Review Article

Intestinal Microbiota Metabolism and Atherosclerosis

Tian-Xing Liu[1], Hai-Tao Niu[2], Shu-Yang Zhang[1]

[1]Department of Cardiology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, China

[2]Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100021, China

Abstract

Objective: This review aimed to summarize the relationship between intestinal microbiota metabolism and cardiovascular disease (CVD) and to propose a novel CVD therapeutic target.

Data Sources: This study was based on data obtained from PubMed and EMBASE up to June 30, 2015. Articles were selected using the following search terms: "Intestinal microbiota", "trimethylamine N-oxide (TMAO)", "trimethylamine (TMA)", "cardiovascular", and "atherosclerosis".

Study Selection: Studies were eligible if they present information on intestinal microbiota metabolism and atherosclerosis. Studies on TMA-containing nutrients were also included.

Results: A new CVD risk factor, TMAO, was recently identified. It has been observed that several TMA-containing compounds may be catabolized by specific intestinal microbiota, resulting in TMA release. TMA is subsequently converted to TMAO in the liver. Several preliminary studies have linked TMAO to CVD, particularly atherosclerosis; however, the details of this relationship remain unclear.

Conclusions: Intestinal microbiota metabolism is associated with atherosclerosis and may represent a promising therapeutic target with respect to CVD management.

Key words: Atherosclerosis; Cardiovascular; Intestinal Microbiota Metabolism; Trimethylamine; Trimethylamine N-oxide

Introduction

Cardiovascular disease (CVD) remains the leading cause of morbidity and mortality worldwide. Atherosclerotic CVD is a chronic inflammatory disease that primarily involves the large arteries and the medium-sized arteries. Multiple conventional risk factors for atherosclerosis have been identified, including hypertension, dyslipidemia, advanced age, and smoking.[1] Red meat (beef, mutton, and pork) consumption has also been linked to CVD.[2,3] It is believed that saturated and trans-fatty acids, which are abundant in red meat, are to blame.[4-6]

Recent researchers have identified components in meat other than fatty acids that may play important roles in the development of CVD. Phosphatidylcholine (also known as lecithin), choline, betaine, and L-carnitine, which are abundant in both red meat and dairy products, participate in intestinal microorganism metabolism and are converted to trimethylamine (TMA). TMA is subsequently metabolized by flavin monooxygenase (FMO), a liver enzyme family, to trimethylamine N-oxide (TMAO),[7,8] which is associated with obesity,[9-12] metabolic syndrome,[13,14] fatty liver disease,[15,16] and cancer.[17] Gut bacteria metabolism and TMAO were recently linked to CVD.[8,18-22] In this review, we will discuss how gut bacteria metabolism is related to CVD and propose novel therapeutic targets that may be useful in the management of CVD.

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Trimethylamine N-oxide Metabolism and Cardiovascular Disease

Trimethylamine-containing nutrients are essential for survival and may be cardioprotective

Lecithin, choline, betaine, and L-carnitine each feature the same TMA moiety and play a central role in human

Address for correspondence: Prof. Shu-Yang Zhang, Department of Cardiology, Peking Union Medical College Hospital, Chinese Academy of Medical Sciences and Peking Union Medical College, Beijing 100730, China
E-Mail: shuyangzhang103@163.com

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metabolism. They are particularly abundant in both red meat and milk.

Lecithin is a vital component of the cell membrane and also plays an important role in immune modulation, anti-platelet aggregation, and lipid metabolism.[23] Choline is a moiety found in lecithin, which plays a role in both membrane structure and lipid metabolism. It may be oxidized to betaine. Both choline and betaine have been linked to lower plasma homocysteine levels and reduced inflammation.[24,25] As dyslipidemia, inflammation, hyperhomocysteinemia, and platelet aggregation have been linked to cardiovascular events, both lecithin and choline supplementation is recommended for cardiovascular protection.[23,25] Indeed, it has been proven that dietary intake of choline and betaine does not render individuals susceptible to developing CVD and may actually prevent CVD by attenuating inflammation.[24,26,27]

L-carnitine transports fatty acids into the mitochondria, particularly in cardiomyocytes, thereby facilitating fatty acid breakdown and energy derivation.[28] A meta-analysis including 13 studies (n = 3629) demonstrated that L-carnitine reduced the incidence of angina by 40% and the all-cause mortality associated with acute myocardial infarction (AMI) by 27%.[29] A randomized controlled trial involving 129 patients suffering from AMI, who were followed for 28 days, determined that carnitine reduces infarction size, cardiac enzyme levels, angina pectoris symptoms, and cardiac death.[30] Another study involving patients receiving hemodialysis determined that although oral carnitine supplementation increases plasma TMA and TMAO levels, the markers of vascular injury were decreased after 6 months.[31]

Therefore, it appears that each of these TMA-containing nutrients may represent a promising drug with respect to the management of angina and CVD. However, there have been no large-scale observation studies assessing the safety and the effectiveness of the supplementation of these TMA-containing compounds.

Trimethylamine-containing compounds are a source of trimethylamine N-oxide

TMAO metabolism has been well studied. Gut microbes take in TMA-containing compounds (lecithin, choline, betaine, and L-carnitine) and release TMA, which is subsequently metabolized by the FMO enzyme family in the liver to form TMAO [Figure 1].[32] L-carnitine may also be transformed to γ-butyrobetaine by gut bacteria before being converted to TMA and TMAO.[33] Hence, it is reasonable to predict that TMA-containing compound intake is linked with increased TMAO levels. Studies in both rodents and humans have proven this hypothesis. When either humans or mice were fed isotope-labeled lecithin, choline or L-carnitine, tracer-labeled TMAO was subsequently noted in both the plasma and the urine.[8,18]

Increased trimethylamine N-oxide level, not increased level of trimethylamine-containing compounds, is an independent risk factor for atherosclerosis and cardiovascular disease in the general population

It has been observed that aortic root atheroma sizes in mice and clinical plaque burdens increase in parallel with TMAO levels; however, lipid, lipoprotein, and glucose levels are unchanged.[8] Mice fed extra choline, carnitine or TMAO also exhibited a greater atherosclerotic burden.[8,18]

In both retrospective and prospective studies, researchers have observed that plasma levels of TMAO, choline, lecithin, betaine, and L-carnitine are associated with coronary artery disease (CAD), cerebrovascular events and peripheral artery disease.[8,18] In a large scale (n = 4007)

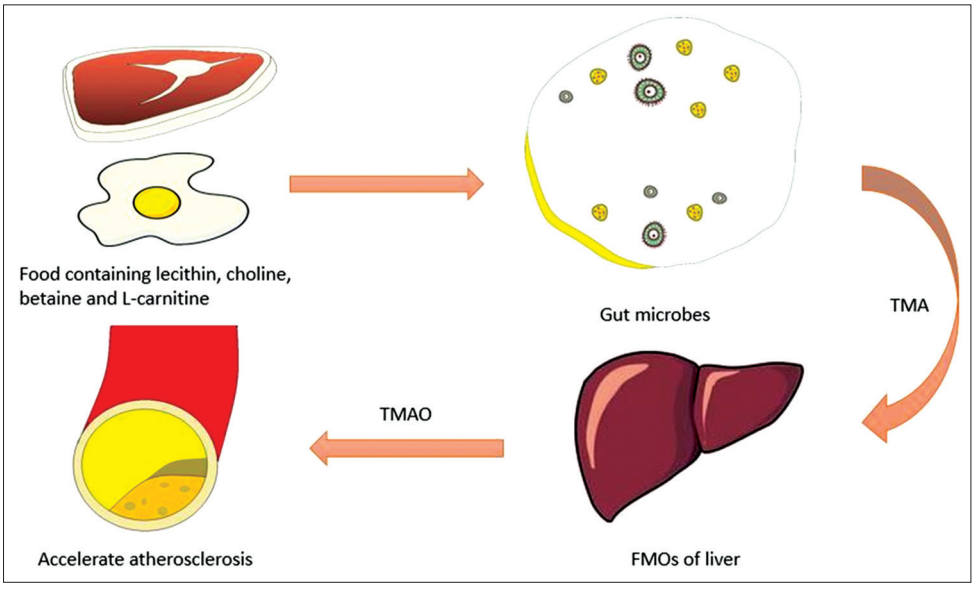

Figure 1: Gut microbes-trimethylamine N-oxide pathway. Gut microbes take in trimethylamine-containing compounds (lecithin, choline, betaine, and L-carnitine) and release trimethylamine (TMA), which is subsequently metabolized by the flavin monooxygenase (FMO) enzyme family in the liver to form trimethylamine N-oxide (TMAO). Trimethylamine N-oxide has been linked to atherosclerosis.

cohort study, researchers observed that higher fasting plasma TMAO levels were associated with major adverse cardiovascular events (MACE), a relationship that remained robust even following adjustments for traditional risk factors.[19]

Two cohort studies involving patients followed for 3 years concluded that elevated choline, betaine, and carnitine levels predict MACE only when TMAO levels are elevated. When TMAO levels were added to the adjustment model, the correlations involving choline, carnitine, and MACE disappeared. Following the administration of antibiotics to carnitine-fed mice, plasma carnitine levels increased independently of atherosclerosis, indicating that TMAO promotes CVD rather than its TMA-containing precursors.[18,22] Indeed, as stated previously, some studies have determined that these TMA-containing compounds exert cardioprotective effects.

Higher TMAO concentrations have also been linked to higher SYNTAX scores.[34] Higher carnitine concentrations are predictive of multiple-vessel lesions in the setting of CAD.[18] However, the relationship between TMAO and mortality was not observed among patients receiving dialysis.[35]

Potential mechanisms underlying the involvement of trimethylamine N-oxide in cardiovascular disease

TMAO and atherosclerosis are related; however, it is unclear whether TMAO causes atherosclerosis or is merely a biomarker of the disease. If the former is true, what is the underlying mechanism of this phenomenon? Currently, limited amount evidence suggested that TMAO is a pro-atherosclerotic molecule.

The fundamental pathological change associated with atherosclerosis is the formation of lipid-rich macrophages (also known as "foam cells") within the arterial wall. The beginning of atherosclerosis entails the uptake of oxidized low-density lipoprotein by macrophages within the arterial wall, a process mediated by macrophage "scavenger" receptors. High-density lipoprotein particles are able to transport lipids out of the arterial wall, thereby eliminating atherosclerotic plaques, a process termed "reverse cholesterol transport (RCT)." Theoretically, TMAO may cause lipid accumulation by facilitating lipid influx, enhancing *in situ* lipid synthesis, and suppressing lipid clean-up.

TMAO facilitates foam cell formation. When Apoe$^{-/-}$ mice were supplied with choline, betaine or TMAO, the levels of two atherosclerosis-related macrophage scavenger receptors (SAs), cluster determinant 36 (CD36) and SR-A1, were increased. Consequently, lipid-rich macrophages (foam cells) formed. However, oral antibiotics inhibited TMAO-dependent foam cell formation.[8]

TMAO also inhibits cholesterol efflux by suppressing RCT, as well as by blocking bile acid secretion. RCT was suppressed in mice receiving either choline or carnitine supplementation. However, following antibiotic administration, the suppression of RCT was reversed. Mice provided TMAO also exhibited decreased RCT; therefore, TMAO is produced by gut microbes and inhibits RCT. However, the levels of cholesterol transporters in macrophages did not change significantly following TMAO treatment.[18] It remains unclear how TMAO alters RCT. Moreover, the levels of bile acid synthetic enzymes (Cyp7a1 and Cyp27a1) and bile acid transporters (Oatp1, Oatp4, Mrp2, and Ntcp) in the liver were reduced when the mice were provided TMAO.[18] As the bile acid pathway plays a major role in cholesterol elimination, blocking this pathway may have accelerated atherosclerosis.

However, TMAO does not affect cholesterol synthesis. Researchers have cultured macrophages with cholesterol and observed that regardless of whether TMAO was added, the messenger RNA (mRNA) levels of the low density lipoprotein receptor, cholesterol synthesis genes, and inflammatory genes did not change.[18]

Taken together, these findings indicate that TMAO accelerates atherosclerosis by facilitating cholesterol influx and inhibiting cholesterol efflux. However, it does not alter cholesterol synthesis [Figure 2].

Additional mechanisms underlying the atherogenic ability of TMAO may exist and are awaiting discovery. Several targets are worth studying, including the effects of TMAO on endothelial function, lipid and glucose profiles and the immune system. Further studies are warranted.

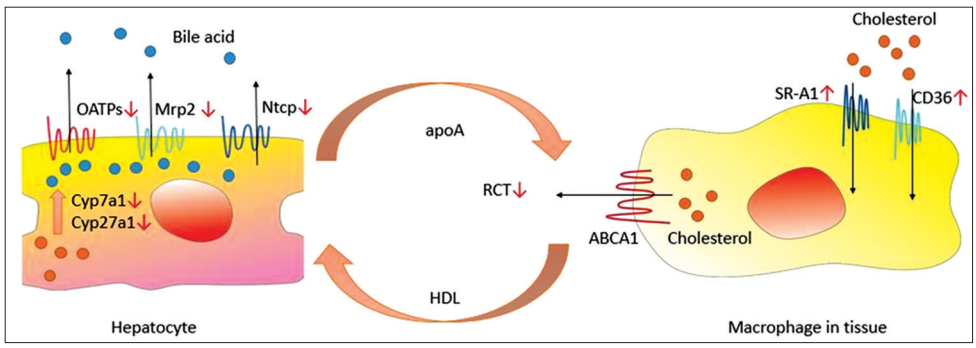

Figure 2: Mechanisms of the pro-atherosclerotic ability of trimethylamine N-oxide. Trimethylamine N-oxide facilitates cholesterol influx into macrophages and inhibit cholesterol efflux by hindering reverse cholesterol transport (RCT) and bile acid excretion.

Flavin monooxygenase 3 expression correlates positively with trimethylamine N-oxide levels and atherosclerotic burdens

FMO3 is the member of the FMO enzyme family with the highest activity level and is capable of oxidizing TMA to TMAO. The expression of *Fmo3* is controlled by androgens and bile acids.[36] A significant positive correlation was observed between *Fmo3* expression and TMAO levels, as well as atherosclerosis lesion sizes, in mice.[8] In patients undergoing liver biopsies, a positive correlation was also observed between hepatic *Fmo3* expression and TMAO levels.[8] Taken together, FMO3 is essential for the formation of TMAO. It has also been found that loss of function (LOF) mutations in *Fmo3* are associated with reduced atherosclerotic burdens in humans.[37]

However, another group observed that although the blockade of FMO3 resulted in decreased TMAO concentrations, increased atherosclerosis was also observed.[38] The mechanism underlying this phenomenon remains unclear.

The Relationship between Microbiota and Trimethylamine N-oxide

Specific intestinal microbiota is associated with trimethylamine N-oxide generation

The human body contains a vast and diverse microbial ecosystem of 10^{14}–10^{15} microorganisms, which begin colonizing the human intestine shortly following the birth and remain there throughout life. Both the species and the quantities of the gut microbiota are closely related to human health.[39] Each of us possesses hundreds of microbial species. The majority of these bacteria fall within the following four phyla: *Actinobacteria*, *Firmicutes*, *Proteobacteria*, and *Bacteroides*.[40,41] It is believed that gut microbiota influence the risk of specific diseases. The composition of gut flora fluctuates in accordance with the food we eat.[42] Gut flora participates in a variety of physiological process, including digesting nutrients, regulating epithelial function, and guiding immune responses.[43] Intestinal flora may cause atherosclerosis by secreting lipopolysaccharides, commonly known as endotoxins.[44]

To verify the hypothesis that gut microbes are essential for TMAO generation, researchers administered antibiotics and observed that gut flora were completely suppressed and that TMA and TMAO were decreased in both chow-fed mice and mice receiving TMA-containing compounds. Meanwhile, plaque burdens in the aorta were decreased, and the numbers of macrophages in the plaques also decreased following antibiotic administration. In another gut flora-free model, germ-free mice were utilized. These mice did not produce TMAO following a carnitine challenge. Following feeding with normal food, intestinal bacteria colonized, and plasma TMAO level rose accordingly.[8,18] Human trials have yielded similar results. Following the administration of broad-spectrum antibiotics, TMAO levels were decreased in volunteers' plasma and urine L-carnitine samples. The reformation of TMAO was subsequently observed when the antibiotics were discontinued.[18,19] Collectively, these observations demonstrated that gut microbes are essential for TMAO generation.

These TMA-producing bacteria could be transplanted in conjunction with atherosclerotic susceptibility. Microbes from atherosclerosis-prone and high TMAO-producing strains enhanced the development of atherosclerosis in mouse recipients.[45]

To identify the microbes responsible for TMA generation, the microbial species localized to the human intestinal tract were cultured with choline *in vitro*; *Anaerococcus hydrogenalis, Clostridium asparagiforme, Clostridium hathewayi, Clostridium sporogenes, Escherichia fergusonii, Proteus penneri, Providencia rettgeri,* and *Edwardsiella tarda* consumed choline and generated TMA. However, none was able to consume carnitine. Surprisingly, when these bacteria were colonized in germ-free mice, only a small proportion (0.15%) was enough to consume choline in the cecum and increase TMA and TMAO levels in the serum, respectively. When a large proportion of TMA-producing bacteria were colonized in the germ-free mice, the choline, TMA and TMAO levels remained unchanged.[46]

Gut flora taxa are influenced by dietary habits

Compared with vegans and vegetarians, omnivores exhibited higher TMAO levels in both the plasma and urine at baseline. Even following an L-carnitine challenge, the vegans exhibited no significant elevations with respect to TMAO levels.[18] The observed differences may be attributed to gut flora taxa. By sequencing bacterial 16S rRNA in fecal samples, several bacterial taxa have been linked with both dietary habits (vegetarians vs. omnivores) and TMAO levels. Among them, *Clostridiaceae* and *Peptostreptococcaceae* are more abundant in omnivores, whereas *Lachnospira* and *Sporobacter* are less abundant in omnivores. Additionally, individuals with enterotype two (enriched of *Prevotella*) exhibited increased TMAO concentrations.[18] We concluded that some components in meat modulated gut flora taxa, thereby influencing the ability of microbes to generate TMAO. TMA-containing nutrients are likely to induce TMA production.

In order to verify this hypothesis, the mice were fed with high L-carnitine chow and several bacterial taxa, including *Anaeroplasma* and *Porphyromonadaceae*, were linked with L-carnitine intake and changes in TMAO concentrations.[18] However, another group observed that choline does not affect the amount of transplanted TMA-producing bacteria in germ-free mice,[46] which may be explained by the fact that the transplanted model is too simple to simulate human gut flora.

Microorganisms produce trimethylamine via several enzymes

The identification of the bacterial enzymes responsible for TMA production may provide more precise therapeutic

targets, as the genes encoding these enzymes may be transferred between bacteria. A two-component enzyme, composed of both an oxygenase (CntA) and a reductase (CntB), is associated with TMA formation from carnitine. A "bridging" glutamate within CntA facilitates electron transfer and is essential for TMA generation.[47] Another group of choline specific TMA-producing enzymes, choline utilization C (CutC) and choline utilization D (CutD), has also been identified. Only the coexpression of CutC and CutD is associated with TMA production, as opposed to the expression of either enzyme alone.[48] The genes encoding these enzymes may be transferred to non-TMA-producing bacteria.[47,48] Additional TMA-producing enzymes must be identified, however.

The genetic influence on trimethylamine N-oxide levels

In addition to environmental factors, the genetic impact on TMAO levels was also analyzed. However, previous researchers failed to identify the locus responsible for the elevated TMAO levels observed in human.[49] It appears that food and gut microbes are the primary determinants of TMAO levels.

Future research and potential therapeutic targets

TMAO, a novel independent cardiovascular risk factor, has drawn the interest of scientists. TMA-containing nutrients, gut flora, and hepatic FMO enzymes are the three indispensable components of this equation. However, many questions regarding this pathway have yet to be elucidated. It is our hope that clarifying these problems may provide us with potential therapeutic targets with respect to the management of CVD.

It is necessary to determine whether the supplementation of TMA-containing compounds is cardioprotective or proatherogenic. According to the gut microbes-TMAO pathway, compounds containing TMA moieties are likely to be metabolized to TMAO, which subsequently accelerates the development and progression of atherosclerosis. However, whether limiting the intake of these compounds are necessary remains unclear. As stated previously, these TMA-containing compounds do not exert direct atherogenic effects. Additionally, they also exert cardioprotective effects. Additional clinical trials utilizing different doses, durations and routes should be considered to determine whether the cardiovascular benefits of TMA-containing compounds outweigh the risks posed by TMAO formation. It is essential to include intake recommendations regarding these TMA-containing compounds in dietary guidelines, as limiting the intake of TMAO precursors fundamentally alters the production of TMAO.

Additionally, TMAO levels may be modulated by manipulating intestinal microbiota. It is necessary to determine which types of bacteria are associated with elevated TMAO levels and whether specific types of bacteria are atherogenic. We may eventually utilize narrow spectrum antibiotics to manage CVD. Some antibiotics may have potential as anti-atherosclerotic medications, as they reduce TMAO levels and plaque sizes. However, many clinical trials using antibiotics for the secondary prevention of CVD ended in failure.[50,51] It appears that only long-term antibiotic use effectively decreases TMAO levels. However, the prolonged administration of antibiotics may be complicated by other problems such as drug tolerance and organ injury. In addition to antibiotics, several other promising methods may be useful with respect to the manipulation of gut microbe taxa. Most recently, an archaea strain known as *Methanomassiliicoccus luminyensis* B10 was found to be able to catabolize TMA.[52] Prebiotics are chemicals that facilitate the growth of certain bacteria and are beneficial for individual's health. However, TMA and TMAO levels increased when mice were fed with prebiotics.[53] The effects of archaea, probiotics, and prebiotics on the cardiovascular system have not yet been elucidated. The direct modulation of gut microbes through the use of antibiotics, probiotics, prebiotics, and other interventions represents a novel treatment method with respect to the management of CVD, as gut microbes are essential for the formation of TMAO.

The administration of medications that decrease TMAO levels is promising, as TMAO causes atherosclerosis. Meldonium decreases the plasma levels of TMAO by increasing its urinary excretion and inhibiting the conversion of TMA to TMAO by bacteria. However, meldonium does not affect either the intake or the conversion of carnitine,[54,55] findings somewhat reminiscent of homocysteine, which is associated with both CVD and stroke. However, homocysteine lowering agents such as vitamin B6, vitamin B12, and folic acid do not reduce the risk of cardiovascular events.[56] It is unclear whether meldonium reduces TMAO levels but does not reduce the risk of CVD. Additional observations regarding the effects of meldonium in the setting of CVD are necessary. In addition to meldonium, other agents that reduce TMAO levels await discovery. The mechanisms underlying TMAO's effects may include either increasing TMAO secretion or suppressing TMAO generation.

The relationship between *Fmo3* mutations and CVD remains unclear and warrants additional clinical research. LOF mutations in *Fmo3* may be cardioprotective, as TMAO levels decrease. Indeed, it was found that LOF mutations involving the *Fmo3* gene in humans were associated with less atherosclerosis.[37] Furthermore, the knockdown of *Fmo3* using antisense oligonucleotide in mice also attenuated atherosclerosis.[57] The knockdown of *Fmo3* not only resulted in decreased TMAO levels but also regulated both lipid metabolism and inflammation, thereby attenuating atherosclerosis.[58] However, individuals without functional FMO3 often exhibit a fishy odor, which is caused by trimethylaminuria.[59] Taken together, these findings indicated that the suppression of FMO3 may represent a potential therapeutic target, a hypothesis that warrants additional basic and clinical research to determine the effects of silencing FMO3 and to weigh the pros and cons of this approach. In addition to using antisense oligonucleotides, another group observed that 17β-estradiol also reduces

Fmo3 mRNA concentrations. However, the effects on atherosclerosis were not evaluated.[60]

TMAO is associated with both heart failure and atherosclerosis. The concentrations of TMAO among patients with heart failure are higher compared with healthy individuals, and TMAO is an independent risk factor for all-cause mortality among patients suffering from heart failure.[20,21,61] Additional studies are necessary to elucidate the correlation among TMAO, CVD and risk factors such as hypertension, dyslipidemia, arrhythmia, and glucose intolerance.

Conclusions

CVD poses a serious threat to human health. Although significant progress has been made regarding both the underlying mechanisms and the treatment of CVD, we remain far from a cure. In addition to the traditional risk factors for CVD, the influence exerted by intestinal microbe metabolism on the pathogenesis of CVD has only recently been recognized. Recent observations indicated that intestinal metabolism may represent a promising therapeutic target with respect to the management of CVD. Measuring TMAO levels in asymptomatic populations to identify individuals with an elevated cardiovascular risk, as well as monitoring changes in TMAO levels to determine the severity of CAD and manipulating gut flora to improve patients' cardiovascular risk, is anticipated in the future.

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Conflicts of interest

There are no conflicts of interest.

References

1. Mendelsohn AR, Larrick JW. Dietary modification of the microbiome affects risk for cardiovascular disease. Rejuvenation Res 2013;16:241-4.
2. Polychronopoulos E, Pounis G, Bountziouka V, Zeimbekis A, Tsiligianni I, Qira BE, *et al.* Dietary meat fats and burden of cardiovascular disease risk factors, in the elderly: A report from the MEDIS study. Lipids Health Dis 2010;9:30.
3. Pan A, Sun Q, Bernstein AM, Schulze MB, Manson JE, Stampfer MJ, *et al.* Red meat consumption and mortality: Results from 2 prospective cohort studies. Arch Intern Med 2012;172:555-63.
4. McAfee AJ, McSorley EM, Cuskelly GJ, Moss BW, Wallace JM, Bonham MP, *et al.* Red meat consumption: An overview of the risks and benefits. Meat Sci 2010;84:1-13.
5. Milicevic D, Vranic D, Mašic Z, Parunovic N, Trbovic D, Nedeljkovic-Trailovic J, *et al.* The role of total fats, saturated/unsaturated fatty acids and cholesterol content in chicken meat as cardiovascular risk factors. Lipids Health Dis 2014;13:42.
6. Lichtenstein AH, Kennedy E, Barrier P, Danford D, Ernst ND, Grundy SM, *et al.* Dietary fat consumption and health. Nutr Rev 1998;56 (5 Pt 2):S3-19.
7. Brown JM, Hazen SL. Metaorganismal nutrient metabolism as a basis of cardiovascular disease. Curr Opin Lipidol 2014;25:48-53.
8. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, *et al.* Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 2011;472:57-63.
9. Ley RE, Bäckhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. Proc Natl Acad Sci U S A 2005;102:11070-5.
10. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: Human gut microbes associated with obesity. Nature 2006;444:1022-3.
11. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. Nature 2006;444:1027-31.
12. Bäckhed F, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. Proc Natl Acad Sci U S A 2007;104:979-84.
13. Li M, Wang B, Zhang M, Rantalainen M, Wang S, Zhou H, *et al.* Symbiotic gut microbes modulate human metabolic phenotypes. Proc Natl Acad Sci U S A 2008;105:2117-22.
14. Delzenne NM, Cani PD. Gut microbiota and the pathogenesis of insulin resistance. Curr Diab Rep 2011;11:154-9.
15. Griffin JL, Scott J, Nicholson JK. The influence of pharmacogenetics on fatty liver disease in the wistar and kyoto rats: A combined transcriptomic and metabonomic study. J Proteome Res 2007;6:54-61.
16. Aron-Wisnewsky J, Gaborit B, Dutour A, Clement K. Gut microbiota and non-alcoholic fatty liver disease: New insights. Clin Microbiol Infect 2013;19:338-48.
17. Ray K. Gut microbiota: Colorectal cancer-driven by inflammation and gut bacteria? Nat Rev Gastroenterol Hepatol 2012;9:558.
18. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, *et al.* Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. Nat Med 2013;19:576-85.
19. Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, *et al.* Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. N Engl J Med 2013;368:1575-84.
20. Tang WH, Wang Z, Shrestha K, Borowski AG, Wu Y, Troughton RW, *et al.* Intestinal microbiota-dependent phosphatidylcholine metabolites, diastolic dysfunction, and adverse clinical outcomes in chronic systolic heart failure. J Card Fail 2015;21:91-6.
21. Tang WH, Wang Z, Fan Y, Levison B, Hazen JE, Donahue LM, *et al.* Prognostic value of elevated levels of intestinal microbe-generated metabolite trimethylamine-N-oxide in patients with heart failure: Refining the gut hypothesis. J Am Coll Cardiol 2014;64:1908-14.
22. Wang Z, Tang WH, Buffa JA, Fu X, Britt EB, Koeth RA, *et al.* Prognostic value of choline and betaine depends on intestinal microbiota-generated metabolite trimethylamine-N-oxide. Eur Heart J 2014;35:904-10.
23. Mourad AM, de Carvalho Pincinato E, Mazzola PG, Sabha M, Moriel P. Influence of soy lecithin administration on hypercholesterolemia. Cholesterol 2010;2010:824813.
24. Rajaie S, Esmaillzadeh A. Dietary choline and betaine intakes and risk of cardiovascular diseases: Review of epidemiological evidence. ARYA Atheroscler 2011;7:78-86.
25. Zeisel SH, da Costa KA. Choline: An essential nutrient for public health. Nutr Rev 2009;67:615-23.
26. Dalmeijer GW, Olthof MR, Verhoef P, Bots ML, van der Schouw YT. Prospective study on dietary intakes of folate, betaine, and choline and cardiovascular disease risk in women. Eur J Clin Nutr 2008;62:386-94.
27. Bidulescu A, Chambless LE, Siega-Riz AM, Zeisel SH, Heiss G. Usual choline and betaine dietary intake and incident coronary heart disease: The Atherosclerosis Risk in Communities (ARIC) study. BMC Cardiovasc Disord 2007;7:20.
28. Pekala J, Patkowska-Sokola B, Bodkowski R, Jamroz D, Nowakowski P, Lochynski S, *et al.* L-carnitine – Metabolic functions and meaning in humans life. Curr Drug Metab 2011;12:667-78.
29. DiNicolantonio JJ, Lavie CJ, Fares H, Menezes AR, O'Keefe JH. L-carnitine in the secondary prevention of cardiovascular disease: Systematic review and meta-analysis. Mayo Clin Proc 2013;88:544-51.
30. Singh RB, Niaz MA, Agarwal P, Beegum R, Rastogi SS, Sachan DS. A randomised, double-blind, placebo-controlled trial of L-carnitine in suspected acute myocardial infarction. Postgrad Med J 1996;72:45-50.
31. Fukami K, Yamagishi S, Sakai K, Kaida Y, Yokoro M, Ueda S, *et al.* Oral L-carnitine supplementation increases trimethylamine-N-oxide but reduces markers of vascular injury in hemodialysis patients. J Cardiovasc Pharmacol 2015;65:289-95.
32. Zhang AQ, Mitchell SC, Smith RL. Dietary precursors of trimethylamine in man: A pilot study. Food Chem Toxicol 1999;37:515-20.

33. Koeth RA, Levison BS, Culley MK, Buffa JA, Wang Z, Gregory JC, *et al.* γ-Butyrobetaine is a proatherogenic intermediate in gut microbial metabolism of L-carnitine to TMAO. Cell Metab 2014;20:799-812.
34. Senthong V, Li X, Coughlin J, Hudec T, Neale S, Li L, *et al.* Higher plasma trimethylamine-n-oxide is associated with greater atherosclerotic burden quantified by the syntax score. J Am Coll Cardiol 2015;65:A1676.
35. Kaysen GA, Johansen KL, Chertow GM, Dalrymple LS, Kornak J, Grimes B, *et al.* Associations of trimethylamine N-Oxide with nutritional and inflammatory biomarkers and cardiovascular outcomes in patients new to dialysis. J Ren Nutr 2015;25:351-6.
36. Bennett BJ, de Aguiar Vallim TQ, Wang Z, Shih DM, Meng Y, Gregory J, *et al.* trimethylamine-N-oxide, a metabolite associated with atherosclerosis, exhibits complex genetic and dietary regulation. Cell Metab 2013;17:49-60.
37. Nohara A, Okazaki S, Yoshida M, Mori M, Nakanishi C, Tada H, *et al.* Impact of fmo3 gene loss-of-function variants on coronary artery disease in Japanese. Circulation 2014;130:A18143.
38. Shih DM, Che N, Meng Y, Wu J, Wang Z, Hasin Y, *et al*. Decreased fmo3 expression is associated with increased atherosclerosis and impaired liver function in apolipoprotein e null mice. Circulation 2013;128 Suppl 1:22.
39. Savage DC. Microbial ecology of the gastrointestinal tract. Annu Rev Microbiol 1977;31:107-33.
40. Bäckhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI. Host-bacterial mutualism in the human intestine. Science 2005;307:1915-20.
41. Gill SR, Pop M, Deboy RT, Eckburg PB, Turnbaugh PJ, Samuel BS, *et al.* Metagenomic analysis of the human distal gut microbiome. Science 2006;312:1355-9.
42. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R. Diversity, stability and resilience of the human gut microbiota. Nature 2012;489:220-30.
43. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, Sargent M, *et al.* Diversity of the human intestinal microbial flora. Science 2005;308:1635-8.
44. Konev IuV, Lazebnik LB. Endotoxin (LPS) in the pathogenesis of atherosclerosis. Eksp Klin Gastroenterol 2011;11:15-26.
45. Gregory JC, Buffa JA, Org E, Wang Z, Levison BS, Zhu W, *et al.* Transmission of atherosclerosis susceptibility with gut microbial transplantation. J Biol Chem 2015;290:5647-60.
46. Romano KA, Vivas EI, Amador-Noguez D, Rey FE. Intestinal microbiota composition modulates choline bioavailability from diet and accumulation of the proatherogenic metabolite trimethylamine-N-oxide. MBio 2015;6:e02481.
47. Zhu Y, Jameson E, Crosatti M, Schäfer H, Rajakumar K, Bugg TD, *et al.* Carnitine metabolism to trimethylamine by an unusual Rieske-type oxygenase from human microbiota. Proc Natl Acad Sci U S A 2014;111:4268-73.
48. Craciun S, Balskus EP. Microbial conversion of choline to trimethylamine requires a glycyl radical enzyme. Proc Natl Acad Sci U S A 2012;109:21307-12.
49. Hartiala J, Bennett BJ, Tang WH, Wang Z, Stewart AF, Roberts R, *et al.* Comparative genome-wide association studies in mice and humans for trimethylamine N-oxide, a proatherogenic metabolite of choline and L-carnitine. Arterioscler Thromb Vasc Biol 2014;34:1307-13.
50. Cannon CP, Braunwald E, McCabe CH, Grayston JT, Muhlestein B, Giugliano RP, *et al.* Antibiotic treatment of *Chlamydia pneumoniae* after acute coronary syndrome. N Engl J Med 2005;352:1646-54.
51. Grayston JT, Kronmal RA, Jackson LA, Parisi AF, Muhlestein JB, Cohen JD, *et al.* Azithromycin for the secondary prevention of coronary events. N Engl J Med 2005;352:1637-45.
52. Brugère JF, Borrel G, Gaci N, Tottey W, O'Toole PW, Malpuech-Brugère C. Archaebiotics: Proposed therapeutic use of archaea to prevent trimethylaminuria and cardiovascular disease. Gut Microbes 2014;5:5-10.
53. Martin FP, Wang Y, Sprenger N, Yap IK, Rezzi S, Ramadan Z, *et al.* Top-down systems biology integration of conditional prebiotic modulated transgenomic interactions in a humanized microbiome mouse model. Mol Syst Biol 2008;4:205.
54. Dambrova M, Skapare-Makarova E, Konrade I, Pugovics O, Grinberga S, Tirzite D, *et al.* Meldonium decreases the diet-increased plasma levels of trimethylamine N-oxide, a metabolite associated with atherosclerosis. J Clin Pharmacol 2013;53:1095-8.
55. Kuka J, Liepinsh E, Makrecka-Kuka M, Liepins J, Cirule H, Gustina D, *et al.* Suppression of intestinal microbiota-dependent production of pro-atherogenic trimethylamine N-oxide by shifting L-carnitine microbial degradation. Life Sci 2014;117:84-92.
56. Liakishev AA. Homocysteine lowering with folic acid and B vitamins in vascular disease. Kardiologiia 2006;46:70.
57. Shih DM, Wang Z, Lee R, Meng Y, Che N, Charugundla S, *et al.* Flavin containing monooxygenase 3 exerts broad effects on glucose and lipid metabolism and atherosclerosis. J Lipid Res 2015;56:22-37.
58. Warrier M, Shih DM, Burrows AC, Ferguson D, Gromovsky AD, Brown AL, *et al*. The tmao-generating enzyme flavin monooxygenase 3 is a central regulator of cholesterol balance. Cell Rep 2015;10:326-38.
59. Mitchell SC, Smith RL. Trimethylaminuria: The fish malodor syndrome. Drug Metab Dispos 2001;29 (4 Pt 2):517-21.
60. Esposito T, Varriale B, D'Angelo R, Amato A, Sidoti A. Regulation of flavin-containing mono-oxygenase (Fmo3) gene expression by steroids in mice and humans. Horm Mol Biol Clin Investig 2014;20:99-109.
61. Troseid M, Ueland T, Hov JR, Svardal A, Gregersen I, Dahl CP, *et al*. Microbiota-dependent metabolite trimethylamine-n-oxide is associated with disease severity and survival of patients with chronic heart failure. J Intern Med 2014;8:12328.