

Minireview

Trimethylamine and Trimethylamine *N*-Oxide, a Flavin-Containing Monooxygenase 3 (FMO3)-Mediated Host-Microbiome Metabolic Axis Implicated in Health and Disease

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Received March 22, 2016; accepted May 13, 2016

ABSTRACT

Flavin-containing monooxygenase 3 (FMO3) is known primarily as an enzyme involved in the metabolism of therapeutic drugs. On a daily basis, however, we are exposed to one of the most abundant substrates of the enzyme trimethylamine (TMA), which is released from various dietary components by the action of gut bacteria. FMO3 converts the odorous TMA to nonodorous TMA *N*-oxide (TMAO), which is excreted in urine. Impaired FMO3 activity gives rise to the inherited disorder primary trimethylaminuria (TMAU). Affected individuals cannot produce TMAO and, consequently, excrete large amounts of TMA. A dysbiosis in gut bacteria can give rise to secondary TMAU. Recently, there has been much interest in FMO3 and its catalytic product, TMAO, because TMAO has been implicated in various conditions affecting health, including cardiovascular

disease, reverse cholesterol transport, and glucose and lipid homeostasis. In this review, we consider the dietary components that can give rise to TMA, the gut bacteria involved in the production of TMA from dietary precursors, the metabolic reactions by which bacteria produce and use TMA, and the enzymes that catalyze the reactions. Also included is information on bacteria that produce TMA in the oral cavity and vagina, two key microbiome niches that can influence health. Finally, we discuss the importance of the TMA/TMAO microbiome-host axis in health and disease, considering factors that affect bacterial production and host metabolism of TMA, the involvement of TMAO and FMO3 in disease, and the implications of the host-microbiome axis for management of TMAU.

Introduction

Flavin-containing monooxygenases (FMOs) (EC 1.14.13.8) catalyze the NADPH-dependent oxidative metabolism of a wide array of foreign chemicals, including drugs, dietary-derived compounds, and environmental pollutants (Krueger and Williams, 2005). Humans possess five functional *FMO* genes: *FMO1*, 2, 3, 4, and 5 (Hernandez et al., 2004; Phillips et al., 2007). The main site of expression of *FMO3* is the liver (Dolphin et al., 1996); however, high expression levels have also been observed in the skin of certain individuals (Janmohamed et al., 2001). The *FMO3* gene is switched on in human liver at birth and can take several years for the gene to attain maximum expression as the liver develops to its full functional capacity (Koukouritaki et al., 2002).

Drug substrates for FMO3 include the antipsychotic olanzapine (Söderberg et al., 2013), the antiestrogen tamoxifen (Parte and Kupfer, 2005), the gastroprokinetic agent itopride (Mushiroda et al., 2000), and the H₂-receptor antagonist cimetidine (Cashman et al., 1995). Substrates undergo *N*- or *S*-oxygenation; generally, this is a detoxification route and the products are excreted in urine, but some chemicals are converted to a

more active or toxic form (Krueger and Williams, 2005). Retroreduction of the oxygenated product can occur, creating a pool of drug that can be recycled (Cashman, 2008). The importance of FMO3 in detoxification and bioactivation of xenobiotics has recently been reviewed (Cruciani et al., 2014). The *FMO3* gene is highly polymorphic (Phillips et al., 2007), and genetic variants that are common in the general population are known to influence drug metabolism (Phillips et al., 2007; Söderberg et al., 2013; Zhou et al., 2014).

FMO3 has an important relationship with the gut microbiome, with an abundant nondrug substrate, trimethylamine (TMA), being derived from dietary components by the action of gut microbes (Seim et al., 1985; Al-Waiz et al., 1992). TMA is rapidly absorbed and is converted in the liver to trimethylamine *N*-oxide (TMAO) (Higgins et al., 1972). Of the five functional FMOs of humans (FMOs 1–5), only FMO3 effectively catalyzes the conversion of TMA to TMAO (Lang et al., 1998). FMO3 is thus an excellent example of a protein that participates in host-gut microbiome metabolic interactions (Nicholson et al., 2012). Rare genetic variants of the *FMO3* gene that abolish or severely impair activity of the enzyme give rise to the inherited disorder primary trimethylaminuria (TMAU) (Phillips and Shephard, 2008) because of inefficient conversion of microbiome-derived odorous TMA to nonodorous TMAO.

Recently, there has been much interest in FMO3 and its catalytic product TMAO. This is because TMAO has been implicated in various

The work was funded in part by a grant from the Medical Research Council, UK [Grant MR/K015494/1].

dx.doi.org/10.1124/dmd.116.070615.

ABBREVIATIONS: *cnt*, carnitine utilization gene cluster; *cut*, choline utilization gene cluster; FMO, flavin-containing monooxygenase; LIRKO, liver insulin receptor-knockout; MA, methylamine; TMA, trimethylamine; TMAO, trimethylamine *N*-oxide; TMAU, trimethylaminuria.

conditions affecting health, including cardiovascular disease, reverse cholesterol transport, and glucose and lipid homeostasis (Wang et al., 2011; Bennett et al., 2013; Koeth et al., 2013; Tang et al., 2013; Gao et al., 2014; Obeid et al., 2016). The microbial origin of TMA is of great interest as we begin to fully appreciate the importance of our microbiomes for health and well-being. Knowledge of the microbial species that give rise to TMA would contribute greatly to our understanding of the consequences for human health of the complex interrelationships of diet, the microbiome, and the capacity for FMO3-catalyzed conversion of TMA to TMAO.

In this review, we first describe the origins and metabolic fate of TMA and outline the basis and consequences of TMAU. We then consider the dietary components that can give rise to TMA, the gut bacteria involved in the production of TMA from dietary precursors, the metabolic reactions by which bacteria produce and use TMA, and the enzymes that catalyze the reactions. Also included is information on bacteria that produce TMA in the oral cavity and vagina, two key microbiome niches that can influence health, and on noncommensal bacteria that produce TMA. Finally, we discuss the importance of the TMA/TMAO microbiome-host axis in health and disease, considering factors that affect bacterial production and host metabolism of TMA, the involvement of TMAO and FMO3 in disease and the implications of the host-microbiome axis for management of TMAU.

Origins and Metabolic Fate of TMA

The discovery and properties of TMA have been recently reviewed (Mitchell and Smith, 2016). TMA is an indicator of food spoilage, particularly of fish and milk. As early as the 1930s Beatty showed that TMA was produced during fish spoilage (Beatty, 1938). The TMA is derived from bacterially mediated reduction of TMAO (Barrett and Kwan, 1985), which is present in large quantities in marine fish (Zhang et al., 1999).

The requirement of bacterial action for the production of TMA from the diet in vivo is demonstrated by the failure of germ-free rats fed carnitine (Seim et al., 1985), or of germ-free or antibiotic-treated mice fed a normal diet, to excrete TMA in their urine (Al-Waiz et al., 1992). TMA is derived from the diet by microbial degradation of precursors found in, for example, marine fish, eggs, offal, soya beans, peas, and red meat (Zhang et al., 1999; Zeisel et al., 2003). Once liberated, TMA is rapidly absorbed through the gut wall and transported to the liver, where it is converted to nonodorous TMAO, which is then rapidly cleared in the urine (Al-Waiz et al., 1987a). Analysis in vitro of heterologously expressed FMOs revealed that, at physiologic pH, *N*-oxygenation of TMA is catalyzed by FMO3, with a K_M of 28 μM and an apparent k_{cat} of $>30 \text{ minutes}^{-1}$ (Lang et al., 1998). Other FMOs are far less effective in catalyzing this reaction: FMO1, FMO2, and FMO4, none of which is expressed in adult human liver (Phillips et al., 2007), exhibit apparent k_{cats} of 0.1, 1.0, and 0.1 minute^{-1} , respectively, whereas FMO5, the only other form of FMO expressed in the adult human liver (Phillips et al., 2007), is unable to catalyze the reaction (Lang et al., 1998). FMO1, which is expressed in human kidney (Dolphin et al., 1991), can catalyze TMA *N*-oxygenation, with an apparent k_{cat} of 5 minutes^{-1} , but only at substrate concentrations of 5 mM (Lang et al., 1998), which are unlikely to be relevant for metabolism of TMA in vivo. The importance of FMO3 for the metabolism of TMA in vivo is demonstrated by the marked reduction in the ability to *N*-oxygenate TMA of individuals homozygous or compound heterozygous for mutations that severely impair FMO3 activity (Dolphin et al., 1997, 2000; Treacy et al., 1998).

In addition to *N*-oxygenation, rat liver microsomes carry out demethylation of TMA, but the latter reaction is much less efficient (Gut and Conney, 1991). Demethylation of TMA in rat liver was shown

to be catalyzed by a different FMO from that which catalyzes production of the *N*-oxide (Gut and Conney, 1993). This FMO is probably FMO1, whose gene is expressed in the liver of rodents and other mammals, but not in adult human liver (Dolphin et al., 1991). In human volunteers, the demethylation product was found in low amounts and only in those dosed with high amounts of TMA (Al-Waiz, et al., 1987b). Consequently, in humans FMO3-catalyzed *N*-oxygenation is by far the most important route of metabolism of TMA.

Since microbial diversity studies have entered the era of high-throughput sequencing and in silico analysis, more insight has been gained into the composition of the human gut microbiota; however, studies based on bacterial cultivation are still the main source for information about the metabolic capacities of the microbiota. Combining what is being uncovered about the composition of the human microbiota with what is known of the functional activity of its components enables identification of microbial metabolic pathways by which TMA can be produced or metabolized in the human gut.

TMAU, a Deficiency of FMO3

In humans, mutations that abolish or severely impair the activity of FMO3 cause the inherited disorder primary TMAU (Dolphin et al., 1997; Hernandez et al., 2003). Affected individuals have a severely reduced ability to convert TMA to TMAO and, consequently, excrete large amounts of odorous TMA in their urine, sweat, and breath (Ayesh et al., 1993). Although the disorder has no overt physiologic effects on patient health, it can have profound psychologic and social consequences, resulting in a severe loss of quality of life, in extreme cases giving rise to clinical depression and suicidal tendencies (Mitchell and Smith, 2001; Shephard et al., 2015, [Phillips IR and Shephard EA. Primary trimethylaminuria. GeneReviews at GeneTests: Medical Genetics Information Resource (database online): University of Washington, 1997—2015 available at <http://www.ncbi.nlm.nih.gov/books/NBK1103/>, updated 2015]. In terms of drug metabolism, TMAU individuals have impaired metabolism of the FMO3 drug substrate benzydamine (Mayatepek et al., 2004). Since the first discovery of a mutation known to cause TMAU (Dolphin et al., 1997), many different causative mutations have been identified (Phillips et al., 2007; Yamazaki and Shimizu, 2013). A catalog of variants of the *FMO3* gene and their effect on the ability of FMO3 to catalyze the oxygenation of TMA and drug substrates of the enzyme can be accessed at the *FMO3* locus-specific mutation database (<http://databases.lovd.nl/shared/genes/FMO3>).

Production of large amounts of TMA, as a result of gut microbial action, exacerbates the symptoms of primary TMAU. In addition, overproduction of TMA, as a consequence of a dysbiosis of the gut microbiome, can give rise to a nongenetic form of the disorder, known as secondary TMAU (Mitchell and Smith, 2001). Therefore, a better understanding of the bacterial species that produce TMA in the gut may provide insights into why some individuals develop secondary TMAU in the absence of impaired *N*-oxygenation of TMA (Shimizu et al., 2014) and offers the potential to develop improved strategies for the management and treatment of both primary and secondary forms of the disorder.

Dietary Precursors of TMA and the Bacteria Involved in its Production in the Gut

TMAO. TMAO, the oxygenated product of TMA, is itself a dietary constituent that can give rise to TMA in the gut. Marine fish contain the highest amounts of TMAO (up to 3 mg/g) of any food source (Mitchell et al., 2002). The TMAO is thought to act as an osmolyte that allows adaptation to changes in salinity (Pang et al., 1977) and hydrostatic pressure (Zerbst-Boroffka et al., 2005). It is estimated that about 50% of

consumed TMAO is not metabolized and passes through the body unchanged to be excreted in the urine (Zhang et al., 1999). In the human gut, the remainder of the TMAO is reduced to TMA by bacterial TMAO reductase (EC 1.7.2.3) (Kwan and Barrett, 1983) (Fig. 1). Examples of bacteria that produce TMA from TMAO are given in Table 1.

Choline. Choline is an essential dietary nutrient, which can function as a precursor for the synthesis of phospholipids, including phosphatidylcholine, that are essential components of membranes, and of the neurotransmitter acetylcholine. It is present in high quantities in a variety of foodstuffs, for example, beef liver, cauliflower, and peanuts (Zeisel et al., 2003), and high concentrations of free choline are present in human milk and in soya bean-derived milk formulae (Zeisel, 1990). Choline is usually ingested as lecithin (also known as phosphatidylcholine), however, in which the choline moiety is covalently bound to a phosphatidate (a phosphoglycerol attached to two fatty acids) (Hirsch et al., 1978). Interconversion of lecithin and choline is bidirectional, conversion of lecithin to choline being catalyzed by phospholipase D (EC 3.1.4.4) and the first step in the conversion of choline to lecithin by choline kinase (EC 2.7.1.32) (Fig. 1).

Free choline is absorbed throughout the small intestine and is subsequently integrated into cell membranes or actively taken up by the liver, where it can be converted to betaine, phosphocholine, or lecithin (Zeisel, 1990). High amounts of choline may exceed the absorptive capacity and pass through to the large intestine, where it is metabolized to methylamines by microbial action (Zeisel et al., 1983). Choline is a quaternary ammonium compound containing a trimethylammonium moiety. Thus, it can act as a precursor for TMA (Zeisel et al., 1989; Chalmers et al., 2006). The bacterial conversion of choline to TMA involves the cleavage of the carbon-nitrogen bond of choline, producing TMA and acetaldehyde (Hayward and Stadtman,

1959). Craciun and Balskus (2012) proposed that a glyceryl radical enzyme CutC (EC 4.3.99.4), encoded by the bacterial choline utilization gene cluster (*cut*), might act as a choline TMA-lyase to catalyze this initial step in choline degradation (Fig. 1). This was confirmed by demonstrating that deletion of *cutC* in *Desulfovibrio desulfuricans* abolished the ability of the organism to produce TMA from choline. Bioinformatics analysis revealed *cutC* homologs in 89 bacterial genomes. The homologs are not distributed evenly among the major bacterial phyla of the human gut, being present in *Firmicutes*, *Actinobacteria*, and *Proteobacteria* spp. but absent from *Bacteroidetes* (Craciun and Balskus, 2012). Table 1 shows bacteria known to be associated with formation of TMA via choline degradation.

Carnitine. Carnitine plays a key role in metabolism, being involved in the transport of long-chain fatty acids from the cytosol into mitochondria. Carnitine is a quaternary ammonium compound that can be synthesized in the body from methionine and lysine, with its immediate precursor being γ -butyrobetaine (Feller and Rudman, 1988). L-carnitine is present in red meat and dairy products (Feller and Rudman, 1988), and it is estimated that the average nonvegetarian American consumes 100–300 mg/day (Feller and Rudman, 1988). Of dietary carnitine, about half is absorbed from the intestine, and the other half is metabolized by gut flora, eventually resulting in the excretion of TMAO and γ -butyrobetaine in urine and feces, respectively (Rebouche and Chenard, 1991). Gut bacteria are thought to cleave the 3-hydroperoxybutyryl moiety from L-carnitine to produce TMA (Meadows and Wargo, 2015). This pathway has been observed in several bacteria, including *Serratia marcescens* and *Acinetobacter calcoaceticus* (Meadows and Wargo, 2015). Using bioinformatics approaches, Zhu et al. (2014) identified a two-component oxygenase/reductase Rieske-type enzyme, encoded by *cntAB*, that catalyzed the formation of TMA from carnitine (see Fig. 1).

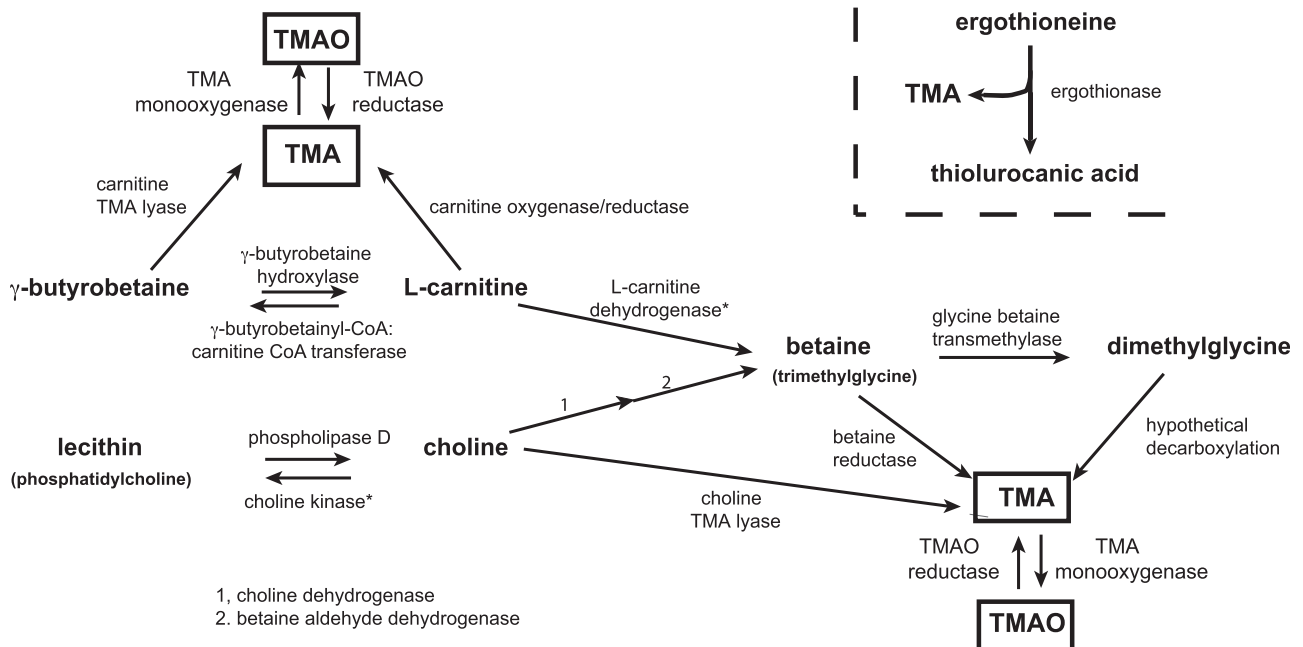


Fig. 1. Metabolic pathways for the production and metabolism of TMA by the human microbiota. TMA can be produced from TMAO in a reaction catalyzed by TMAO reductase. Choline, either in its free form or released from lecithin (phosphatidylcholine), contributes to the formation of TMA directly, via the action of choline-TMA lyase, encoded by the choline utilization cluster (*cutC*), or potentially indirectly, via conversion to betaine. Similarly, L-carnitine, present in red meat or derived from γ -butyrobetaine, contributes to TMA formation directly via the action of a Rieske-type carnitine reductase/oxidase (*cntAB*), or potentially indirectly, via conversion to betaine or to γ -butyrobetaine. Betaine can potentially contribute to TMA formation directly, via the action of a betaine reductase, or indirectly, via conversion to dimethylglycine, which could be metabolized to TMA via a hypothetical decarboxylation. TMA can be produced from ergothioneine via the action of ergothionase. TMA itself can be oxidized to TMAO, via the action of TMA monoxygenase. Asterisks represent multistep pathways. Bold text, TMA and its precursors and metabolites. Plain text, enzymes that catalyze reactions in the pathways. Based on Zeisel, 1990; Kleber, 1997; Serra et al., 2002; Wood et al., 2010; Craciun and Balskus, 2012; Caspi et al., 2014; Zhu et al., 2014.

TABLE 1
Bacteria that produce trimethylamine (TMA) from various substrates

Phylum	Genus or Species	References
TMA N-oxide		
<i>Actinobacteria</i>	<i>Micrococcus</i>	Robinson et al., 1952
	<i>Mobiluncus</i>	Cruden and Galask, 1988
<i>Firmicutes</i>	<i>Bacillus</i>	Robinson et al., 1952
	<i>Clostridium</i>	Robinson et al., 1952
	<i>Staphylococcus</i>	Robinson et al., 1952
	<i>Sarcina</i>	Robinson et al., 1952
	<i>Streptococcus</i>	Robinson et al., 1952
<i>Proteobacteria</i>	<i>Alcaligenes</i>	Robinson et al., 1952
	<i>Campylobacter</i>	Sellars et al., 2002
	<i>Citrobacter</i>	Lin and Hurng, 1989
	<i>Escherichia</i>	Robinson et al., 1952; Ishimoto and Shimokawa, 1978; Cox and Knight, 1981; Takagi et al., 1981; Easter et al., 1982; Lin and Hurng, 1989; Denby et al., 2015
	<i>Proteus</i>	Robinson et al., 1952; Strøm et al., 1979; Stenberg et al., 1982
	<i>Pseudomonas</i>	Robinson et al., 1952; Lee et al., 1977; Easter et al., 1982; Chen et al., 2011
Choline		
<i>Actinobacteria</i>	<i>Mobiluncus</i>	Cruden and Galask, 1988
	<i>Olsenella</i>	Craciun and Balskus, 2012; Martínez-del Campo et al., 2015
<i>Bacteroidetes</i>	<i>Bacteroides</i>	Cruden and Galask, 1988
<i>Firmicutes</i>	<i>Anaerococcus</i>	Craciun and Balskus, 2012; Romano et al., 2015
	<i>Clostridium</i>	Robinson et al., 1952; Bradbeer, 1965; Fiebig and Gottschalk, 1983; Möller et al., 1986; Craciun and Balskus, 2012; Martínez-del Campo et al., 2015; Romano et al., 2015
	<i>Desulfitobacterium</i>	Craciun and Balskus, 2012
	<i>Enterococcus</i>	Simenhoff et al., 1976
<i>Proteobacteria</i>	<i>Streptococcus</i>	Robinson et al., 1952; Simenhoff et al., 1976; Chao and Zeisel, 1990; Craciun and Balskus, 2012; Martínez-del Campo et al., 2015
	<i>Desulfovibrio</i> ^a	Hayward and Stadtman, 1959, 1960; Baker et al., 1962; Bradbeer, 1965; Fiebig and Gottschalk, 1983; Craciun and Balskus, 2012
	<i>Edwardsiella</i>	Romano et al., 2015
	<i>Enterobacter</i> ^b	Eddy, 1953; Craciun and Balskus, 2012
	<i>Escherichia</i>	Craciun and Balskus, 2012; Martínez-del Campo et al., 2015; Romano et al., 2015
	<i>Klebsiella</i> ^b	Eddy, 1953; Craciun and Balskus, 2012; Kuka et al., 2014; Kalnins et al., 2015; Martínez-del Campo et al., 2015
	<i>Proteus</i>	Seim et al., 1982a; Craciun and Balskus, 2012; Kuka et al., 2014; Martínez-del Campo et al., 2015; Romano et al., 2015
	<i>Providencia</i>	Craciun and Balskus, 2012; Romano et al., 2015
	<i>Pseudomonas</i>	Robinson et al., 1952; Kleber et al., 1978
	<i>Yokenella</i>	Craciun and Balskus, 2012
Carnitine		
<i>Proteobacteria</i>	<i>Acinetobacter</i>	Kleber et al., 1977; Seim et al., 1982b; Miura-Fraboni et al., 1982; Ditullio et al., 1994; Zhu et al., 2014
	<i>Citrobacter</i>	Zhu et al., 2014
	<i>Escherichia</i>	Zhu et al., 2014
	<i>Klebsiella</i>	Kuka et al., 2014; Zhu et al., 2014
	<i>Proteus</i>	Seim et al., 1982a
	<i>Pseudomonas</i>	Kleber et al., 1978; Miura-Fraboni et al., 1982
Betaine		
<i>Firmicutes</i>	<i>Clostridium</i>	Naumann et al., 1983; Möller et al., 1986
	<i>Eubacterium</i>	Zindel et al., 1988; Hormann and Andreesen, 1989
	<i>Sporomusa</i>	Möller et al., 1986; Hormann and Andreesen, 1989
Ergothioneine		
<i>Proteobacteria</i>	<i>Alcaligenes</i>	Yanasugondha and Appleman, 1957; Kelly and Appleman, 1961
	<i>Escherichia</i>	Wolff, 1962

^aSynonym: *Vibrio cholincus*.

^bInitially observed as *Aerobacter* but reassigned to genera *Enterobacter* and *Klebsiella*.

Both components are required for catalysis of electron transfer and degradation of carnitine to TMA. Deletion of *cntAB* in *A. calcoaceticus* rendered the microbe incapable of producing TMA. As is the case for *cutC* in choline metabolism (Craciun and Balskus, 2012), *cntAB* is distributed unevenly among the major bacterial phyla, being present in *Proteobacteria* (mainly *Gammaproteobacteria*) and *Firmicutes* and absent from *Bacteroidetes* spp. (Zhu et al., 2014).

In addition to generating TMA directly from L-carnitine, it has been suggested that gut bacteria can also convert γ -butyrobetaine to TMA. Most studies consider γ -butyrobetaine to be an intermediary metabolite produced during the biosynthesis of L-carnitine, by a reaction catalyzed by γ -butyrobetaine hydroxylase (EC 1.14.11.1); however, the gut microbiota may also be able to produce γ -butyrobetaine from catabolism of L-carnitine by a reaction catalyzed by γ -butyrobetainyl-CoA:

carnitine-CoA transferase (EC 2.8.3.21) (Rebouche and Seim, 1998; Caspi et al., 2014). Koeth et al. (2014) showed that γ -butyrobetaine, obtained through oral γ -butyrobetaine or L-carnitine supplementation, can be metabolized by microbes to produce TMA and identified the gene pair *yeaW/yeaX* as encoding an enzyme that catalyzes this reaction in vitro. The enzyme, termed a carnitine TMA lyase (see Fig. 1), can also catalyze production of TMA directly from choline, betaine, and carnitine (Koeth et al., 2014). Table 1 shows bacteria known to be associated with the formation of TMA via carnitine degradation.

Betaine. Betaine, or trimethylglycine, plays an important role as a methyl donor in the betaine homocysteine methyltransferase pathway (Obeid, 2013). It is present in high concentrations in various foodstuffs, for example, wheat bran, wheat germ, and spinach (Zeisel et al., 2003). The daily intake of betaine varies from ~30–400 mg/d (Obeid, 2013). Betaine

can also be biosynthesized, in both the human host and in gut bacteria, by oxidation of choline or carnitine. The oxidation of choline to betaine involves an intermediate conversion to betaine aldehyde, catalyzed by choline dehydrogenase (EC 1.1.99.1), which in turn is converted to betaine in a reaction catalyzed by betaine aldehyde dehydrogenase (EC 1.2.1.8) (Andresen et al., 1988) (Fig. 1). L-carnitine can be converted to betaine via a multistep pathway, the first reaction of which is catalyzed by L-carnitine dehydrogenase (EC 1.1.108) (Meadows and Wargo, 2015) (Fig. 1).

There are several catabolic pathways for betaine (Fig. 1). One involves demethylation of betaine to dimethylglycine, in a reaction catalyzed by glycine betaine transmethylase (EC 2.1.1.5) (Wood et al., 2010; Caspi et al., 2014). Dimethylglycine could be metabolized to TMA via a hypothetical decarboxylation (Wood et al., 2010). Betaine can also function as an electron acceptor in a coupled reduction-oxidation reaction (i.e., Stickland reaction), catalyzed by betaine reductase (EC 1.2.1.4.4), in which it is reduced and cleaved to produce TMA and acetate (Naumann et al., 1983). Table 1 shows bacteria associated with the formation of TMA through betaine degradation.

Ergothioneine. Ergothioneine, a biogenic amine, is a derivative of histidine. Production of ergothioneine is common among fungi and in certain bacteria, such as *Actinobacteria*, *Cyanobacteria*, *Bacteroidetes*, and *Proteobacteria* spp. (Seebeck, 2010; Pfeiffer et al., 2011). Ergothioneine is also present in mammals, where it is concentrated in specific tissues or cells, such as kidney, liver, and erythrocytes (Cheah and Halliwell, 2012). Mammals lack the ability to synthesize ergothioneine, however, and humans derive the compound exclusively from dietary sources such as mushrooms (bolete and oyster), some meat products (kidney and liver), and some plant products (e.g., black beans, red beans, oat bran) (Ey et al., 2007). The biologic role of ergothioneine in humans is unknown, but it is thought to have antioxidant and cytoprotectant properties (Cheah and Halliwell, 2012). When grown on ergothioneine, some bacteria are able to use the compound as a sole source of nitrogen, carbon, or energy. The degradation of ergothioneine, catalyzed by ergothionase (EC 4.3.3.-), yields TMA and thiolurocanic acid (Muramatsu et al., 2013) (Fig. 1). Table 1 shows bacteria associated with the production of TMA from ergothioneine.

Metabolism of TMA by Gut Bacteria

Two pathways for microbial metabolism of TMA have been proposed (Colby and Zatman, 1973). One involves the sequential oxygenation of TMA and demethylation of its oxygenated form to yield dimethylamine and formaldehyde (Fig. 2). This pathway is mediated by a trimethylamine monooxygenase (EC 1.14.13.148) and a trimethylamine-oxide aldolase (EC 4.1.2.32) and has been demonstrated to occur in *Pseudomonas aminovorans* (Large et al., 1972; Boulton et al., 1974), *Bacillus* (Myers and Zatman, 1971), and methylotrophs such as *Hyphomicrobium* (Meiberg et al., 1980; Barrett and Kwan, 1985). The other pathway involves an energetically more favorable direct *N*-demethylation of trimethylamine to give dimethylamine and formaldehyde, catalyzed by trimethylamine dehydrogenase (EC 1.5.8.2) (Colby and Zatman, 1973) (Fig. 2). In methylotrophs, dimethylamine is further metabolized to methylamine (MA) and then ammonia, each step producing formaldehyde, by reactions catalyzed by dimethylamine dehydrogenase (EC 1.5.8.1) and MA dehydrogenase (amicyanin) (EC 1.4.9.1), respectively (Asatoor and Simenhoff, 1965; Colby and Zatman, 1973; Barrett and Kwan, 1985) (Fig. 2).

Oral and Vaginal Microbiota

The gut, although the major site of TMA production, is not the only niche with resident bacterial species capable of producing TMA. Patients

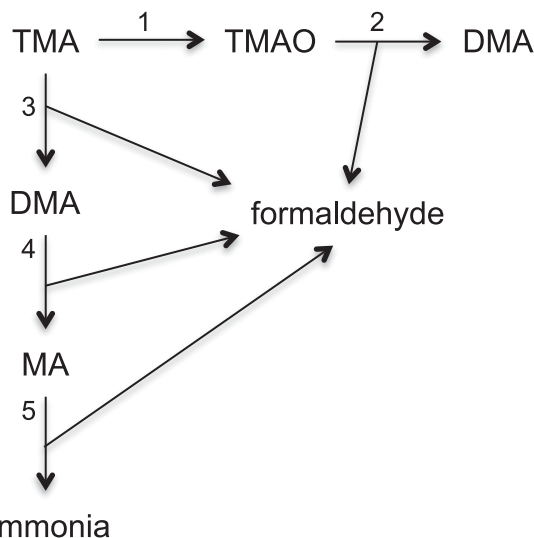


Fig. 2. Metabolism of TMA by gut bacteria. TMA can be metabolized to TMAO, which is subsequently converted to dimethylamine (DMA) and formaldehyde. TMA can be converted by direct *N*-demethylation to DMA and formaldehyde. In methylotrophs, DMA can be further converted to MA and then ammonia, each step producing formaldehyde. Reactions are catalyzed by 1, TMA monooxygenase; 2, trimethylamine-oxide aldolase; 3, trimethylamine dehydrogenase; 4, dimethylamine dehydrogenase and 5, methylamine dehydrogenase (amicyanin). Based on Colby and Zatman (1973). Names of enzymes are those currently recommended by the Enzyme Commission, and some differ from those given in the original papers.

with TMAU report a strange taste in their mouth and may suffer from bad breath (Mitchell, 2005; Wise et al., 2011). Halitosis is primarily the result of microbial production of volatile sulfur compounds, short-chain fatty acids, and diamines (Scully and Greenman, 2012); however, TMA can also contribute to foul breath odor, either being produced in situ from choline (Chao and Zeisel, 1990) or as a result of TMA circulatory overload (Mitchell, 2005). One specific member of the oral microbiota, *Streptococcus sanguis I*, has been shown to form TMA from choline, presumably as a result of enzymatic action on the carbon-nitrogen bond of choline (Chao and Zeisel, 1990).

In bacterial vaginosis, the vaginal discharge has a prominent fishy odor owing to elevated amounts of TMA (Brand and Galask, 1986; Oakley et al., 2008). Vaginal TMA can arise from the degradation of carnitine and choline or from the reduction of TMAO produced by some methylotrophs (Barrett and Kwan, 1985) (see previous discussion). Normally, *Lactobacillus* and *Atopobium* spp. are the predominant members of the vaginal microbiota. In cases of bacterial vaginosis, *Lactobacillus* appears to be replaced by genera such as *Gardnerella*, *Pseudomonas*, *Mobiluncus*, *Bacteroides*, *Prevotella*, *Porphyromonas*, and *Streptococcus* (Cruden and Galask, 1988; Hyman et al., 2005; Armougom and Raoult, 2009). Thus, compared with a healthy vaginal microbiota, bacterial vaginosis is associated with a higher proportion of *Actinobacteria* and *Bacteroidetes* phyla and a lower proportion of *Firmicutes* (Oakley et al., 2008). *Mobiluncus* strains isolated from patients with bacterial vaginosis can produce TMA by reduction of TMAO (Cruden and Galask, 1988; Wolrath et al., 2002) and, to a lesser extent, by degradation of choline (Cruden and Galask, 1988). One *Bacteroides* isolate has also been shown to produce TMA from choline (Cruden and Galask, 1988).

As described already, methylotrophs can produce MA, their main source of energy, from TMA (Barrett and Kwan, 1985). Thus, excess production of TMA in the mouth and vagina in halitosis and bacterial vaginosis, respectively, would provide environments favorable to methylotrophs. Several methylotrophic strains have been shown to be present in the microbiota of the human mouth (Anesti et al., 2005).

Noncommensal Bacteria Able to Produce TMA

In humans, TMA-producing bacteria are not limited to the commensal flora. Some noncommensal bacteria, including pathogens such as *Aeromonas*, *Burkholderia*, *Campylobacter*, *Salmonella*, *Shigella* and *Vibrio*, are able to form TMA (Wood and Keeping, 1944; Lerke et al., 1965; Kwan and Barrett, 1983; Muramatsu et al., 2013) from TMAO, carnitine, choline or ergothioneine. Noncommensal bacteria that produce TMA are shown in Table 2.

TMA/TMAO Host-Microbiome Metabolic Axis in Health and Disease

TMA is produced from dietary precursors by the action of gut bacteria and is metabolized to TMAO in the liver by a reaction catalyzed by FMO3 (Fig. 3A).

Factors Affecting the Production of TMA from Dietary Precursors. Production of TMA by microbial gut action is dependent on the type and amount of TMA precursors in the diet, the foodstuffs in which they are present, and the identity and relative abundance of bacterial species present in the gut.

A study of healthy male volunteers found that, in each individual, there were striking differences in the amount of TMA produced from various dietary precursors (15 mmol) administered orally in pure form, as judged by urinary analysis of TMA and TMA *N*-oxide (Zhang et al., 1999). TMAO was the best source, with almost 80% of the dose being excreted in urine as TMA or TMA *N*-oxide, followed by choline (about 60%) and carnitine (about 30%); however, betaine, creatinine and lecithin, although containing a trimethylamino moiety, elicited no significant increase in urinary excretion of either TMA or TMAO in any of the individuals studied. In addition, comparison of the amounts of TMA released by chemical hydrolysis of foods in vitro with those

obtained from biologic digestion of the foods in vivo revealed that marine fish and seafood are by far the best source of dietary-derived TMA, with up to 85% of the TMA content of the food being liberated by biologic digestion. In contrast, red meat, despite its high content of carnitine, a good source of TMA when ingested in its pure form, is a relatively poor source of TMA, with only 5% and 12% of the TMA content of lamb and beef, respectively, being released by biologic digestion (Zhang et al., 1999; Mitchell et al., 2002). These studies, although on a relatively small number of individuals, indicate that amounts of TMA derived from digestion of a particular food cannot be accurately predicted on the basis of the content of the trimethylamino moiety in the food.

The human gut microbiome displays considerable interindividual and intraindividual variation (Caporaso et al., 2011; Yatsunen et al., 2012). Such variation would be expected to influence the relative yield of TMA produced from various foodstuffs. The identity and relative abundance of gut microbes can be investigated by bacterial cultivation; however, this approach is limited by the inability to culture some members of the microbiota (Zoetendal et al., 2008). Although sequence determination of 16S rDNA is able to identify most major phyla, it is thought to underestimate *Actinobacteria* (Totter et al., 2013), which are known to produce TMA from TMAO and choline (Table 1). The development of next-generation deep-sequencing methods will provide more accurate knowledge of the identity and relative abundance of bacterial species present in the human gut.

In addition to the identification of bacteria, it is necessary to establish their function with respect to their ability to produce or metabolize TMA. There are several pathways resulting in the formation of TMA (Fig. 1), and production of TMA is not exclusive to any one bacterial phylum. Current taxonomic classification is based on DNA sequence rather than on bacterial function. Thus, it is possible for a bacterial species

TABLE 2
Noncommensal bacteria that produce trimethylamine (TMA) from various substrates

Phylum	Genus or Species	References
TMA <i>N</i>-oxide		
Bacteroidetes <i>Proteobacteria</i>	<i>Flavobacterium</i>	Robinson et al., 1952
	<i>Achromobacter</i>	Campbell and Williams, 1951; Lerke et al., 1965
	<i>Aeromonas</i>	Lerke et al., 1965; Lin and Hurng, 1989
	<i>Alteromonas</i>	Lerke et al., 1965; Lee et al., 1977; Easter et al., 1982, 1983; Ringø et al., 1984
	<i>Photobacterium</i>	Dalgaard, 1995
	<i>Rhodopseudomonas</i>	Madigan and Gest, 1979; Cox et al., 1980; Madigan et al., 1980
	<i>Salmonella</i>	Kwan and Barrett, 1983
	<i>Shewanella</i> ^a	Dalgaard, 1995; López-Caballero et al., 2001; Shaw and Shewan, 1968
	<i>Shigella</i>	Wood et al., 1943; Campbell and Williams, 1951
	<i>Vibrio</i>	Lerke et al., 1965; Unemoto et al., 1965; Shaw and Shewan, 1968; Easter et al., 1982
Betaine		
<i>Firmicutes</i> <i>Proteobacteria</i>	<i>Haloanaerobacter</i>	Mouné et al., 1999
	<i>Desulfuromonas</i>	Heijthuisen and Hansen, 1989
Carnitine		
<i>Proteobacteria</i>	<i>Achromobacter</i>	Zhu et al., 2014
	<i>Serratia</i>	Unemoto et al., 1966
	<i>Shigella</i>	Zhu et al., 2014
Choline		
<i>Proteobacteria</i>	<i>Achromobacter</i>	Campbell and Williams, 1951
	<i>Shigella</i>	Wood and Keeping, 1944
Ergothioneine		
<i>Proteobacteria</i>	<i>Burkholderia</i>	Muramatsu et al., 2013
	<i>Campylobacter</i>	Muramatsu et al., 2013
	<i>Stigmatella</i>	Muramatsu et al., 2013
	<i>Sinorhizobium</i> ^b	Muramatsu et al., 2013

^aBasionym: *Alteromonas purefaciens*.

^bBasionym: *Rhizobium meliloti*.

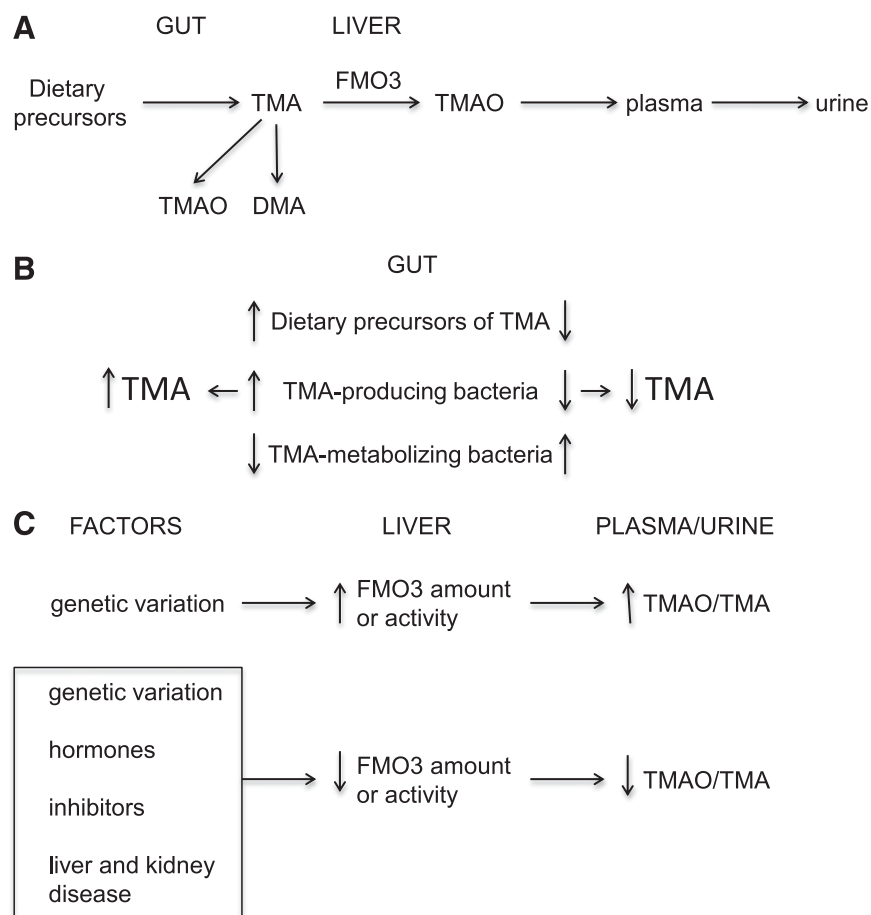


Fig. 3. The origin of TMA and TMAO and factors that influence their production. (A) The production and metabolic fate of TMA. TMA is produced from dietary precursors by the action of gut bacteria. TMA can be metabolized by gut bacteria to TMAO and dimethylamine (DMA) (see Fig. 2) or absorbed and converted in the liver to TMAO in a reaction catalyzed by FMO3. TMAO and any unmetabolized TMA will enter the plasma and subsequently will be excreted in urine. (B) Factors affecting the production of TMA in the gut. The amount of TMA produced is dependent on the nature and quantity of dietary precursors and the relative abundance of TMA-producing and TMA-metabolizing bacteria. (C) Factors influencing TMA metabolism in the host. The metabolism of TMA to TMAO in liver is dependent on the amount and activity of FMO3. The abundance and activity of FMO3 can be increased as a consequence of genetic variation, resulting in an increase in the ratio of TMAO:TMA. The amount and activity of FMO3 can be decreased by genetic variation, hormones, inhibitors, and disease, resulting in a decrease in the ratio of TMAO:TMA.

belonging to one phylum to share metabolic activity with species belonging to other phyla but not with other members of the same phylum. Based on DNA sequence analysis, *Bacteroidetes* lack the *cutC* and *cntAB* genes (Craciun and Balskus, 2012; Zhu et al., 2014) and, thus, would appear unlikely to produce TMA from choline or carnitine; however, in vivo cultivation studies show that some members of the *Bacteroidetes* phylum are able to produce TMA from choline (Cruden and Galask, 1988). In addition, not all strains that encode CutC are able to produce TMA from choline, and even within the same species, strains may differ in the ability to convert choline to TMA (Romano et al., 2015). Thus, one should be cautious when using phylogeny as the sole predictor of microbial TMA production or as an indicator of metabolic capacity in general.

The lack of any increase in urinary TMA or TMAO, in response to ingestion of betaine, in any of the individuals studied (Zhang et al., 1999; Mitchell et al., 2002) (see preceding) indicates that the metabolic reactions that produce TMA from betaine, either directly or via dimethylglycine (Fig. 1), or the bacteria in which these reactions occur, are not prevalent, at least in healthy British men. The inability to generate TMA from betaine also indicates that the potential routes for production of TMA from choline or carnitine, via their initial conversion to betaine (Fig. 1), do not make significant contributions to TMA production.

Factors affecting the production of TMA in the gut are summarized in Fig. 3B.

Factors Influencing TMA Metabolism in the Host. Individuals who consume a 'typical' Western diet will produce, via the action of gut bacteria, about 50 mg of TMA/day (Walker and Wevers, 2012), most of which (~95%) is converted to TMAO, which is excreted in

the urine (Mitchell and Smith, 2001). Thus, FMO3 is efficient in dealing with a relatively high substrate load; however, even individuals with good FMO3 catalytic capacity can become overloaded when challenged with large amounts of TMA precursors (Al-Waiz et al., 1989; Mitchell and Smith, 2016). Dietary components such as indoles, found in members of the *Cruciferae* family of vegetables, have been shown to inhibit the activity of FMO3 in vitro and, in a human study, to decrease the amount of TMAO produced as a percentage of total TMA (TMA + TMAO) excreted (Cashman et al., 1999).

Gender-specific effects in humans also influence the amount of urinary TMA. In a study of men over a 30-day period, only a slight variation in the amount of TMA excreted in the urine was seen when expressed as a percentage of total TMA (TMA + TMAO) (Mitchell and Smith, 2010). In contrast, women excreted increased urinary TMA around the time of menstruation, and this increase lasted for ~7 days (Mitchell and Smith, 2010). This increase in TMA excretion is the result of a reduction in its metabolism to TMAO, which is thought to be due to the action of female sex hormones decreasing the expression of the *FMO3* gene (Coecke et al., 1998). In men, high levels of testosterone also have been found to reduce FMO3 capacity (Ayesh et al., 1995).

Primary TMAU is known to be due to mutations that result in impaired catalytic activity of FMO3 and, hence, increased urinary excretion of TMA; however, other situations also cause an increase in TMA excretion (Mitchell and Smith, 2016), for example, impaired liver function (Mitchell et al., 1999), impaired renal function (Bain et al., 2006; Hur et al., 2012; Hao et al., 2013), and pancreatic cancer (Navaneethan et al., 2014).

The expression of the *FMO3* gene in human liver is switched on between birth and 2 years of age and increases during childhood and adolescence, reaching its maximum level in adults (Koukouritaki et al., 2002). Thus, in comparison with adults, children, especially infants, have lower amounts of FMO3. Consequently, they are less able to metabolize TMA to TMAO and, thus, may exhibit symptoms of transient childhood TMAU, particularly if they are homozygous for two common polymorphic variants of *FMO3*, c.472G>A [p.(Glu158Lys)], and c.923A>G [p.(Glu308Gly)], which moderately reduce catalytic activity (Zschocke and Mayatepek, 2000). One polymorphic variant of *FMO3*, c.1079T > C [p.(Leu360Pro)], increases catalytic activity (Lattard et al., 2003); however, the variant has been detected only in African populations and then at low frequency. Polymorphic promoter-region variants of the *FMO3* gene have been identified that severely reduce transcription in vitro, whereas others increase transcription (Koukouritaki et al., 2005), but the impact of these variants on expression of *FMO3* in vivo has not been validated. Factors influencing the metabolism of TMA to TMAO in the liver are summarized in Fig. 3C.

Potential Involvement of TMAO and FMO3 in Disease. Recently, TMAO has been implicated in a number of disease states as a cause, consequence, or biomarker of the disease. It has been suggested that an increase in the plasma concentration of TMAO, as a consequence of production of TMA from dietary supplements of choline, carnitine, or TMAO by gut bacteria and its subsequent oxygenation by FMO3 in the liver, increases the risk of cardiovascular disease (Wang et al., 2011; Bennett et al., 2013; Koeth et al., 2013; Tang et al., 2013; Obeid et al., 2016). TMAO has also been implicated in chronic kidney disease (Tang et al., 2015), colorectal cancer (Xu et al., 2015), and in impaired glucose tolerance in mice fed a high-fat diet and a TMAO supplement (Gao et al., 2014); however, lower plasma concentrations of TMAO have been associated with inflammatory bowel disease and with active versus inactive ulcerative colitis (Wilson et al., 2015).

A number of roles for FMO3 itself in health and disease have been suggested, based on studies using mice. Knockdown of FMO3 mRNA by antisense oligonucleotides in female low-density lipoprotein receptor-knockout mice suggests a role for FMO3 in modulating glucose and lipid homeostasis (Shih et al., 2015). FMO3 has also been identified as a target of insulin (Miao et al., 2015): *Fmo3* expression was more than 1000-fold higher in the livers of male liver insulin receptor-knockout (LIRKO) mice compared with littermate controls, whereas knockdown of FMO3 in LIRKO mice reduced expression of the transcription factor forkhead box O1 and prevented the development of hyperglycemia, hyperlipidemia, and atherosclerosis. Other suggested roles for FMO3 include involvement in cholesterol metabolism and reverse cholesterol transport (Bennett et al., 2013; Warriar et al., 2015).

The implication that TMAO itself is a causative factor for cardiovascular and other diseases is controversial. A recent review considers in detail the physiologic roles of TMAO and the evidence for and against TMAO being detrimental for health (Ufnal et al., 2015). The link between TMAO levels and atherosclerosis seems counterintuitive considering that consumption of marine fish, the richest dietary source of TMAO (Zhang et al., 1999), is firmly associated with a reduction in the incidence of cardiovascular disease (Takata et al., 2013). Other studies found no correlation of TMAO levels with biomarkers of cardiovascular disease. For instance, elevated levels of TMAO caused no increase in C-reactive protein or low-density lipoprotein, in either mouse or human plasma (Miller et al., 2014), and a study of oral carnitine supplementation of patients undergoing hemodialysis showed that, although TMAO was increased, markers of vascular injury were reduced, and, consequently, supplementation with carnitine is thought to be beneficial for these patients (Fukami et al., 2015). Indeed, carnitine

has proven efficacious for treatment of cardiovascular disease (Flanagan et al., 2010).

Many of the studies that implicate TMAO as a causative factor in cardiovascular disease are based on studies of mice and involve chronic administration of pure TMA precursors in amounts that far exceed those present either in a normal diet or in therapeutic supplements. Indeed, in the case of carnitine, significant increases in dietary-derived TMA in humans are obtained only with high chronic dose regimens (Holmes et al., 1997). Consequently, normal dietary conditions or moderate supplementation are unlikely to elicit the production of the amounts of TMAO that have been associated with cardiovascular disease.

When designing experimental studies on mice and extrapolating the results of these experiments to humans, it is important to appreciate that there is a species-specific gender difference in the expression of FMO3 between humans and mice. At about 6 weeks of age, expression of the *Fmo3* gene is switched off in the liver of male, but not of female, mice (Falls et al., 1995; Janmohamed et al., 2004). After this age, male mice produce no hepatic FMO3 protein; thus, in comparison with female mice, produce far less TMAO and, consequently, excrete larger amounts of TMA in their urine. Despite this marked gender-specific difference, there is no obvious difference in the propensity of male and female wild-type mice to develop cardiovascular disease. A study of adult C57BL/6 wild-type mice found no difference in plasma glucose between male and female mice and showed that females, which express hepatic FMO3 and thus produce TMAO, have lower plasma cholesterol than males (Gonzalez Malagon et al., 2015). A further complication is that humans do not express *FMO1* in liver (Dolphin et al., 1991; Phillips et al., 1995; Koukouritaki et al., 2002), in marked contrast to all other mammals investigated, in which FMO1 is a major form of FMO in this tissue (Hernandez et al., 2004).

Evidence has shown that the *FMO3* gene of humans has been the subject of balancing natural selection (Allerston et al., 2007). Three alleles appear to be targets for selection: the ancestral allele, an allele encoding an enzyme with moderately reduced catalytic activity, and one associated with increased promoter activity. Heterozygotes for a loss-of-function mutation of *FMO3*, despite having a 50% reduction of FMO3 activity, are able to convert ~95% of a normal dietary load of TMA to TMAO and, in this respect, are indistinguishable from individuals homozygous for a functional *FMO3* gene (Al-Waiz et al., 1987c). This finding indicates that it is unlikely that the decreased capacity to produce TMAO was the evolutionary advantage that drove selection of the allele encoding a FMO3 with reduced activity.

An alternative explanation for high levels of TMAO associated with cardiovascular disease is that the concentrations are indicative of dysfunction(s) elsewhere and that TMAO is not the mediator but is merely a marker of the disease (Ufnal et al., 2015). In mice, TMAO increases in response to a high-fat diet (Yang et al., 2014). High-fat diets change the composition of the gut microbiome to one associated with obesity and its related health problems. These changes increase the relative proportion of *Firmicutes* (Ley et al., 2006), a phylum whose members contribute to TMA production, and decrease that of *Bacteroidetes*, a phylum that contributes very little to TMA production (Table 1). Elevated plasma and urinary TMAO, therefore, may be a marker for a gut microbiome that has deleterious effects on health and not itself a causative factor. Indeed, atherosclerosis indices were increased in mice in response to a high-fat diet, but dietary supplementation with TMAO had the opposite effect, significantly decreasing atherosclerosis indices (Gao et al., 2014). It is important to note that no health benefits have been reported for TMAU individuals, who produce very low amounts of TMAO because of their impaired FMO3 activity.

Implications for TMAU. One disorder known unambiguously to be associated with FMO3 is primary TMAU, caused by mutations in the

FMO3 gene (Dolphin et al., 1997) (see previous discussion), a condition characterized by secretion of excessive amounts of odorous TMA (Mitchell and Smith, 2001). There is no cure for the disorder, and current treatment is aimed at management of the symptoms. The main approach is restriction of dietary precursors of TMA. Although humans may possess gut bacteria capable of producing TMA from choline and carnitine when administered in pure form, they are much less able to access these precursors from foodstuffs such as red meat and peanuts (Zhang et al., 1999; Mitchell et al., 2002). In contrast, the precursor TMAO is efficiently extracted in the gut from marine fish and seafood (Zhang et al., 1999; Mitchell et al., 2002). These results have implications for dietary management of TMAU, indicating that the most important foodstuffs to avoid are marine fish and seafood and those that contain free choline, such as soya bean-derived milk, whereas red meat would contribute less to TMA load.

Another strategy would be manipulation of gut flora to reduce the proportion of bacteria that produce TMA and increase the proportion of those that metabolize TMA. Although TMA-producing species are widely distributed across bacterial phyla, they are more common in *Firmicutes* and relatively scarce in *Bacteroidetes* (Table 1). Consequently, decreasing the ratio of *Firmicutes* to *Bacteroidetes* would be expected to reduce production of TMA from dietary precursors. A low ratio of *Firmicutes* to *Bacteroidetes* is associated with a healthy microbiome (Ley et al., 2006); thus, such alteration of the gut microbiome would promote general health as well as aiding in the management of TMAU.

One approach to altering the composition of the gut microbiome is exogenous supplementation with beneficial species (probiotics); however, a recent study found that the multi-strain probiotic VSL#3 had no effect on the increase in plasma concentration of TMAO in individuals fed a high-fat diet (Boutagy et al., 2015). Another proposal is the therapeutic use of methylotrophic strains of archaea (archaeobiotics) that use TMA as an energy source (Brugère et al., 2014), although this has yet to be tried.

A further possibility is the selective inhibition of bacterial enzymes that catalyze reactions involved in the production of TMA. Candidates are TMA reductase, CutC, and CntAB, which respectively catalyze TMA production from TMAO, choline and carnitine (Fig. 1). For instance, 3,3-dimethyl-1-butanol, a structural analog of choline, inhibits microbial CutC and reduces the plasma concentration of TMAO in mice fed diets rich in choline or carnitine (Wang et al., 2015); however, most of the choline eaten by humans is in the form of lecithin, which is not a good dietary source of TMA (Zhang et al., 1999); thus, the efficacy of the inhibitor for reducing TMA production in human gut is unclear.

Conclusions

TMA is derived from dietary precursors via the action of gut bacteria. The amount of TMA absorbed by humans is determined by the type and quantity of dietary precursors and the composition of the gut microbiome, in particular with respect to bacteria that produce or consume TMA. TMAO, choline, and carnitine, but not betaine, creatinine, and lecithin, are good sources of TMA in their pure form. Marine fish and seafood are the best food sources of TMA, but red meat, despite its high content of carnitine, is a poor source. Consequently, it is not possible to predict the amount of TMA that will be derived from a particular food on the basis of the content of the trimethylamino moiety in the food. Bacteria from several phyla produce TMA from dietary precursors but are more common in *Firmicutes* and scarce in *Bacteroidetes*.

FMO3, its microbiome-derived substrate TMA and the product of FMO3 catalysis, TMAO, have been implicated in a number of disease

states. That mutations of *FMO3* cause the inherited disorder primary TMAU, through a failure to convert TMA to TMAO, is well established; however, TMAO has attracted increasing notoriety as a molecule that might be harmful for health. The evidence for this is controversial, and there are studies for and against the importance of TMAO in conditions affecting health. No known health benefits have been reported as a consequence of the lack or low production of TMAO in primary TMAU patients. Large, well-designed clinical studies are needed to examine whether changes in the plasma concentration of TMAO in non-TMAU patients are a consequence of the diet of an individual or of the bacterial species that populate their gut, which may well change in response to the health status of an individual. It is intriguing that consumption of marine fish, the richest dietary source of TMAO, is beneficial, not harmful, to cardiovascular health.

In addition, FMO3 has a wide range of drug substrates. This, together with its intimate relationship with the microbiome-produced FMO3 substrate TMA, indicates that changes in composition of the gut microbiome might influence both the metabolism and efficacy of therapeutic drugs.

Authorship Contributions

Contributed to the writing of the manuscript: Fennema, Phillips, Shephard.

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