	contradictory results reported with one study showed an inverse association between TMAO concentrations and GDM, but another study showed a direct association	164, 165
kidney diseases	13	plasma, serum, and urine	increasing TMAO concentration with advancing chronic kidney disease (CKD) stage but was not replicated in a study involving children; functions as an independent predictor for mortality and secondary diseases, e.g., CVD; post-kidney-transplant, the level of TMAO decreased to normal range; concentration of TMA, however, is not affected by CKD; renal toxicity with chemotherapy also increased the level of TMAO	132, 166−173
cancer	15	plasma, serum, tissue, and urine	contradictory results regarding the association of TMAO and cancer; in general, higher TMAO in cancer but a few smaller-scale studies ($N < 100$) showed lower TMAO with breast cancer, pancreatic cancer, and hepatocellular cancer	174−188
liver disease	2	plasma and serum	lower TMAO concentrations in liver disorders may be explained by impaired liver metabolism of TMA to TMAO	150, 189
neurological disorders	6	cerebrospinal fluid, urine, and plasma	contradictory results regarding the influence of TMAO and Alzheimer's disease; higher TMAO was observed for autism spectrum disorders, Parkinson's disease, and bipolar depression disorders	190−195
miscellaneous disorders	10	follicular fluid, plasma, serum, and urine	high TMAO levels in female-specific disorders, e.g., polycystic ovary syndrome and pre-eclampsia treatment; metabolic syndrome, thrombosis risk, stroke, and inflammatory markers were found to show higher levels of TMAO, but low levels were found in irritable bowel disease	150, 196−208

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secretions in snakes are thought to serve an antipredatory function.[114]

TMA is both water- and fat-soluble and can thus be rapidly dispersed through the body. Additionally, it passes through the skin, and once it is exposed to air at the skin surface, it has a vapor pressure of 2.86 atm, allowing it to volatilize easily.[9] However, because TMA is twice as dense as air, it does not diffuse quickly, and it is therefore associated with the organism excreting it for longer than most other compounds, making it a suitable chemical messenger.

The attraction to TMA in mice is dependent on trace amine-associated receptor 5 (TAAR5), and the response is not present in TAAR5 knockout mice.[115] TAAR5 expression occurs in multiple sections of the brain including the hippocampus, amygdala, anterior olfactory nucleus, thalamus, and piriform cortex, pointing to functionality beyond simple odor sensing and regulation of emotional behaviors.[116] Espinoza et al. showed that TAAR5 knockout mice exhibited lower anxiety than wild-type mice and that the serotonin metabolism was altered with lower 5-hydroxytryptamine and 5-hydroxyindoleacetic acid levels in the hippocampus.[116]

The clear physiological roles of TMAO as a cellular protectant and TMA as a chemical messenger are often overlooked or downplayed in the investigation of their contribution to cardiovascular disease (CVD) and other pathologies. A holistic picture of the contribution of diet and variation of circulating concentrations in relation to normal physiological levels and rhythms should be taken into account to understand the true impact of TMAO on increased CVD risk and other pathologies

7. NATURAL VARIATION AND BIOLOGICAL RHYTHMS OF TMA AND TMAO

Biofluid and tissue concentrations and fluctuations of TMA and TMAO in humans are influenced by age, sex, hormonal status, circadian rhythm, diet, and the composition of the gut microbiome. Studies reporting physiological and pathological conditions that affect TMA or TMAO levels are summarized in Table 2 and Table S3. In healthy adults, the urinary concentrations of TMA and TMAO vary, with most studies reporting urinary concentrations of TMAO (range 6.8−181.2 μmol/mmol creatinine) to be many times higher than those of TMA (range 0.21 to 0.98 μmol/mmol creatinine),[26] with a further substantial increase in the TMAO/TMA ratio after a fish meal. In terms of the temporal stability of urinary TMA and TMAO, Li et al. found no statistically significant day-to-day variation over a 30 day period when samples were stored at −20 °C and also demonstrated a minimal impact of five freeze−thaw cycles.[26] Fasting plasma levels of TMAO for a healthy Western population from Europe and America were found to be in the range of 3.4 to 4.5 mM[21,117,118] and were relatively stable over a 12 month period, independent of diet, lifestyle, or routine biochemistry analysis, including blood lipid measurements.[117] Despite the interest in the association between the microbiome and TMAO, information on the normal range of fecal TMA and TMAO concentrations is lacking. Fiori et al. reported fecal concentrations of 0.080 to 0.146 μmol/g for TMA and 0.015 to 0.058 μmol/g for TMAO in two healthy individuals.[37]

7.1. Age

The evolutionary codevelopment of humans and their gut microbiome is apparent in the relationship with TMA/TMAO synthesis and the variation in the levels of these chemicals with age through life. The production of TMA and, consequently, TMAO is dependent on the microbial conversion of dietary choline, and numerous studies have shown a depletion of TMAO in the urine or plasma following antibiotic administration.[119] Nevertheless, TMAO is excreted in infant urine and decreases over the first 3 or 4 years of life,[120] which would suggest that the early infant microbiome is adapted to process the relatively high choline content in breast milk. One study showed that the mean ratios of TMAO, taurine, and myo-inositol to creatinine were 14.1, 4.3, and 10.1 times higher, respectively, in preterm compared with full-term infants, which may suggest that TMAO serves as an osmolyte to balance the incomplete physiological development of the nephrons.[121]

In adults, aging is associated with an increase in urinary TMAO. In a study in healthy men and women, the mean concentration of TMAO and dimethylamine (DMA) in the <45 years of age group was 62.5 ± 59.0 mmol/mol creatinine and 34.0 ± 6.5 mmol/mol creatinine, respectively, compared with 81.5 ± 66.8 mmol/mol creatinine and 40.5 ± 10.6 mmol/mol creatinine in the >45 years age group.[122] Because there is no age-associated difference in FMO3 abundance,[123] the association between age and increased excretion of TMAO and DMA more likely reflects the inefficiency of renal osmolyte systems associated with aging[122] or alternatively may reflect age-related changes in the microbiome and the consequent production of TMA.

7.2. Sex and Hormonal Influence

Distinctive sex-related differences in both TMA and TMAO urinary concentrations are found for multiple animal species. In vertebrates, TMA is found in higher concentrations in the urine of males. One possible explanation for this is that it acts as a pheromone.[209] Conversely, TMAO concentrations have been consistently found to be higher in females in rats,[210] mice,[211,212] and humans.[126] These observations are in keeping with the fact that hepatic concentrations of FMO3, which converts TMA to TMAO, are modestly higher in women.[123] Conversely, two studies found no significant sex differences in TMAO excretion.[122,125] The contribution of hormones to observed sex differences in TMA and TMAO concentrations is unclear but in humans, TMAO levels have been found to be higher during menstruation and menopause,[213,214] and in female rats higher levels are present in the diestrus phase of the estrus cycle.[215] Conversely, in men, testosterone is associated with the downregulation of FMO3 expression,[216] which may account for the higher ratio of TMA to TMAO in men.

A further indication of hormonal involvement in TMA/TMAO levels is the increase in both circulating and urinary levels of TMAO and TMA during pregnancy,[217] with increased circulating TMAO being associated with pre-eclampsia[202] and correlated with systemic inflammation, endothelial dysfunction, and increased serum lipopolysaccharide levels, consistent with altered microbiome or intestinal barrier integrity.[203] The relationship between plasma TMAO and gestational diabetes (GDM) is more controversial, with a study reporting a direct association of TMAO with GDM[165] and another finding an inverse association.[164] In contrast, concentration of circulating TMA has been found to be associated with adverse pregnancy events including risk of GDM[164] and fetal growth restriction.[218] Although other studies have failed to find any association between TMA and fetal growth,[219] the relationship is more clear in animal models where the administration of

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0.75 mM TMA to pregnant mice caused inhibition of embryo growth by ~30% together with neural tube defects in 73% of embryos.[220] When added to embryos in culture, ethylamines, in general, have a toxic effect in terms of embryo survival, size, and DNA and RNA content, with the order of toxicity descending from TMA to DMA to monomethylamine (MMA).[221]

7.3. Ethnicity

Whereas population differences in biofluid TMA and TMAO concentrations have been reported, most of these differences are largely explained by dietary behaviors rather than ethnicity per se. For example, the higher urinary concentrations of TMAO in Swedish[222] and Japanese[223] populations compared with a British population are mostly related to the higher fish consumption in those populations.

7.4. Diurnal Rhythms

Some evidence of diurnal rhythm has been found for TMA and TMAO excretion. In a mouse model, samples collected in the morning contained higher levels of TMA but lower levels of TMAO relative to samples collected in the afternoon or evening.[224,225] In humans, the circulating TMAO concentrations were found to be higher in the evening chronotype than the morning chronotype.[226] Sleep deprivation has also been associated with increased urinary excretion of TMAO and other microbial metabolites.[227]

8. DIETARY ASSOCIATIONS WITH TMAO CONCENTRATION

The main dietary precursors of TMA and, consequently TMAO, are choline and carnitine, with trimethyllysine and ergothionine making minor contributions to the dietary TMA pool (Figure 2). Both urine and plasma concentrations of methylamines reflect dietary intake with consumption of fish, red meat, eggs, dairy products, soy, legumes, and some green leafy vegetables increasing the concentration of TMAO. Many of these dietary associations have been extensively summarized in recent reviews[228,229] and hence are only briefly covered here, with the main observations summarized in Table 3 and Table S4. However, in considering the impact of diet on circulating and excreted TMAO levels, it should be borne in mind that comparisons across diet studies can be difficult due to the lack of standardization of TMAO arising from other dietary sources or specification as to which type of fish was consumed and the time of sampling. The interindividual variation in terms of circulating and excreted TMAO levels following dietary challenges can be as large as 10-fold, which is suggestive of variation in the individual's gut microbiome composition in addition to kidney function[167,230] and genetic polymorphisms in the FMO3 enzyme.[231]

The consumption of marine fish has the strongest dietary effect on urine and plasma TMA and TMAO concentrations and is associated with the amount of TMAO per gram of fish ingested and the species and environment of the fish.[1,24,25,27,35] The fold increase in TMAO reported post-ingestion ranges from 4.72- to 50-fold for TMAO, with a similar range in TMA.[239] An increase in the plasma methylamines (TMAO, TMA, and DMA) occurs within 15 min of consumption, peaks within 2 h, and remains elevated for at least 6 h post-ingestion.[232] The second largest dietary component contributing to urinary and circulating TMAO levels is meat products, in particular, red meat such as beef, which is rich in carnitine.[240] Wang et al. showed that fasting urinary and plasma TMAO concentrations were elevated after a 4-week period of consumption of a diet containing red meat, whereas isocaloric diets based on white meat or vegetable protein had no effect on TMAO levels, which is consistent with the association of circulating TMA and TMAO with increased risk of CVD.[242] Direct supplementation of the diet with 1.5 g/day of L-carnitine for 24 weeks resulted in a 10-fold increase in plasma TMA when compared with placebo, but this was not associated with any change in inflammatory markers such as C-reactive protein, tumor necrosis factor α, or interleukin (IL) 6.[60,272]

Other lesser sources of dietary choline or carnitine include eggs[231,244] and dairy products[118,267] but in lower levels than fish and red meat. Contrasting results have been published regarding the impact of dairy products on TMA/TMAO levels. In one study, the consumption of dairy products correlated directly with plasma TMAO concentrations,[118] whereas other studies have reported lower postprandial plasma and urinary TMAO following the ingestion of dairy food products.[247,248] These studies highlight the challenge in comparing dietary studies where the methodology, study population, and sample collection are not harmonized. As with dairy products, a similar dichotomy in the literature exists for ascertaining the impact of egg consumption on TMAO concentrations, with some studies reporting an increase in both plasma and urinary TMAO concentrations[231,244,273] and other studies reporting no significant difference in urinary TMAO excretion after a six eggs/week diet versus an egg-free diet.[245] A limited number of studies have shown that some components of vegetarian diets such as resistant starch have been associated with increased circulating TMAO concentrations.[254]

The impact of whole diets on systemic and excreted TMAO levels have been assessed, and, consistent with the fish and red meat studies, high-protein diets have been shown to result in increased circulating and excreted TMAO.[274] Similarly, several studies have shown that omnivores have higher levels of urine and plasma TMAO than vegan or vegetarians.[241,275] Consensus on the impact of high-fat diets is more controversial (Table S4), with one 5 day feeding trial reporting a transient increase in postprandial circulating TMA within the first 4 h.[256] Weight-loss diets have also reported reductions in plasma TMAO,[269,276] and in one study, the reduction in serum TMAO concentrations was found to be correlated with choline TMA-lyase-encoding genera such as *Ruminococcus* spp., *Parabacteroides* spp., and *Bacteroides* spp.[277] However, without taking the choline and carnitine content of the diets into account, it is difficult to draw reliable conclusions from such studies.

9. DISEASE STATES AND DISORDERS

Blood and urine concentrations of TMAO and TMA have been directly associated with multiple pathological conditions including CVD,[135,278,279] diabetes,[157] cancer,[280] autoimmune diseases such as psoriatic arthritis,[197] polycystic ovary syndrome,[181] and autism[192] and inversely associated with other conditions such as dementia[190,191] and inflammatory bowel disease.[199] For many of these diseases, however, the literature is inconsistent, highlighting the complexity of the interaction of humans with their microbiome. The most studied group of chronic diseases in relation to TMAO production includes CVD,[278,279] diabetes,[157] and renal diseases,[281,282] with particular attention being given to the observed correlation between plasma TMAO levels and CVD

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Table 3. Summary of Diet and Relationship of TMAO and TMA[a]

category	number and type of studies (number of participants)	biological matrix	summary	reference
Food				
fish	11 interventions (n = 190) 4 observational studies (n = 1742)	plasma, urine	except for one observational study,[118] all studies find increased urinary excretion and/or increased circulating TMAO levels with consumption of fish and seafood	28, 118, 223, 228, 229, 232−240
meat (red meat and processed meat)	9 interventions (N = 226) 2 observational studies (n = 568)	plasma, urine	all studies find increased urinary excretion and/or increased circulating TMAO levels with consumption of red meat (except one observational study[118])	118, 228, 229, 232, 236, 239−243
egg	6 interventions (n = 145) 2 observational studies (n = 560)	plasma, urine	contradictory results were observed from intervention study on the intake of egg, and no changes were reported in observational studies	118, 228, 231, 232, 240, 244−246
dairy	5 interventions (n = 61) 2 observational studies (n = 568)	plasma, urine	all studies find that ingestion of cheese and milk result in no significant increase in urinary and plasma TMAO; however, a significant association was found between TMAO and milk intake in one observational study[118]	118, 228, 240, 247, 248
starchy foods	3 interventions (n = 73) 2 observational studies (n = 594)	plasma, urine	inconsistent results were observed, with no significant association with TMAO level after ingestion of biscuits or bread in one intervention; a positive association between cereals and cereal products was reported in an observational study the change in plasma TMAO concentrations at 12 weeks in the whole-grain cereal group was significantly higher in the refined cereal group	228, 240, 249
fruit and vegetable	1 intervention (n = 6)	urine	no increase in levels of urinary TMA and TMAO after intake of fruit and vegetables including apple, banana, carrots, cauliflower, orange, peanut, pear, peas, pineapple, potato, soybean, and tomato	240
nuts and seeds	1 intervention (n = 39)	urine	lower TMAO excretion after 57 g/day of pistachios daily for 4 months compared with control diet	250
legumes and pulses	1 observational study (n = 50)	serum, urine	nonpulse consumers (≤4 g/day of pulse intake) had lower TMAO and DMA levels compared with habitual pulse consumers (≥25 g/day of pulse intake)	251
tea	1 intervention (n = 8)	urine	decrease in TMAO excretion after dictamnus tea ingestion	252
Nutrients				
protein	1 intervention (n = 77)	urine	TMAO excretion positively correlated with urinary nitrogen (biomarker of protein intake); participants in high protein diet group (23−28% energy) had higher excretion of TMAO compared with those on low protein diet (10−15% of energy)	253
starch	3 interventions (n = 64)	feces, plasma	resistant starch correlated with increased plasma TMAO, but no change in feces was observed	254, 255
fat	2 interventions (n = 123)	plasma	no association between fats and plasma TMAO levels	242, 256
vitamin D	1 observational study (n = 104)	plasma	individuals with vitamin D deficiency (<20 ng/mL) had higher TMAO levels compared with those with normal (≥30 ng/mL) or insufficient (21−29 ng/mL) levels of vitamin D	257
energy-reduced diet	1 intervention (n = 264)	plasma	no significant differences in changes in TMAO, choline, or L-carnitine across 4 different diet groups among overweight and obese adults	258
Supplements				
carnitine supplement	6 interventions (n = 166)	plasma, urine	all studies found increased urinary excretion and/or increased circulating TMAO after carnitine challenge tests with higher excretion in omnivores compared with long-term (>1 year) vegans/vegetarians	60, 228, 259, 260
choline supplement	1 intervention (n = 6)	urine	increased total TMA and TMAO urinary excretion after supplementation	228
TMAO supplement	2 interventions (n = 14)	urine	increased total TMA and TMAO urinary excretion after supplementation	127, 228
TMA supplement	1 intervention (n = 8)	urine	increased total TMA and TMAO urinary excretion after supplementation	127
insulin supplementation	1 intervention (n = 18)	plasma	fasting and postprandial TMAO and TMA concentrations did not significantly change in either the insulin or the control group	261
probiotic supplement	1 intervention (n = 8)	serum	no significant difference in TMAO between probiotic supplementation group and controls	262
Diets				
paleolithic diet (PD)	1 intervention (n = 39) 1 observational study (n = 91)	serum	no change in TMAO between paleolithic diet and Australian Guide to Healthy Eating diet groups for the intervention study;[263] however, an observational study reported that TMAO was higher in strict PD compared with pseudo-PD and usual diet for >1 year[264]	263, 264
vegetarian diet	1 intervention (n = 12)	urine	lower urinary TMAO in vegetarian when compared with that in nonvegetarian	241
lactovegetarian diet	1 observational study (n = 161)	urine	significantly lower TMAO excretion in long-term (>5 years) lactovegetarians compared with in control group having omnivorous diet	274
vegan diet	1 observational study (n = 105)	plasma	no difference in the plasma concentrations of TMAO between long-term (>10 years on average) vegans (n = 38) and lacto-ovo-vegetarians (n = 67)	265
plant-based diet	1 intervention (n = 36)	serum	TMAO concentrations were significantly lower in the plant-based diet phase than in the animal-based diet phase	266

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Table 3. continued

category	number and type of studies (number of participants)	biological matrix	summary	reference
Diets				
polyphenols and long-chain n-3 fatty acid diet	1 intervention (n = 78)	plasma	changes in TMAO were statistically significant at 8 weeks for the diets rich in long-chain n-3 fatty acids; no difference was found for the diets rich in polyphenols	249
habitual dietary patterns	1 intervention (n = 125)	urine	individuals reporting a dietary pattern consistent with high red meat, red-meat dishes, and meat product content but low in vegetables excreted higher TMAO concentrations than individuals who reported higher consumption of whole-meal bread, dairy products, and ice cream	267
Mediterranean dietary pattern	1 observational study (n = 119)	urine	higher Mediterranean dietary score (>10) was associated with higher TMAO excretion compared with lower score (≤7)	268
lifestyle intervention program	1 intervention (n = 16)	plasma, urine	exercise combined with a reduced calorie diet was associated with reduced plasma and urinary TMAO concentrations	269, 270
	1 observational study (n = 34)			
new Nordic diet	1 intervention (n = 181)	urine	TMAO levels were higher in individuals following new Nordic diet compared with control	271

[a]DMA, dimethylamine; TMA, trimethylamine; TMAO, trimethylamine *N*-oxide.

risk. The role of TMAO in driving CVD has been widely debated, and whereas the majority of published literature focuses on TMAO as a causal agent, several research groups have proposed that a more balanced consideration of the role of this molecule in human pathology is required.[283,284] Because there are several comprehensive reviews defining the association of TMA and TMAO with CVD,[283,285,286] we provide here only a brief overview of the association of TMAO and TMA in a range of pathologies and focus on the continuum of conserved metabolism across the prokaryotes and the animal kingdom, emphasizing the bacterial mediation of pathology in mammals via TMA and TMAO metabolism.

9.1. Fish Odor Syndrome (Primary Trimethylaminuria) and Transient Trimethylaminuria

Excessive excretion of TMA in the urine, sweat, and breath, resulting in an unpleasant body odor reminiscent of rotting fish, is characteristic of a condition known as primary trimethylaminuria (TMAU, OMIM 602079). This metabolic disorder was first described by Humbert in 1970[287] and is caused by mutations in the FMO3 gene resulting in a reduction in the oxidation of TMA to TMAO.[128,130,131] Currently more than 40 mutations have been identified that abolish or severely reduce FMO3 activity.[128,130,131,213,288] Diagnosis is made on the basis of genetic testing or a ratio of TMAO to TMA. Any individual excreting >10% as the free amine, that is, TMA/(TMA + TMAO), has a high likelihood of having fish odor syndrome.[289] In patients diagnosed with TMAU, the levels of TMA are typically increased by almost 300-fold (5.3−230 μmol/mmol creatinine) compared with those of normal individuals, whereas the levels of TMAO are slightly raised (0.36−607 μmol/mmol creatinine).[33]

The incidence of heterozygous carriers in the white British population is 0.5 to 1.0%,[127] giving an estimated frequency of the disorder in this population of about 1 in 40 000,[290] but carrier incidence may be higher in other populations,[291] with a more marked effect in individuals with fish odor syndrome.[292,293] Symptoms of TMAU are usually present from birth and may worsen during puberty[294,295] and during menstruation in women.[214,292] Although no physical symptoms are associated with TMAU, the unpleasant odor characteristic of the disorder often results in psychological and social problems. Administration of TMA to individuals with TMAU results in 70% of the dose being excreted unmodified instead of being converted to TMAO.[296] Currently, TMAU is primarily treated with dietary restriction of TMA and precursors of TMA including choline, lecithin, and TMAO, and patients are advised to avoid foods like cow's milk, egg, liver, kidney, legumes, brassicas, and fish/seafood,[297] although antibiotic treatment[298,299] and riboflavin[300] supplementation have also been used to reduce TMA levels.

9.2. Cardiometabolic Diseases and the Role of the Gut Microbiome

9.2.1. Cardiovascular Disease. TMAO has been established as one of the key villains in CVD risk and etiology and has been the focus of numerous reviews.[134−137] In summary, serum/plasma TMAO concentrations have been found to be associated with, or predictive of, mortality risk for heart failure,[155] myocardial infarction,[155,273,301,302] cardiorenal mortality,[303] peripheral artery disease,[273] and stroke,[302] independent of traditional risk factors. Some of these studies have also suggested an association with circulating TMAO levels and downstream mortality.[135,302,303] Two large meta-

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analyses, each involving over 10 000 participants, found that elevated plasma TMAO concentrations were associated with a 23−58% higher risk of major adverse cardiovascular events and a 55% higher risk of all-cause mortality.[134,136,137]

Currently, there are two main general hypotheses regarding the potential mechanistic links between TMAO and the development of atherosclerosis.[61,216,286] The first hypothesis is that TMAO may be a marker of disease that reflects the effects of CVD and atherosclerotic renal artery stenosis, which results in a reduction in TMAO urinary excretion with a consequent accumulation in the plasma.[216] The second and more widely favored hypothesis proposes that TMAO plays a causal role in disease pathogenesis due to its functional role in promoting macrophage foam cell accumulation, thereby inhibiting the reverse cholesterol transport that affects aspects of bile and sterol metabolism, resulting in platelet hyperactivity that promotes atherosclerotic plaque formation.[60,273] In support of this causal relationship, Zhu et al. showed that direct exposure of platelets to TMAO can enhance stimulus-dependent platelet activation.[304] The gut microbiome is central to the causal association of TMAO with CVD because the production of TMAO is dependent on the gut microbial transformation of dietary sources of choline and phosphatidylcholine into TMA. Germ-free mice do not produce TMAO from dietary sources and do not develop atherosclerotic plaques; similarly, suppression of the gut microbiome via orally administered antibiotics suppresses both TMAO production and the formation of diet-dependent atherosclerotic plaques.[60,204,273] Fecal microbial transplantation from prothrombotic mice (C57BL/6J) with high circulating TMAO to germ-free mice also results in an increase in circulating TMAO and an enhanced thrombosis potential.[204]

The influence of the microbiome on mediating the TMAO−CVD association is further reinforced by the impact of TMAO on vascular inflammation via the promotion of the nucleotide-binding oligomerization domain-like receptor family pyrin domain-containing 3 (NLRP3) inflammasome,[305] resulting in the release of the inflammatory cytokines IL-1β and IL-18 and the inhibition of nitric oxide production via nitric oxide synthase.[306] However, some studies have failed to find an association between plasma TMAO and inflammatory markers such as C-reactive protein.[231] Similarly, the host−microbiome partnership underpins the connection between TMAO and the induction of the transcription factor FoxO1, a key driver of metabolic disease, via binding to the endoplasmic reticulum stress kinase PERK. Manipulation of the gut microbiota to reduce TMAO levels reduces both PERK activation and hepatic FoxO1 levels.[307]

Clearly, the involvement of TMAO in atherogenesis, causal or otherwise, involves a complex interaction between diet, the microbiome, and the host-signaling processes. The role of the diet as a source of circulating TMAO (Table 3 and Table S4) has been widely debated, contrasting the mounting evidence of a direct association of TMAO with coronary heart disease (CHD) risk with the fact that the consumption of fish, a rich source of TMAO, has long been associated with reduced CVD risk.[223,308,309] Whereas several studies report evidence of a direct relationship between TMAO precursors such as L-carnitine, choline, or betaine and major adverse cardiovascular events,[135] other studies have failed to find any association between these dietary sources with CVD, CHD, stroke, or total CVD incidence,[310] and some have suggested that the link with CVD risk factors is mediated via TMA and not its oxide. For example, in a rat model, TMA was shown to induce a significant increase in mean arterial blood pressure, whereas TMAO had no effect.[311] Several review articles have pointed out the inconsistencies in the theories around TMAO and its involvement with CVD.[312−314] Ufnal et al. proposed that the association between TMAO and CVD lies in a compensatory response to the increased hydrostatic and osmotic pressure placed on the heart and kidneys by cardiac dysfunction.[315]

9.2.2. Obesity. Obesity is a risk factor for CVD and type 2 diabetes (T2D), with inflammation being an underpinning mechanism responsible for the translation to adverse clinical outcomes. Multiple studies have reported an association between body mass index (BMI) and serum or plasma concentrations of TMAO,[151,316] whereas others have found no correlation.[144,153] Serum TMAO has also been associated with other measures of obesity including the visceral adiposity index and the fatty liver index,[150] whereas the urinary excretion of TMA has been directly associated with obesity.[152,154]

Few studies have addressed the simple relationship between BMI or adiposity and TMA/TMAO levels in "healthy" individuals independent of confounders such as diet. Animal models have been a popular alternative or adjunct to help us understand the obesity−microbiome−inflammation axis but have yielded mixed results. In a comparison of ob/ob mice, with a genetic predisposition for obesity, versus C57BL/6J mice, urinary concentrations of TMA were found to be lower in the ob/ob mice,[317] a result that was mirrored in the db/db obese mouse model.[318] This contrasts with observations in humans that find a direct association between TMA excretion and obesity.[319] Decreased serum and urinary levels of TMAO were also characteristic of the Zucker rat model of obesity/diabetes[320] and obese pigs.[321] Furthermore, in a high-fat-diet mouse model of obesity, dietary supplementation with TMAO was found to significantly decrease atherosclerosis indices.[322]

Because obesity is one of the biggest risk factors for diabetes and CVD, weight-loss strategies have been evaluated with respect to evaluating metabolic correlates associated with weight loss and the consequent reduction in inflammation and improvement of metabolic health. A range of dietary interventions and weight-loss trials have demonstrated that weight loss is associated with a reduction in circulating TMAO and cardiometabolic disease risk and a reduction in visceral adipose tissue.[269] However, Zhou et al. have shown that TMAO may protect against bone mineral density reduction during weight loss, and this is irrespective of the type of diet, containing varying amounts of macronutrients, suggesting that TMAO may have a protective effect against osteoporosis,[258] although Lin et al. found an inverse association between serum TMAO and bone mineral density.[205] When considering the array of discordant outcomes with respect to the role of TMA/TMAO in weight loss, it should be borne in mind that many of the published weight-loss studies fail to take total/dietary TMAO into account, whereas studies involving the chronic administration of pure TMA precursors often supplement in amounts that far exceed those present either in a normal diet or in therapeutic supplements, thereby contributing to the lack of clarity regarding the role of TMAO in cardiometabolic disease.

Bariatric surgery is used as a therapeutic strategy to reduce weight in individuals with morbid obesity, when diet- and exercise-based approaches have failed. One of the hallmark effects of bariatric surgery is the marked shift in both microbial composition and metabolism postsurgery, with the shift

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occurring prior to weight loss. Fecal concentrations of methylamines, including MMA and TMA, are consistently elevated postsurgery in fecal samples[323] and have been shown to correlate with the cytotoxic effects of fecal slurry obtained from rats post-gastric-bypass surgery.[324] One of the most consistent observations across human and animal bariatric surgery studies is a significant and sustained increase in TMAO excretion postsurgery,[144,145,149] which correlates with a switch from a *Firmicutes*- and *Bacteroidetes*-dominated microbiome to one in which Gamma Proteobacteria, in particular, *Enterobacteriaceae* feature strongly. This is consistent with *Enterobacteriaceae* being one of the main bacterial families involved in TMA production.[149,325,326] Given the hypothesis that circulating TMAO is correlated with CHD but that bariatric surgery reduces the risk of CHD, it would appear that the TMAO story is complex with multiple inconsistent threads.

9.2.3. Type 2 Diabetes. Similarly to CVD, there is a general consensus in the literature that insulin resistance and T2D are also associated with high circulatory TMAO. In a meta-analysis of 12 studies involving 15 314 participants, high levels of circulating TMAO were associated with an increased risk of diabetes (odds ratio = 1.89) and were also differential in the comparison of diabetic and nondiabetic individuals (odds ratio = 1.54).[157] Serum levels of TMAO have also been observed to be correlated with diabetes duration, markers of gut permeability, lipopolysaccharide endotoxins, and TMA-producing gut bacteria.[159] One of the proposed mechanistic factors underlying this association involves disruption to the insulin signaling pathway caused by TMAO-induced inflammation of the adipose tissue.[327] For diabetics, TMAO has been shown to be a strong risk marker for cardiovascular events.[155] In a high-fat-diet mouse model, a dietary supplement of 0.2% TMAO over a 3 week period caused an increase in fasting insulin levels, aggravated glucose tolerance, and increased the pro-inflammatory cytokine MCP-1 while reducing IL-10, an anti-inflammatory cytokine.[322]

9.3. Renal Disease

Abnormal levels of urinary and circulating TMAO have been described in different renal diseases including chronic kidney disease (CKD),[166,167] nephropathy,[169] interstitial nephritis,[173] and renal papillary necrosis. Uremic toxicity is a condition in which solutes that are normally excreted via the kidneys accumulate in the renal cells and tissues and can adversely interact with endogenous molecular functions in either the kidney or other organs. TMA,[328] and, more recently, other methylamines including TMAO, DMA, dimethylglycine, and MMA,[329] have been included in the uremic toxin database (https://database.uremic-toxins.org/soluteList.php). High circulating levels of TMAO can indicate uremic toxicity, but, equally, low levels can also be indicative of other types of renal dysfunction.[166,330] Gessner et al. showed that for every increase of 1 mL/min in the estimated glomerular filtration rate (eGFR), a reduction of 0.005 (0.3%) log TMAO plasma value was observed.[330]

CKD is the second fastest growing cause of death worldwide[331] and is a strong risk factor for CVD. The Hemodialysis (HEMO) Study, involving 1232 dialysis patients, found serum TMAO levels to be 10 to 20 times higher than those with normal kidney function.[332] In CKD, circulating TMAO levels are typically ∼30 μmol/L (∼15 times higher than those of healthy controls)[169] and have been found to correlate with serum urea and creatinine and also with a reduction of renal function.[333] Renal dialysis is the core strategy for removing uremic toxins in patients in renal failure, and blood concentrations of TMA and TMAO have been reported to decrease post-dialysis,[334] although the post-dialysis levels remain higher than those of healthy controls.[335] In contrast with nondialysis patients, plasma TMAO levels in dialysis patients were not found to be predictive of hospitalization or all-cause mortality,[335,336] suggesting that despite circulating TMAO levels being several times higher than those of the general population, the extent of vascular endothelial damage attributed to CKD overshadows the CVD outcome. Similar to renal dialysis, successful renal transplantation also normalizes serum TMAO levels,[167] although high circulating TMAO levels can be indicative of graft dysfunction.[337,338] CKD is associated with an abnormal microbiome with elevated *Enterobacteriaceae*, *Halomonadaceae*, *Pseudomonadaceae*, *Moraxellaceae*, and *Brachybacterium* and depleted *Lacobacillaceae* and *Prevotellaceae* families in comparison with healthy controls.[339] Many of these bacteria have microbial genes related to TMA production.[169] Urinary TMA and DMA excretion is characteristic of glomerulonephropathy, a common feature of CKD.[340] Because TMAO serves as an osmolyte in renal cells, damage to the kidney causes marked changes in TMAO levels. For example, in an ischemic renal reperfusion kidney transplant in a rat, renal dysfunction resulted in a three- to five-fold increase in plasma TMAO,[341] whereas unilateral-ureteral-obstruction-induced renal interstitial fibrosis was associated with higher serum levels of TMAO, changing to comparatively lower levels in later stages of the condition.[342] Increased urine levels of TMAO can be directly associated with damage to the cells in the renal cortex or medulla,[343,344172,173] whereas the reduced urinary excretion of TMAO can be indicative of renal papillary necrosis caused by nonsteroidal anti-inflammatory drug such as indomethacin.[344]

9.4. Cancer

Cancer is globally one of the most significant causes of human mortality, and its etiology has been linked to both genetic and environmental factors including diet, obesity, pollutants and other environmental contaminants, stress, and the intestinal microbiome. The association between TMAO levels and cancer risk has sparked much interest, and several studies are summarized in a review by Chan et al.[345] The literature is largely consistent, and the consensus from multiple studies concludes that cancer is typically associated with lower urinary concentrations of TMAO. For example, urinary excretion of TMAO was shown to be decreased in breast cancer patients in comparison with controls[179] and in hepatocellular carcinoma patients compared with either healthy or hepatitis B controls for Bangladeshi,[188] Nigerian,[346] and Egyptian cohorts.[187] In mice implanted with human gastric adenocarcinoma, relative urinary concentrations of TMAO were found to decrease significantly within the first 5 days post-implantation, whereas TMA levels increased.[347] Treatment with the anticancer drug adriamycin subsequently restored TMAO and TMA levels.

The association of circulating TMAO levels with cancer is more variable and is dependent on cancer type. Low serum concentrations of TMAO have been associated with nasopharyngeal carcinoma patients[178] and renal cell carcinoma, with levels increasing post-nephrectomy.[186] In contrast, higher levels of serum or plasma TMAO have been reported for primary liver cancer patients (sex- and age-adjusted odds ratio of 3.43),[180] oral squamous cell carcinoma,[348] and colorectal

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cancer.[174,175] In the case of pancreatic cancer, contradictory results have been published, with one study finding significantly lower serum TMAO concentrations in patients[182] and another reporting that higher concentrations of TMAO were associated with risk for pancreatic cancer.[181]

Mechanistically, the link between TMAO and cancer is unclear. In vitro experiments have shown that TMAO can stimulate the production of reactive oxygen species in cells by activation of thioredoxin-interacting protein (TXNIP)-NLRP3 inflammasome signaling.[349] TMAO can also act in a protective role with respect to carcinogenesis by preserving protein folding or correcting folding defects in mutant proteins.[350] Contrary to this hypothesis, TMAO has been suggested to disrupt the function of α-casein, which serves as a chaperone protein and tumor suppressor that helps protect against breast cancer.[345] In the case of pancreatic, liver, and colorectal cancers, there is strong evidence of an association of the gut microbiome in the pathogenesis, with all three cancers causing dysregulation of the microbiome.[280]

High concentrations of TMAO and sorbitol within the tumor tissue have been reported for brain tumors[351] and renal carcinomas.[183,185] Specifically, in glial brain tumors, TMA was suggested to be a marker of proliferating tumor tissue and was found in significantly higher levels in high-grade tumors compared with low-grade tumors (2.65 ± 0.86 mM and 1.67 ± 0.32 mM, respectively).[184] Although several in vivo NMR studies refer to TMA as a potential cancer biomarker, in several cases, the chemical shift of the peak is more consistent with that of TMAO.

When considering the role of methylamines in cancer, the direct cytotoxic effects of the methylamines should not be overlooked. It has been proposed that high concentrations of dietary amines and amides, such as TMAO, can undergo transformation to TMA and DMA, which are precursors of dimethylnitrosamine, a known carcinogen.[352] Thus seafood containing high levels of methylamines, such as squid, shark, and cod, and other foods, such as fermented soybean products, that contain DMA and TMA may contribute to the formation of *N*-nitroso compounds in vivo. The association of TMAO and colon cancer is unsurprising given that foods rich in choline and carnitines, such as red meat, are metabolized to TMAO.[353] As with many of the previously discussed pathological conditions, most studies (Table 2 and Table S3) have tended to be inadequately powered, and a systematic study of the association of TMA/TMAO levels with clinical diagnosis stratified by cancer type and grade is warranted.

10. CONCLUSIONS

Strong evolutionary pressures have resulted in the widespread distribution of TMA and TMAO in organisms present in multiple aquatic and terrestrial environments. The production of methylamines via abiotic, prokaryotic, and eukaryotic organisms coupled to their ubiquitous presence and multiple functionalities in living organisms suggests that these simple compounds were seconded to perform fundamental chemical and physiological processes early in evolution. Although TMAO is directly dependent on the bacterial production of TMA, they have divergent biological roles. Thus TMAO predominantly serves as a protein stabilizer or disruptor, whereas TMA plays a stronger role as a pheromone or acts as a hormonal signal.

Recently TMAO has been labeled as a "villain" in cardiometabolic diseases, with some elegant mechanistic experiments showing potential etiopathological contributions, yet there are many inconsistencies. The assessment of either molecule as wholly deleterious would be perverse given that there is no question that TMAO performs major physiological functions, as does TMA. But like many biochemical systems, it is the pattern of distribution and the concentration that determine the overall physical effect. The metabolic origins of TMA and TMAO are mostly within the gut microbiome, which has coevolved with its human host, yet the majority of the literature evaluates TMAO concentration without consideration of its microbial precursor, TMA. Other major deficiencies in the existing body of literature include the failure of many studies to take the background dietary contribution to TMAO production into account, and some of the previous studies have required the reduction of TMA to TMAO prior to measurement. The broader anomalies, such as why the Japanese do not have a higher incidence of CVD when their intake of dietary TMAO is so high and why bariatric surgery dramatically increases TMAO excretion if the procedure is protective against CVD (associated with higher systemic levels of TMAO), remain unanswered.

The diverse occurrence of TMAO and TMA in multiple biological systems on different scales of evolution intrinsically tells us that they are essential to most forms of life. Their regulation is based on a wide range of genetic and environmental factors, signposting the complex gene-environmental interplay and reflecting the need for further consideration of their chemical and biological properties at the systems level. Nevertheless, abnormalities of TMA/TMAO metabolism are associated with numerous human pathologies. Understanding these processes may also be fundamental to the understanding of diverse disease mechanisms

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jproteome.1c00851.

Table S1. Summary of different analytical methods used to analyze TMAO and TMA together with their LOD and quantification. Table S2. Summary of marine and mammalian bacteria species involved in the metabolism of TMA and TMAO. Table S3. Exemplar studies that show the effects of TMAO and TMA in normal physiology and different pathologies that were used to summarize the information in Table 2. Table S4. Exemplar interventional and observational diet studies investigating the relationships of diet with TMAO and TMA. These studies were used to summarize the information in Table 3 (PDF)

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