REVIEW ARTICLE

Trimethylamine N-oxide: breathe new life

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Association between elevated levels of systemic trimethylamine N-oxide (TMAO) and increased risk for adverse cardiovascular events have been proposed in recent years. Increasing experimental and clinical evidence in the last decade has implicated TMAO as an important contributor to the pathogenesis of cardiovascular diseases. TMAO, the oxygenated product of trimethylamine (TMA), belongs to the class of amine oxides. Most of the TMA derived from the metabolism of choline and L-carnitine by gut bacteria is absorbed into the bloodstream and gets rapidly oxidized to TMAO by the hepatic enzyme, flavin-containing monooxygenase-3. Here, we discussed the biosynthesis of TMAO and clinical studies that have assessed TMAO as a biomarker for various cardiovascular and other diseases such as kidney failure, thrombosis, atherosclerosis, obesity, diabetes and cancer. We also summarized the interaction of TMAO with synthetic and traditional molecules that together affect circulating TMAO levels.

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Abbreviations

CAC, coronary artery calcium; cIMT, carotid intima-media thickness; CVD, cardiovascular disease; FMO3, flavin-containing monooxygenases; IBD, inflammatory bowel disease; HFD, high-fat diet; HR, hazard ratio; MDA, malondialdehyde; TF, tissue factor; TMA, trimethylamine; TMAO, trimethylamine N-oxide; UC, ulcerative colitis; VCAM-1, vascular cell adhesion molecule 1

Introduction

Our understanding of the pathophysiological mechanisms underlying cardiovascular diseases (CVD) has increased exponentially over the past two decades. Current advances in research have led to the accumulation of more evidence to support this claim, specifically the link between the gut flora and 'new age' disorders, such as obesity (Musso *et al.*, 2010), insulin resistance (Musso *et al.*, 2010; Diamant *et al.*, 2011; Miele *et al.*, 2015; Tai *et al.*, 2015), atherosclerosis (Chistiakov *et al.*, 2015) cancer (Lewis, 1971; Ray, 2012; Erdman and Poutahidis, 2015; Dey and Ciorba, 2016) and neurological complications (Cryan and Dinan, 2012). In recent years, an association of elevated levels of systemic trimethylamine N-oxide (TMAO) with increased risk for adverse cardiovascular events has been proposed (Wang *et al.*, 2011; Koeth *et al.*, 2013; Kaysen *et al.*, 2015; Miele *et al.*, 2015; Randrianarisoa *et al.*, 2016; Zheng *et al.*, 2016). TMAO, the oxygenated product of **trimethylamine (TMA)**, belongs to the class of amine oxides (Ufnal *et al.*, 2015). Most of the TMA derived from the metabolism of **choline** and **L-carnitine** by bacteria in the gut is absorbed into the bloodstream, and then TMA is rapidly oxidized to TMAO by the hepatic enzyme, **flavin-containing monooxygenase-3 (FMO3)** (Zeisel, 1981; Zeisel *et al.*, 1983; Wang *et al.*, 2011). Saltwater fish contain the highest amounts of TMAO (~3 g·kg⁻¹) of all food sources (Mitchell *et al.*, 2002).

Many bacterial species are involved in producing TMA including *Clostridia*, *Proteus*, *Shigella* and *Aerobacter* (Zeisel *et al.*, 1983). The composition of the human gut flora changes with age. For instance, *Proteobacteria* predominates in the gut of neonates (~16%) but then substantially reduces in adulthood (~4.6%) (Gritz and Bhandari, 2015). Although gut microbiota appears to have both positive and negative effects on our health, most of those which exist in the large intestine, process many indigestible components of foods and convert them into 10% of our daily energy supply. 'New Age' foods rich in calories with reduced fibre, facilitate the aberrant expansion of *Proteobacteria*, as observed in both inflammatory bowel disease (IBD) and metabolic disorders (Wilson *et al.*, 2015; Wilson *et al.*, 2016). Particularly, an increased ratio of *Firmicutes/Bacteroidetes* has been associated with a high risk of cardiovascular disease (CVD; Abdallah Ismail *et al.*, 2011). During the onset of high-fat diet (HFD)-induced insulin resistance, the systemic levels of *Escherichia coli* increased several-fold, until a diabetic state was established, a finding strongly implying that dietary factors affect glucose homeostasis (Amar *et al.*, 2011). Furthermore, treatment with the probiotic *Bifidobacterium animalis* improved insulin sensitivity and this effect correlated with decreased bacterial translocation. Thus, the probiotic intervention could reverse the HFD-induced adverse metabolic phenotype (Amar *et al.*, 2011). This link was further supported by a study in which a group of mice fed a HFD together with *Akkermansia muciniphila* were protected from developing insulin resistance, had improved gut integrity and less tissue inflammation compared to those fed with the HFD alone. However, when the mice were fed the HFD with heat killed *A. muciniphila*, the protective effects were also lost (Everard *et al.*, 2013). In contrast, few research findings suggest that

TMAO is a metabolic waste product that causes a pro-inflammatory responses and a renal toxicity. Also, the higher *Firmicutes* to *Bacteroidetes* ratio among men exhibiting a greater response to dietary TMAO precursor indicates that TMAO production is a function of individual differences in the gut microbiome (Cho *et al.*, 2016). Based on the recent health studies, the Nutrition Board of the Institute of Medicine (<http://www.ncbi.nlm.nih.gov/books/NBK114310>) and the United States Department of Agriculture have provided the recommended levels of dietary TMAO (Patterson *et al.*, 2008) (Table 1).

Importantly, accumulating data suggest that the microbial metabolite TMAO is linked to cardiovascular and metabolic diseases, though the molecular mechanisms remain elusive. Here, based on the recent findings, we reviewed the biosynthesis and distribution of TMAO and how this affects the health system for the development of cardiovascular and metabolic diseases.

Biosynthesis, distribution and elimination of TMAO

Red meat, eggs, dairy products and salt-water fish are rich in choline, lecithin and L-carnitine and are a potential source of TMAO (Cho and Caudill, 2017; Koeth *et al.*, 2014). TMAO is derived primarily from dietary choline and carnitine through the action of gut microbiota, which metabolizes these constituents to TMA. TMA is absorbed and travels *via* the portal circulation to the liver, where it is oxidized by flavin-containing monooxygenases, primarily FMO3, to TMAO (Bennett *et al.*, 2013; Koeth *et al.*, 2014; Wang *et al.*, 2011) (Figure 1). Systemic concentration of TMAO in normal healthy individuals ranges from 0.5–5 µM (Ufnal *et al.*, 2015; Wang *et al.*, 2011). Similar levels have been reported in

Table 1

Gender-based recommendations for dietary intake of choline per day

Gender-based recommendation	Intake of choline per day
Nutrition Board of the Institute of Medicine	
Men	550 mg
Women	425 mg
Food	Intake in 100 g of food
USDA Database	
Egg yolk	Approx. 250 mg
Meat/fish	Approx. 75 mg
Whole grains	Approx. 75 mg
Cereals	Approx. 50 mg
Fruits/vegetables	Approx. 30 mg
Milk	Approx. 10 mg
Fat and oils	Approx. 5 mg

Figure 1

Biochemical pathways for the production and metabolism of TMA and TMAO and pathological complications. Red meat, eggs, dairy products and saltwater fish are rich in choline, lecithin and L-carnitine and are a potential dietary source of TMAO. TMAO is derived primarily from choline and carnitine in food through the action of gut microbiota, which metabolizes these constituents to TMA. TMA is absorbed and travels via the portal circulation to the liver, where it is oxidized by flavin-containing monooxygenases, primarily FMO3, to TMAO. Biochemically, TMA is produced from TMAO by TMAO reductase. Choline, either in its free form or released from lecithin (phosphatidylcholine), contributes to the formation of TMA, via the action of choline TMA-lyase. Similarly, L-carnitine (derived from red meat) contributes to TMA formation directly via the action of a carnitine reductase/oxidase. Increased plasma concentration of TMAO leads to cardiovascular complications such as atherosclerosis and thrombosis; 3,3-dimethyl-1-butanol blocks the production of TMA. TMAO is mostly eliminated unchanged in urine within 24 h.

rodents (Ufnal *et al.*, 2015; Wang *et al.*, 2011). High Concentrations and dose-dependence of distribution volumes, along with the tissue-to-blood partition ratio suggest that the methylamines (TMA and TMAO) are distributed homogeneously throughout the body and to an extent translocate and accumulate at high concentrations in some tissues (Cho and Caudill, 2017; Smith *et al.*, 1994; Tang *et al.*, 2013; Velasquez *et al.*, 2016). In most subjects, 50% of the TMAO does not get metabolized and is excreted unchanged within 24 h through urine, sweat and breath (Ayesh *et al.*, 1993; Smith *et al.*, 1994; Zhang *et al.*, 1999). The remaining TMAO is reduced to TMA by the bacterial TMAO reductase in the human gut (Fennema *et al.*, 2016; Kwan and Barrett, 1983) (Figure 1).

Association of TMAO with heart failure

Hazen and coworkers found a correlation between elevated levels of TMAO in plasma and CVD risk in an untargeted metabolomics study involving patients undergoing elective cardiac evaluation (Wang *et al.*, 2011). Many studies have reported an association between plasma TMAO and choline levels in patients with heart failure (Tang *et al.*, 2014; Troseid *et al.*, 2015; Tang *et al.*, 2015b; Troseid *et al.*, 2016). Enhanced translocation of gut bacteria and the resultant increased inflammatory responses and oxidative stress is a consequence

of ischaemia and congestion within the intestines induced by heart failure. Moreover, dietary intake that influence gut microbiome and TMAO levels have been associated with heart failure outcomes (Verbrugge *et al.*, 2013; Nagatomo and Tang, 2015). Especially, elevated levels of plasma TMAO is associated with diastolic dysfunction (Tang *et al.*, 2015b) and poor survival (Troseid *et al.*, 2015). In an experimental study, the severity of heart failure was significantly enhanced in mice (C57BL6/J) fed diets supplemented with either the gut bacterial metabolite TMAO, or its precursor choline (Organ *et al.*, 2016). Also, TMAO-fed C57BL/6J mice showed increased levels of lung oedema, **natriuretic peptide** levels and ventricular remodelling which included fibrosis, chamber dilation, thinning of the walls and reduced ejection fraction (Organ *et al.*, 2016).

TMAO enhances the risk of inflammation, obesity, and atherosclerosis

Recent preclinical and clinical studies have demonstrated that TMAO is involved in metabolic diseases such as obesity and atherosclerosis. In experimental studies, supplementation with dietary choline, carnitine, precarnitine or even TMAO alone was sufficient to enhance macrophage

cholesterol load and atherosclerotic plaque formation (Wang *et al.*, 2011; Koeth *et al.*, 2013; Koeth *et al.*, 2014). TMAO also has significant effects on cholesterol metabolism in the **bile acid** compartments (Wang *et al.*, 2011; Koeth *et al.*, 2014). Wilson and colleagues studied the plasma TMAO levels in patients with IBD and ulcerative colitis (UC) and found them to be lower in the IBD patients (2.27 μM) compared with the healthy individuals. Similarly, lower TMAO levels were observed in patients with active UC (1.56 μM) versus inactive disease (3.40 μM) (Wilson *et al.*, 2015; Wilson *et al.*, 2016). In an experimental study, Van Hecke and colleagues studied the effect of white versus red meat consumption on oxidative stress, TMAO and inflammation in Sprague–Dawley rats. Compared with the chicken diets, consumption of beef diets resulted in higher urinary trimethylamine (4.5-fold) and TMAO (3.7-fold) concentrations. Also, rats on the fat beef diet had higher renal malondialdehyde (MDA; +24.4%) and heart MDA (+12.9%) and lower liver vitamin E (−26.2%) compared with all other diets such as lean beef and lean chicken diets (Van Hecke *et al.*, 2016). Interestingly, a study in a group of German adults concluded that consumption of meat, eggs or fish was not associated with plasma TMAO, choline or betaine concentrations. However, plasma TMAO levels were increased after consumption of milk and other dairy foods. Also, the increased plasma TMAO level was associated with the increased **TNF- α** , **IL-6** and C-reactive protein (Rohrmann *et al.*, 2016). In a clinical study, serum TMAO concentrations in patients undergoing haemodialysis (28–67 μM) were elevated compared to normal healthy individuals (1.41 $\pm$ 0.49 μM). Furthermore, TMAO was directly correlated with concentrations of serum albumin, pre-albumin and creatinine and inversely correlated with log concentration of C-reactive protein. However, higher serum concentrations of TMAO did not significantly associated with time to death or time to cardiovascular hospitalization or cardiovascular death (Kaysen *et al.*, 2015). In an elegant study, Trøseid *et al.* compared the TMAO levels in obese patients and patients after bariatric surgery. TMAO levels were not elevated in obese patients or reduced by lifestyle interventions although, after bariatric surgery, TMAO levels increased approximately twofold. However, carnitine and choline, which are abundant in red meat and eggs, and not products of the microbiota, were reduced after lifestyle interventions and rebounded after bariatric surgery (Trøseid *et al.*, 2016).

Obeid *et al.* assessed cardiometabolic risk factors and pathways associated with TMAO concentrations in humans. High TMAO and choline concentrations were associated with an advanced cardiometabolic risk profile. Diabetes was related not only to higher plasma TMAO concentrations but also to alterations in interrelated pathways such as lipids, phospholipids and methylation. Elevated plasma TMAO concentrations were likely to reflect a specific metabolic pattern characterized by low high-density lipoprotein (HDL) and phospholipids, in addition to hypomethylation (Obeid *et al.*, 2016).

Senthong and colleagues studied the association between plasma levels of TMAO and peripheral artery disease (PAD). Elevated TMAO levels were associated with 2.7-fold increased mortality risk (hazard ratio; HR, 2.86, 95% CI 1.82–3.97). Similar prognostic value for elevated TMAO was seen in patients with carotid artery, non-carotid artery or lower

extremity PAD (Senthong *et al.*, 2016). In line with the previous reports, plasma TMAO levels were significantly higher in patients with ruptured plaque (8.6 $\pm$ 4.8 μM) compared to those without plaque rupture (4.2 $\pm$ 2.4 μM). These data suggest that circulating TMAO level may reflect coronary plaque vulnerability and progression (Fu *et al.*, 2016).

In contrast, Meyer and colleagues studied the associations between TMAO and coronary artery calcium (CAC) and carotid intima-media thickness (cIMT) in a study of coronary artery risk development in young adults (CARDIA). In this population-based study, TMAO did not show an association with measures of atherosclerosis such as CAC incidence, CAC progression or cIMT. These data indicate that TMAO may not contribute significantly to the progression of early atherosclerotic disease risk among healthy early–middle-aged adults (Meyer *et al.*, 2016). TMAO promotes the early pathological process of atherosclerosis by accelerating endothelial activation and dysfunction, including decreasing endothelial self-repair and increasing monocyte adhesion by activation of a **PKC/NF- κB /vascular cell adhesion molecule 1 (VCAM-1)** pathway; (Ma *et al.*, 2017).

Gut microbiota and the effects of TMAO on glucose homeostasis and diabetes

Evidence suggests that TMAO negatively regulates glucose metabolism, which leads to diabetes mellitus. In a clinical study, Zheng and colleagues examined the association of dietary consumption of **phosphatidylcholine**, from eggs, red meat and fish, and CVD mortality in two cohorts of US women and men. The study outcome concluded that the association of phosphatidylcholine with all-cause and CVD mortality was stronger in diabetic than in healthy individuals (Zheng *et al.*, 2016). Also, increased levels of serum TMAO levels were associated with increased cIMT, independently of established cardiovascular risk markers, including insulin resistance, visceral obesity and fatty liver. Moreover, the reduction of cIMT during lifestyle intervention was related to the decrease in TMAO levels (Randrianarisoa *et al.*, 2016). Dambrova and colleagues studied the association between plasma TMAO and diabetes and proposed that age, diabetes and body mass index were associated with higher plasma TMAO levels, independently of L-carnitine (Dambrova *et al.*, 2016). Diabetes was related not only to higher plasma TMAO concentrations but also to alterations in interrelated pathways such as lipids, phospholipids and methylation (Obeid *et al.*, 2016). An observational clinical study demonstrated that elevated plasma TMAO levels were a strong risk marker in diabetes. High TMAO levels in diabetic patients was a marker of adverse cardiovascular outcomes, such as death (HR 2.7; 1.1–7.1), myocardial infarction (HR 4.0; 1.6–9.8), heart failure (HR 4.6; 2.0–10.7), unstable angina (HR 9.1; 2.8–29.7) and other cardiovascular events (HR 2.0; 1.1–3.6) (Lever *et al.*, 2014). Similarly, within patients with type-2 diabetes, higher plasma TMAO levels were associated with a significant 3.0-fold increased 3 year major adverse cardiac event risk ($P < 0.001$) and a 3.6-fold increased 5 year mortality risk ($P < 0.001$) (Tang *et al.*, 2016). In contrast, McEntyre *et al.*, in an observational study, found that the TMAO levels were highly variable in the plasma and urine of overweight

individuals with type-2 diabetes over a two-year period. Furthermore, given the high variability of TMAO levels, TMAO readout alone was suggested to have low diagnostic value (McEntyre *et al.*, 2015). Thus, the clinical, observation and diagnostic studies demonstrate that the TMAO levels are elevated during diabetic state. Although the mechanisms behind the risk are still undefined, TMAO could be a useful marker to predict the risk of diabetes and its complications. To date, there is very limited experimental evidence demonstrating role of TMAO in diabetes mellitus. Interestingly, Xu *et al.* indicated that the tissues of diabetic mice have lesser amounts of TMAO and choline than those from control mice (Xu *et al.*, 2009). Furthermore, treatment of rats with TMAO neutralized endoplasmic reticulum stress *via* enhanced protein folding (Lupachyk *et al.*, 2013a; Lupachyk *et al.*, 2013b).

TMAO is a renal toxin as well as prognostic marker

A high consumption of some red meat and eggs contribute to elevated TMAO levels because these foods contain large amounts of choline and L-carnitine (Collins *et al.*, 2016; Zhu *et al.*, 2016). TMAO is excreted in the urine (Fogelman, 2015; Tang *et al.*, 2015a; Mafune *et al.*, 2016) and Missailidis and colleagues evaluated the link between serum TMAO and renal function. Serum levels of TMAO significantly affect the GFR ($\beta = -0.41$). Also, renal transplantation in five patients with chronic kidney disease reduced levels of TMAO to that of controls. Furthermore, TMAO levels were associated with changes in plasma IL-6 ($r = 0.42$), fibrinogen ($r = 0.43$) and hsC-reactive protein ($r = 0.17$) suggesting that TMAO increased systemic inflammation (Missailidis *et al.*, 2016). An investigation of the contribution of TMAO to the development and progression of renal dysfunction revealed that chronic dietary intakes increased the plasma levels of TMAO directly and contributes to progressive renal fibrosis and dysfunction (Tang *et al.*, 2015a). These clinical and preclinical data demonstrate that the accumulation of TMAO, as a result of low GFR and poor plasma clearance, could be a prognostic marker in patients with chronic renal disease (Bain *et al.*, 2006). Furthermore, TMAO levels were significantly raised during ischaemic kidney damage caused by either cold ischaemia or transplantation (Hauet *et al.*, 2000a; Hauet *et al.*, 2000b; Doucet *et al.*, 2004).

TMAO and cancer

Although the mechanisms of underlying any association of TMAO levels with cancer are not known, recent clinical data showed that colorectal cancer is positively associated with plasma TMAO levels. Similarly, experimental studies proposed that urinary TMAO could be used as a predictor for gastric tumourigenesis (Kim *et al.*, 2010; Bae *et al.*, 2014). TMAO is a red meat-derived metabolite and notably, red meat consumption associates positively with colorectal cancer. High TMAO levels in plasma reflect high intestinal concentrations of TMA and DMA, which are known to be readily converted to nitrosated amines which are potent carcinogens (Lijinsky *et al.*, 1972; Bartsch and Montesano, 1984). In

contrast, some studies have demonstrated that TMAO corrected the mutant protein-folding defect and played a protective role in carcinogenesis (Lunn *et al.*, 2007; Georgescauld *et al.*, 2009).

TMAO triggers thrombosis

Recent studies have revealed that TMAO activates inflammatory pathways in cells of the vasculature leading to increased endothelial cell-leukocyte interactions and atherosclerosis. Both *in vivo* and *in vitro* (cultured human aortic endothelial cells and vascular smooth muscle cells) studies showed that elevated levels of TMAO induced expression of pro-inflammatory cytokines and adhesion molecules mediated by the NF- κ B signalling pathway (Seldin *et al.*, 2016). Zhu *et al.* reported that the plasma levels of TMAO in humans were correlated with the risk of thrombosis. In addition, at physiological doses, under shear stress condition, TMAO showed hyperreactivity of platelets in the collagen-coated flow chamber model (Zhu *et al.*, 2016). Furthermore, mice receiving microbially generated TMAO showed an enhanced prothrombotic phenotype and increased platelet responsiveness. Stimulating platelets with TMAO resulted in the release of Ca^{2+} by altering the inositol 1,4,5-trisphosphate (IP_3) signalling pathway. In addition, in mice, intraperitoneal injection of TMAO showed decreased time to occlusion of the carotid artery (Zhu *et al.*, 2016). In line with this report, Koka and colleagues studied whether TMAO induced **NLRP3** inflammasome formation and activation and thereby contributed to endothelial injury initiating atherogenesis. Mice treated with TMAO significantly increased cell permeability and neointimal formation, which correlated with increased **IL-1 β** production in the intima. IL-1 β is a potent inducer of tissue factor (TF) which indirectly showed the increased risk of atherothrombosis by TMAO (Koka *et al.*, 2016). Furthermore, Seldin *et al.* suggested that activation of NF- κ B and G $\beta\gamma$ signalling is required for TMAO to exert proinflammatory response on endothelial cells (Seldin *et al.*, 2016). TMAO enhances monocyte adhesion by activating endothelial cells to express VCAM-1 (Ma *et al.*, 2017). Interestingly, when we stimulated HUVECs with TMAO, we were able to show a dose-dependent (5–75 mM) increase in mRNA for TF (two-fold) which was confirmed by the observation of increased endothelial TF activity (Subramaniam *et al.*, unpublished data). Taking together previously published data and our findings, we would suggest that TMAO (10–100 μM) induces vascular inflammation, which was manifested as elevated levels of cytokines and increased expression of the endothelial extracellular matrix. Even though TMAO triggers the NF- κ B pathway, we did not see any change in NF- κ B-dependent procoagulant activity over this concentration range. However, the specific cell surface receptor by which TMAO regulates proinflammatory and procoagulant activity, as well as hyperreactivity of platelets, is still to be identified.

TMAO and brain diseases

The role of TMAO in the CNS has not been fully explored. There are very few studies have demonstrated the association

Table 2

Association of TMAO with various diseases

Species/cells	Effects and proposed mechanisms	References
TMAO and heart failure		
Human	-↑ Heart failure -Diastolic dysfunction and poor survival	Tang <i>et al.</i> , 2014; Trosid <i>et al.</i> , 2015; Tang <i>et al.</i> , 2015b; Trosid <i>et al.</i> , 2016
Mouse	-↑ Lung oedema -↑ Natriuretic peptide levels -↑ Ventricular remodelling -↑ Fibrosis, chamber dilation and thinning of the walls -↓ Ejection fraction	Organ <i>et al.</i> , 2016
TMAO and thrombosis		
Mouse and human cells	-↑ Pro-inflammatory cytokines -↑ Adhesion molecules	Seldin <i>et al.</i> , 2016
Human and mouse	-Hyperreactivity of platelets <i>via</i> unknown platelet receptor -Alters inositol-1,4,5-trisphosphate signalling pathway	Zhu <i>et al.</i> , 2016
Mouse	-↑ Cell permeability -↑ Neointimal formation	Koka <i>et al.</i> , 2016
Mouse and human aortic endothelial cells	Proinflammatory response and adhesion molecule (VCAM-1) expression <i>via</i> -Activation of NF- κ B pathway -Activation of G β γ signalling pathway	Seldin <i>et al.</i> , 2016; Ma <i>et al.</i> , 2017
TMAO and brain diseases		
Human	-Blood brain barrier disruption by ↓ tight junction proteins (claudin-5 and ZO-1)	Liu and Huang, 2015
Human	-↓ Stress-induced alteration of organic anion transport in choroid plexus (beneficial effect)	Galland, 2014; Villalobos and Renfro, 2007
TMAO and cancer		
Mice	-A predictor for gastric tumorigenesis (Diagnostic marker)	Kim <i>et al.</i> , 2010
Human	-A predictor for colorectal cancer and carcinogenesis (diagnostic marker)	Lijinsky <i>et al.</i> , 1972; Bartsch and Montesano, 1984; Bae <i>et al.</i> , 2014
Human	-Corrects the mutant protein-folding defect (beneficial effect) -Protects against carcinogenesis (beneficial effect)	Lunn <i>et al.</i> , 2007; Georgescauld <i>et al.</i> , 2009
TMAO and renal system		
Human	-Excreted in the urine in 24 h	Mafune <i>et al.</i> , 2016; Tang <i>et al.</i> , 2015a; Fogelman, 2015
Human and mouse	-TMAO significantly affects the glomerular filtration rate. -TMAO levels were associated with kidney function -A prognostic marker in the patients with chronic renal diseases	Missailidis <i>et al.</i> , 2016; Bain <i>et al.</i> , 2006
Human	-Progressive renal fibrosis and dysfunction	Tang <i>et al.</i> , 2015a
Human	-Ischaemic kidney damage increases circulating TMAO levels	Doucet <i>et al.</i> , 2004; Hauet <i>et al.</i> , 2000a; Hauet <i>et al.</i> , 2000b
TMAO effects on glucose homeostasis and diabetes		
Human	-↑ cIMT, ↑ insulin resistance and ↑ visceral obesity -Fatty liver.	Randrianarisoa <i>et al.</i> , 2016; Zheng <i>et al.</i> , 2016
Human	-Age, diabetes, and body mass index modulates plasma TMAO levels	Obeid <i>et al.</i> , 2016
Human	-↑ Myocardial infarction and ↑ heart failure -↑ Risk of unstable angina.	Lever <i>et al.</i> , 2014; Tang <i>et al.</i> , 2016
Human	-Plasma TMAO level is highly variable in the plasma and urine of overweight individuals with type 2 diabetes.	McEntyre <i>et al.</i> , 2015
Mouse	-Less TMAO localization in tissues of diabetes mice	Xu <i>et al.</i> , 2009
Rat	-Neutralizes endoplasmic reticulum stress <i>via</i> enhanced protein folding	Lupachyk <i>et al.</i> , 2013a; Lupachyk <i>et al.</i> , 2013b

continues

Table 2 (Continued)

Species/cells	Effects and proposed mechanisms	References
TMAO and the risk of inflammation, obesity and atherosclerosis		
Human and mouse	-↑ Macrophage cholesterol load -↑ Atherosclerotic plaque formation -↑ Plaque rupture	Koeth <i>et al.</i> , 2014; Koeth <i>et al.</i> , 2013; Wang <i>et al.</i> , 2011; Fu <i>et al.</i> , 2016
Human	-↓ TMAO levels in patients with IBD and UC	Wilson <i>et al.</i> , 2016; Wilson <i>et al.</i> , 2015
Human	-↑ TNF- α , ↑ IL-6 and ↑ C-reactive protein	Rohrmann <i>et al.</i> , 2016
Human	-↑ TMAO levels in patients after the bariatric surgery	Troscid <i>et al.</i> , 2016
THP-1 and HUVECs	-↑ Endothelial dysfunction -↓ Endothelial self-repair -↑ Monocyte adhesion by activation of PKC/NF- κ B/VCAM-1	Ma <i>et al.</i> , 2017

between plasma TMAO levels and brain diseases. Liu and Huang suggested that elevated TMAO levels may contribute to the progression of cerebral small vessel dysfunction in post-stroke patients, through disruption of the blood brain barrier disruption by reducing the expression of tight junction proteins such as claudin-5 and zonula occludens (ZO)-1 (Liu and Huang, 2015). Villalobos and Renfro demonstrated that TMAO suppresses stress-induced alteration of organic anion transport in the choroid plexus (Villalobos and Renfro, 2007; Galland, 2014). To assess the brain-gut-microbiome connections in Alzheimer's disease, Xu and Wang developed a novel network-based approach to model the genetic interactions between all human microbial metabolites and genetic diseases. This integrated computational approach showed a strong positive correlation between TMAO and Alzheimer's (Xu and Wang, 2016).

Blockade of TMAO formation

Owing to the crucial involvement of TMAO in various cardiovascular and metabolic diseases, active investigations are emerging to identify TMAO inhibitors. Direct TMAO inhibitors are not yet available however, two compounds namely, 3,3-dimethyl-1-butanol (DMB) and resveratrol are proposed to inhibit TMAO production and adverse effects. DMB, which inhibits formation of TMA by gut microbes, thereby reducing plasma TMAO levels and also attenuating the development of choline diet-enhanced atherosclerosis (Wang *et al.*, 2015). Furthermore, DMB treatment significantly reduced plasma TMAO levels and prevented cardiac dysfunction in mice fed with a Western diet (Chen *et al.*, 2017). **Resveratrol**, from red wine, attenuated TMAO-induced atherosclerosis by regulating TMAO synthesis and bile acid metabolism *via* remodelling of the gut microbiota (Chen *et al.*, 2016).

Concluding remarks

Clinical findings are constantly suggesting that there is a potential link between the metabolites produced by the gut flora and the risk factors for cardiovascular and other diseases (Figure 1 and Table 2). In contrast to recently reported

harmful effects of TMAO towards cardiovascular diseases, as shown by *in vitro* studies, increased circulating concentrations of TMAO in rats (0.6 to 60 μ M) did not produce any adverse effects. In addition, it is worth mentioning that a high concentration of TMAO is found in saltwater fish and dietary intake of these fish apparently showed beneficial effects on the circulatory system in humans. Interestingly, recent findings reported that a raised systemic concentration of TMAO (100 μ M) showed increased platelet hyperreactivity and platelet aggregation *in vitro* and *in vivo*. Other studies imply that TMAO (100 μ M) directly activates inflammatory pathways including NF- κ B signaling in endothelial and smooth muscle cells. Furthermore, patients with colorectal cancers or gastric tumours have elevated levels of TMAO. These results suggest that TMAO may be a potential prognostic marker for both CVD and gastrointestinal-associated cancers. In contrast, a few reports suggest that TMAO has beneficial effects as it corrects the mutant protein-folding defect and protects against carcinogenesis. These contradictory observations in cancer studies decrease the value of TMAO as a diagnostic marker for cancer. Although recent studies have proposed new mechanisms underlying the effects of TMAO on cardiovascular and metabolic diseases in various cell types *in vitro*, the definitive mechanism is still undefined. Nevertheless, in terms of the plasma level of TMAO and cardiovascular risks, experts in this field consider the microbial metabolite, TMAO, to be a useful prognostic biomarker for CVD. Further experimental studies are most certainly needed to elucidate the mechanistic aspects of TMAO action and to explore pharmacological interventions of the relevant microbial metabolic processes. Experimenting with gnotobiotic models will allow a better understanding of the fundamental role of bacteria and their metabolites in host-associated disorders, as well as homeostasis in humans.

Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in <http://www.guidetopharmacology.org>, the common portal for data from the IUPHAR/BPS Guide to PHARMACOLOGY (Southan *et al.*, 2016), and are permanently archived in the Concise Guide to PHARMACOLOGY 2015/16 (Alexander *et al.*, 2015).

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Conflict of interest

The authors declare no conflicts of interest.

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