SMALL MOLECULE, BIG ROLE:

TRIMETHYLAMINE-N-OXIDE AND ALZHEIMER'S DISEASE

by

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disease considered the most common cause of dementia, with 6.7 million Americans aged 65 and older living with AD, and this number is projected to grow to 12.7 million by 2050. Over 11 million Americans currently provide unpaid care to family or friends with dementia, valued at $340 billion. Manifestation symptoms of AD include a decline in cognitive, motor, behavioral, and, most notably, memory function, and the disease is biologically marked by the accumulation of extracellular amyloid-β plaques and intracellular hyperphosphorylated tau proteins. It has been proposed that the gut microbiome, a community of commensal microorganisms living symbiotically in our bodies, contributes greatly to normal bodily function but can also contribute to AD progression under different circumstances. Recent studies suggest that trimethylamine, a metabolite produced by some members of this gut microbiome, can be converted to TMAO in the liver, where it moves into the bloodstream and has important roles in colorectal cancer, type 2 diabetes, cardiovascular diseases, and AD progression among many others. Much of the understanding of the roles that TMAO plays come from human observational studies or rodent experimental studies, with oxidative stress, blood-brain-barrier interactions, inflammation, and various other mechanisms not as well understood playing significant, overlapping roles in the development of many of these diseases. What is understood is that neuroinflammation appears to play a significant role in the progression of AD, with astrocytes having a lead role in the development of this inflammation. Recent studies have found TMAO crossing the BBB, with subsequent induction of astrocytes into their inflammatory state, contributing to neuroinflammation directly through these astrocytes. TMAO has also been found to play more indirect roles in AD development, with neuronal damage, oxidative stress, and increased inflammation being detected at heightened

TMAO levels in mice, each contributing to cognitive impairment. Additionally, TMAO leads to insulin resistance (IR) via this chronic inflammation and oxidative stress, which is important as it has been determined that IR is heavily associated with AD, with AD even being labeled as type 3 diabetes. Overall, studies to date support a significant relationship between circulating TMAO in plasma and cerebrospinal fluid (CSF) and the progression and pathology of AD, among several other diseases. However, continued research into this relationship is necessary, particularly in human experimental studies, to resolve several limitations, such as TMAO precursors being beneficial to humans, which have been found in the literature. Following a better understanding of the action of TMAO, therapies targeting this gut-microbe-derived metabolite to treat various diseases, particularly AD, may be improved.

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INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disease considered the most common cause of dementia (60-80% of cases). There are currently 6.7 million Americans aged 65 and older living with Alzheimer's disease, with this number projected to grow to 12.7 million by 2050 (Alzheimer's Disease Association, 2023). This rapid growth will require 1.2 million additional direct care workers between 2020 and 2030, which is more new workers than in any other single occupation in the United States (Alzheimer's Disease Association, 2023). Furthermore, greater than 11 million Americans are providing unpaid care for a family member or friend with dementia, which is valued at nearly $340 billion (Alzheimer's Disease Association, 2023). These statistics will continue to rise if curative and preventive measures are not found. Manifestation symptoms of AD include a decline in cognitive, motor, behavioral, and, most notably, memory function (Alzheimer's Disease Association, 2023). It is biologically marked by the accumulation of extracellular amyloid-β plaques and intracellular hyperphosphorylated tau proteins (Liu et al., 2019). The amyloid-β plaques are formed via proteolytic cleavage of the amyloid precursor protein, which aggregates forming the plaques characteristic of AD (Finder and Glockshuber, 2007). The accumulation of these plaques and tangles in the brain results in oxidative stress and neuroinflammation, which leads to synaptic and neuronal loss (Kumar et al., 2015). The mechanisms associated with AD are multifaceted. One of the angles that has garnered strong research interest in the last years has been the possible role of the gut microbiome in potentially modulating neuroinflammation and progression of the disease.

The gut microbiome is a community of commensal microorganisms comprised of trillions of bacteria, archaea, protozoa, viruses, and fungi that live symbiotically in our bodies and have been shown to modulate neuroinflammation in a variety of conditions, including AD

(Chandra et al., 2023; Megur et al., 2021). The microbiota's functions include: fermentation of non-digestible carbohydrates, conversion of primary to secondary biliary acids, maturation of the immune system, and the production of molecules such as vitamin K and B, short-chain fatty acids, and neurotransmitters [GABA, dopamine, serotonin, and norepinephrine] (Mayer et al., 2022; Thursby & Juge, 2017). However, not all gut-derived molecules are beneficial. Trimethylamine-N-Oxide (TMAO) is a gut microbiota-derived molecule that has been linked to cardiovascular diseases and type 2 diabetes mellitus (T2DM) (Thomas & Fernandez. 2021; Gatarek & Kaluzna-Czaplinska, 2021). Recent studies suggest that TMAO has an important role in AD progression, though little is truly understood about this connection (Constantino-Jonapa et al., 2023; Buawangpong et al., 2022). In light of this, the main objective of this review is to discuss the recent evidence for the potential relationship between the gut microbiota and Alzheimer's disease development and progression as modulated by TMAO.

TMAO PRODUCTION

Trimethylamine (TMA) is a molecule produced via microbial metabolism in the gut. A large amount of TMA is synthesized by members of two bacterial phyla in the gut microbiota, Firmicutes and Proteobacteria. Eight particularly important members of these phyla include *Providencia rettgeri, Clostridium asparagiforme, C. sporogenes, C. hathewayi, Proteus penneri, Escherichia fergusonii,* and *Edwardsiella tarda* (Buawangpong et al., 2022). Following production, TMA will then passively diffuse through the gut epithelium before reaching the liver. The major precursors involved in TMA production include choline, glycine betaine, and L-carnitine, found in many foods. Choline is commonly found in animal products such as red meat, fish, and eggs; L-carnitine is commonly found in red meat; and glycine betaine is found mainly

in plants. L-carnitine is converted to TMA by the enzyme carnitine oxidoreductase, choline is converted via TMA lyase, and glycine betaine by betaine reductase, all present in gut bacteria. Interestingly, if members of this consortium of TMA-producing bacteria which contain these enzymes are not present, then these precursors will not be converted to TMA, suggesting that composition of the gut microbiome is fundamental. Upon reaching the liver after conversion to TMA by bacteria through one of these pathways, TMA is converted to trimethylamine-N-oxide (TMAO) via flavin-containing monooxygenase 1 or flavin-containing monooxygenase 3, after which TMAO enters systemic circulation through the hepatic vasculature (Oellgaard et al., 2017; Buawangpong et al., 2022). Once in systemic circulation, a majority of produced TMA and TMAO is excreted by the kidneys into the urine (Mudimela et al., 2022). Previous studies have shown that TMAO is the primary gut microbiota-derived metabolite associated with AD, presenting itself as an intriguing option for better understanding the link between the gut and the brain and the contribution it has to the development of AD (Xu & Wang, 2016). Evidence has indicated that TMAO acts as a potential risk factor for atherothrombosis, vessel wall inflammation, disruption of cholesterol transport, astrocyte formation, and increased production of reactive oxygen species, amyloid-β, and tau proteins (Buawangpong et al., 2022). Each of these outcomes has serious pathophysiological consequences important in a multitude of different diseases, such as AD.

TMAO AND DISEASE

Increased TMAO levels have been found to predict an increased risk of developing kidney disorders, cancer, T2DM, obesity, cardiovascular disease, metabolic syndrome, and neurological diseases, such as AD (Constantino-Jonapa et al., 2023). Unfortunately, the exact

mechanisms for the action of TMAO in the development of most of these diseases has not yet been elucidated, though many mechanisms have been suggested such as inducement of atherothrombosis, vessel wall inflammation, disruption of cholesterol transport, increased production of reactive oxygen species, action as a chaperone protein for amyloid-β and protein tau, and activation of astrocytes (Buawangpong et al., 2022). In a 2016 study conducted by Missailidis *et al*. on patients with chronic kidney disease, it was found that TMAO level was higher in kidney disease patients than in healthy controls, and it was highest in individuals with declining renal function. Additionally, this heightened TMAO was associated with a drop in 5-year survival and a 6.3-fold increase in mortality risk (Missailidis et al., 2016).

Cancer, particularly colorectal cancer, has been found to be correlated with elevated levels of TMAO. Specifically, the Women's Health Initiative Observational Study found a 1.9-fold higher risk of proximal tumors, a 1.8-fold higher risk of local tumors, and a 2.3-fold higher risk of rectal tumors in those with high TMAO levels (Bae et al., 2014). Oxidative stress has been proposed as a primary mechanism behind TMAO-associated increased cancer risk, with oxidative stress leading to DNA damage when antioxidant defense in the body is not sufficiently high. This results in the potential favoring of growth and proliferation of cancer cells, leading to cancer development or worsening of its symptoms (Constantino-Jonapa et al., 2023). Concomitantly with heightened risk due to oxidative stress, TMAO leading to induction of insulin resistance may play a role in heightening cancer risk, as changes in insulin receptors, IRS-1 and IRS-2, are primary markers for colorectal cancer (Esposito et al., 2012; Slattery et al., 2004).

TMAO has also been implicated in the progression of obesity, with elevated TMAO levels being linked to obesity, though the nature of this link has not been determined. What is

known is that obesity is a significant risk factor for multiple diseases, two of the most prominent being T2DM and cardiovascular diseases. TMAO association with T2DM has seen mixed results, with clear indication that TMAO is associated with increased insulin resistance as a result of oxidative and ER stress, which elevates the risk for development of T2DM. In the Cardiovascular Health Study, which involved the observation of 4,442 participants observed for 7 years, no association between TMAO and T2DM incidence was found. There was, however, a relationship with fasting insulin concentration which is a marker of insulin resistance (Lemaitre et al., 2021). What this evidence shows is that there is a clear association between TMAO and insulin resistance, though this increased resistance may not be enough to result in T2DM.

The effects of high TMAO levels have seen the most research in relation to cardiovascular disease. Unsurprisingly, this has resulted in a far better understanding of the mechanisms behind TMAO and the associated elevation in major adverse cardiovascular events, cardiac insufficiency, and mortality as a result of cardiovascular disease (Gao et al., 2020; Zhou et al., 2020; Hayashi et al., 2019). What has been learned is that through calcium release and platelet hyperactivity, induction of apoptosis, ER stress, and foam cell formation and adhesion, TMAO contributes to the development of plaque ruptures which lead to cardiovascular disease (Constantino-Jonapa et al., 2023). This process has been confirmed through observational studies conducted in multiple patient populations around the globe, with positive correlations between TMAO and mortality risk, number of infarcted coronary arteries, major adverse cardiovascular events, and high troponin levels (Shafi et al., 2017; Mafune et al., 2016; Senthong et al., 2021). Additionally, accumulating evidence suggests a connection between TMAO and atherosclerosis risk. Mice fed a high-fat Western-style diet show increased plasma TMAO concentration and increased prevalence of cardiac dysfunction relative to controls (Chen et al., 2017).

The overlap between cardiovascular diseases and AD seems to involve the apoE gene as well as an exaggerated inflammatory response (Constantino-Jonapa et al., 2023). The apoE gene is a significant genetic risk factor for AD, atherosclerosis, and cardiovascular diseases (Mahley & Rall Jr. et al., 2000). Carriers of the ε4 allele have a heightened risk of AD due to ApoE protein interaction with amyloid-β peptides while also having an increased risk of atherosclerosis, hypercholesterolemia, and cardiovascular disease (Mahley & Rall Jr. et al., 2000). On top of the apoE gene, an exaggerated inflammatory response is a critical factor in AD and cardiovascular diseases. Interestingly, as TMAO induces inflammation and oxidative stress, it also elevates the risk for AD and cardiovascular diseases. This is supported by the fact that several gut microbial taxa associated with the production of TMA have been found at increased levels in both AD and cardiovascular disease patients (Vogt et al., 2017).

TMAO AND THE BLOOD BRAIN BARRIER

While the mechanisms behind AD are multifaceted and incompletely understood, neuroinflammation appears to play a critical role in the progression of the disease and neural impairment. Astrocytes play a particularly significant role in the development of inflammation in the brain. Astrocytes, which can take on a toxic “reactive” state when exposed to immune-related stressors, have been increasingly associated with aging and neurodegenerative diseases such as AD as a result of their ability to cause neuroinflammation (Clarke et al., 2018; Csipo et al., 2020; Habib et al., 2020). Following production in the liver and movement to systemic circulation, it has recently been discovered that TMAO is capable of crossing the blood-brain-barrier (BBB) into the cerebrospinal fluid, with concentrations of the metabolite detectable in the cerebrospinal

fluid (CSF) of patients with dementias like AD (Del Rio et al., 2017). The mechanism behind this transport across the BBB is still unknown (Vernetti et al., 2017; Brunt et al., 2021). Once in circulation, TMAO has been demonstrated to activate pro-inflammatory pathways, but a question has been what effect this molecule has on the brain itself when it comes into direct contact upon successful crossing of the BBB (Chen et al., 2017; Ransohoff, 2016). Recently, it has been determined that one effect TMAO has upon entry into the brain is the induction of astrocytes to their "reactive," pro-inflammatory state, influencing the brain via neuroinflammation, which contributes to the decline in mental capacity commonly associated with AD (Brunt et al., 2021).

TMAO'S ROLE IN ALZHEIMER'S DISEASE

While explaining the role that TMAO plays in contributing to other diseases is important, it does not fully account for the role that TMAO may play in AD. One issue with explaining this role, however, is that there are no reports yet linking TMAO receptors with neuronal function, so it is still very uncertain how exactly this metabolite is exerting its effects on the brain (Mudimela et al., 2022). What is known as of yet regarding the exact role of TMAO in AD is, therefore, mostly speculative based on experimental rodent studies and correlational human studies. One of the most significant studies conducted to date in humans regarding TMAO and AD pathology came from a 2018 paper by Vogt *et al.* in which TMAO levels were found to be higher in CSF of individuals with AD dementia and mild cognitive impairment compared with unimpaired individuals (Vogt et al., 2018). Within this same study, it was also found that high TMAO in CSF was correlated with phosphorylated tau protein and neuronal degeneration markers, indicating that TMAO likely plays a role in AD pathology (Vogt et al., 2018). Unfortunately, follow-up studies in humans have not always yielded these same outcomes, with a 2017 study by

Del Rio *et al.* finding that TMAO levels in CSF did not vary among AD, non-AD, and other neurological disorder groups (Del Rio et al., 2017). Likewise, a 2021 study by Zhuang *et al.* conducting a bidirectional Mendelian randomization study on AD in the genetic sequence of 455,258 individuals found that the genetic prediction of TMAO was not correlated with an increased risk of AD, and there was no causal link between TMAO or any of its precursors and AD (Zhuang et al., 2021).

Much more is known regarding the TMAO-AD link in mice than in humans. Zarbock *et al.,* in their 2022 study, examined whether TMAO has an impact on AD pathology by putting TMAO in the drinking water of 5XFAD (AD-model) transgenic mice. At higher TMAO concentrations, it was found that neurite density and dispersion were reduced (Zarbock et al., 2022). This finding is important, as damage to neurons like this is highly correlated with cognitive impairment. Additionally, there was a significant decrease in plaque intensity in these mice, with decreased amyloid-β plaque intensity found to be toxic to surrounding neurons, perhaps contributing to the reduced neurite density and dispersion (Zarbock et al., 2022).

High TMAO also contributes to AD via upregulation of the production of reactive oxygen species, lipid peroxidation, hydrogen peroxide, and more, leading to oxidative stress (Islam, 2017; Seldin et al., 2016). This is problematic, as the progression of AD is thought to be highly associated with oxidative imbalance after very high levels of oxidative stress were found in the beginning stages of AD before even the amyloid-β plaques had built up to high levels (Leyane et al., 2022; Yan et al., 2013). Increased reactive oxygen species levels during aging, alongside increased mitochondrial dysfunction as the result of mitochondrial mutations over many generations in one's lifetime, leads to an amplification of oxidative stress in the onset of AD (Yan et al., 2013). The observed mitochondrial dysfunction means that these neurons are less

capable of altering their energy production dynamics in response to the changing needs of the cells, making them even more dysfunctional as a cell population, contributing to the neuron degeneration observed throughout AD (Swerdlow, 2018). Accumulating evidence also suggests that oxidative stress is significantly correlated with cognitive impairment through gut-liver-brain communication, leading to a lower quality of life. Overall, evidence suggests that aging and TMAO levels are tightly related, with higher TMAO contributing to inflammation and oxidative stress (Brunt et al., 2019). A final major way in which TMAO has been linked to AD is through a pathway often thought of only in terms of T2DM, insulin resistance (IR).

INSULIN RESISTANCE

Much is known about IR and the role it plays in T2DM. Interestingly, IR affects far more than T2DM, with IR and its effect on the pathogenesis of AD being the focus of much research in the last couple of decades (Qiu & Folstein, 2006; Bosco et al., 2011; Craft et al., 2013). IR is the reduction of a response to insulin in the body. When an individual develops IR, the body compensates by releasing more insulin (hyperinsulinemia) to maintain a normal blood glucose level. Despite being released into the bloodstream, apart from the cerebrospinal fluid, the hyperinsulinemia observed with IR also results in an increase in insulin in the brain, as transport across the BBB is heavily dependent upon the concentration in the bloodstream (Sims-Robinson et al., 2015; Mullins et al., 2017). Once inside the brain, insulin is bound for degradation by the insulin-degrading enzyme, which incidentally also degrades the amyloid-β protein (Qiu & Folstein, 2006; Sims-Robinson et al., 2015). Insulin degrading enzyme has been found to have a higher binding affinity for insulin than amyloid-β protein, meaning that as insulin in the brain increases as a result of IR, less amyloid-β protein will be degraded (Qiu & Folstein, 2006; Cater

& Holter, 2022). This ultimately leads to amyloid-β accumulation and aggregation, a hallmark of AD (Qiu & Folstein, 2006; Sims-Robinson et al., 2015; Cater & Holter, 2022). Additionally, it has been found that accumulation of these amyloid-β proteins results in disruption of insulin binding to insulin receptors, causing increased IR and thus feeding a vicious cycle (Bosco et al., 2011; Craft et al., 2013; Qiu & Folstein, 2006; Xie et al., 2002). The insulin-degrading enzyme is not the only mechanism by which IR is associated with AD; the hyperglycemia resulting from IR also negatively impacts neuronal growth and memory, both signature features of AD (Xiang et al., 2015). IR has additionally been implicated in altering neurotransmitter levels, with a reduction in acetylcholine production being one outcome. Low acetylcholine levels are frequently found in the progression of AD (Rivera et al., 2005; Francis, 2005; Rizzo et al., 2022). Another way in which IR directly affects AD is through the increased production of tau hyperphosphorylated proteins (Chatterjee et al., 2019). Interestingly, despite the state of hyperinsulinemia in the bloodstream, insulin deficiency in the brain may result with time as a result of insulin influx across the BBB being inherently limited and saturable (Craft et al., 2013; Sims-Robinson et al., 2015; Mullins et al., 2017; Frank & McNay, 2022). This insulin deficiency in the brain has, in turn, been found to be associated with the progression of AD and has been labeled as type 3 diabetes (de la Monte, 2012; Sędzikowska & Szablewski, 2021).

This connection between IR and AD is important to understanding TMAO because it has been determined that TMAO leads to IR through the shared AD/IR risk factors of chronic inflammation and oxidative stress (Tao et al., 2018; Bosco et al., 2011; Park et al., 2020). Furthermore, it has been demonstrated that TMAO increases levels of N-nitroso compounds which have been shown to lead to IR (Tong et al., 2009; de la Monte et al., 2009). In a study

comparing TMAO-supplemented animals with or without a high-fat diet, it was found that the TMAO-supplemented animals had increased IR relative to these other groups (Li et al., 2012).

POTENTIAL THERAPIES

As the leading factor for the development and progression of AD is oxidative stress and the inflammation which leads to it, antioxidants or other agents which reduce the oxidative imbalance and inflammation present within the brain is a target for AD therapy (Kook et al., 2014). One drug which has been developed with this target in mind is Resveratrol, also used in T2DM treatment, which has been used in clinical studies and was found to delay or prevent many AD indicators such as amyloid-β and protein tau accumulation via anti-inflammatory function, improving mitochondrial function, and anti-oxidation (Moussa et al., 2017; Huang J. et al., 2021).

Insulin resistance, as it has been found to be heavily associated with the development of AD, has also been the target of recent AD therapy. There are a multitude of drugs developed for use in patients with T2DM to address IR, which have seen testing for their efficacy with AD. One of the most commonly prescribed of these T2DM drugs is metformin, though studies have been conflicting. In one clinical study, there was no change in TMAO level after 3 months of treatment with metformin in humans, while in another study, there was a decrease in TMAO level (Velebova et al., 2016; Huo et al., 2009). Another drug targeting insulin resistance in the brain is intranasal insulin. Intranasal insulin bypasses the BBB via the nasal passages to the brain, allowing insulin to enter the CSF directly and treat the insulin deficiency associated with AD as it becomes a type 3 diabetes (Michailidis et al., 2022). Intranasal insulin has been tested in

a handful of clinical trials with mixed results, some appearing to improve memory compared to placebo, some finding no statistically significant effects (Craft et al., 2017; Craft et al., 2020).

Aspirin is another potential treatment for TMAO, acting as an inhibitor of TMA-lyase, which some bacteria use to create TMA. By inhibiting this enzyme, aspirin may inhibit the growth of these bacterial species (Kalagi et al., 2019). Another drug that targets TMA-lyase to reduce TMAO levels is Berberine. Berberine is currently used in clinical studies targeting patients with atherosclerosis (Ma et al., 2022). 3,3-dimethyl-1-butanol, an analog of choline, also targets TMA-lyase to reduce TMAO levels (Wang et al., 2015). Several other drugs have also been developed that target this same enzyme, including iodomethylcholine, fluoromethylcholine, benzoxazole ligand, and betaine aldehyde (Roberts et al., 2018; Orman et al., 2019; Gabr et al., 2020). A problem with most of these drugs, however, is that, while effective in mice, they have seen no use in human studies.

The last category of potential treatments for TMAO is the use of probiotics. One of the most promising of these probiotics appears to be *Methanomassiliicoccus luminyensis.* This bacteria is able to remove TMA through H2-reduction, thereby leading to reduced TMAO in plasma and a reduction in TMAO-associated problems (Brugere et al., 2014). While *M. luminyensis* appears like a promising option, this bacterium has still not seen use in humans. Other probiotics, while used in humans, have not found significant differences between placebo and probiotic groups in a double-blind randomized study (Borges et al., 2019). What this demonstrates is that much further research is necessary to determine the ideal composition of microbes to use in probiotics for TMAO or AD therapy.

LIMITATIONS

While the connection between TMAO and AD appears to be well-backed and robust, there are some pertinent limitations to consider. The first of these is the lack of experimentally-based studies involving humans. Most experimental research has been done to date in animals, with only observational research being conducted in humans (Bae et al., 2014; Chen et al., 2017; Lemaitre et al., 2021; Wang et al., 2022). While murine models are beneficial for gaining preliminary data on what may occur in humans, these are not perfectly representative. Without these human experimental studies, it will be challenging to determine the impact that TMAO has on the human body and its connection to AD progression.

Another important limitation to the proposed TMAO-AD link is that the majority of studies have been conducted at supraphysiological concentrations of TMAO in the bloodstream, leading to a potentially skewed view of the true action of TMAO in the human body (Zhao et al., 2019; Brunt et al., 2021; Su et al., 2021). Lending evidence to this is a 2021 study by Hoyles *et al*. in which it was determined that at physiological concentrations, TMAO exhibits beneficial action upon the BBB in mice following injection with LPS, counteracting the activity of LPS and maintaining BBB integrity (Hoyles et al., 2021). Additionally, this study showed that long-term TMAO exposure acts as a protective agent for murine cognitive function during inflammatory challenge assays (Hoyles et al., 2021). This contradicts the action of TMAO discussed throughout this paper at supraphysiological concentrations, and, as such, more studies will be necessary to determine the true nature of action of TMAO at physiological concentrations.

An additional limitation to TMAO contributing to AD development regards the fact that TMA precursors, such as choline and L-carnitine, are known to be beneficial to humans and mice (Poly et al., 2011; Nurk et al., 2013). In fact, dietary choline intake and cognitive function have been positively correlated in both mice and humans (Nurk et al., 2013; Bartus et al., 1980).

Choline is a crucial molecule in the body, contributing to processes such as fat and cholesterol transport, energy metabolism, and is converted to acetylcholine, an essential neurotransmitter (Inazu, 2019; Zeisel et al., 2009). In a 2020 study by Velazquez *et al*., it was even determined that in AD-model mice, treatment with choline led to reduced amyloid-β pathology, improved memory, and transgenerational reduction in AD pathology as a result of heritable epigenetic mutations, indicating that choline is a largely beneficial molecule (Velazquez et al., 2020). Additionally, the Mediterranean diet, rich in fish and other seafood, has significant quantities of TMAO (Lundstrom & Raicot, 1983), yet this diet is actually associated with decreased cognitive decline and cerebrovascular disease (Zeng et al., 2017; Keenan et al., 2020; Zhao et al., 2019).

A final limitation involves TMAO leading to IR. There is an additional mechanism proposed for this connection, whereby reverse causality is suggested, with increased IR and hyperinsulinemia leading to activation of FMO3, which increases TMAO levels (Miao et al., 2015). Additional research is necessary to determine the true mechanism behind the link between TMAO and IR, but this would suggest TMAO is a product of IR and AD progression, as opposed to a causative factor in their development. While much has been learned regarding TMAO and AD, there is still much unknown that will require future research before many of these limitations may be resolved.

CONCLUSION

Murine experimental studies and human observational studies support a significant relationship between circulating TMAO in plasma and CSF and the progression and pathology of AD, among other diseases. Continued research into this relationship is necessary, particularly in human studies, to resolve many current limitations and shed light on the true nature of TMAO in

the human body. Following a better understanding of the action of TMAO, treatments targeting this gut microbiota-derived metabolite may be desirable.

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