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Meta-Organismal Nutrient Metabolism as a Basis of Cardiovascular Disease

J. Mark Brown, Ph.D. and Stanley L. Hazen, M.D., Ph.D.*

Department of Cellular and Molecular Medicine, Cleveland Clinic Lerner Research Institute, Cleveland, OH 44195, USA

Abstract

Purpose of Review—Atherosclerosis and associated cardiovascular disease (CVD) remains the leading cause of mortality in Western Societies. It is well accepted that consumption of foods abundant in saturated fats and cholesterol, like meats, egg yolk and high fat dairy products, are associated with increased CVD risk. New evidence suggests that trimethylamine-containing nutrients within these foods, including phosphatidylcholine (PC), choline and L-carnitine, can enter into a microbial metabolic pathway that promotes CVD. In this review we highlight the role of gut microbiota-driven nutrient metabolism as a novel pathway promoting CVD.

Recent Findings—Recent studies demonstrate a link between ingestion of dietary PC, choline and L-carnitine and CVD risk. At the center of this pathway is gut microbiota-dependent synthesis of a metabolic intermediate called trimethylamine (TMA), and subsequent host-driven conversion of TMA to trimethylamine-N-oxide (TMAO). Microbiota-dependent generation of TMAO is associated with increased risk of incident major adverse cardiovascular events in humans, and provision of TMAO promotes atherosclerosis in mice.

Summary—Microbial metabolism of trimethylamine containing nutrients can lead to formation of the proatherogenic compound TMAO. Recent insights into this diet-microbe-host interaction provide new clues surrounding the pathogenesis of atherosclerosis, and may serve as a framework for new CVD therapies.

Keywords

atherosclerosis; L-carnitine; cholesterol; trimethylamine; trimethylamineoxide

*Correspondence should be addressed to: Stanley L. Hazen, M.D., Ph.D. 9500 Euclid Avenue, NC-10, Cleveland Clinic, Cleveland, OH 44195. Tel: 216-445-9763; Fax: (216)-636-0392; hazens@ccf.org.

Conflict of Interest:

Dr. Hazen is named as co-inventor on pending and issued patents held by the Cleveland Clinic relating to cardiovascular diagnostics and therapeutics. Dr. Hazen reports he has been paid as a consultant or speaker by the following companies: Cleveland Heart Lab, Inc., Esperion, Liposcience Inc., Merck & Co., Inc., Pfizer Inc., and Proctor & Gamble. Dr. Hazen reports he has received research funds from Abbott, Astra Zeneca, Cleveland Heart Lab, Esperion, Liposcience, Inc., Proctor & Gamble and Takeda. Dr. Hazen has the right to receive royalty payments for inventions or discoveries related to cardiovascular diagnostics from Abbott Laboratories, Cleveland Heart Lab, Inc., Frantz Biomarkers, Liposcience, Inc., and Siemens.

INTRODUCTION

Cardiovascular disease (CVD) continues to represent the leading cause of death and morbidity worldwide. Meat consumption is linked to increased CVD risk, and most studies have attributed this connection to the large amount of saturated fat and cholesterol in meat products [1-4]. However, recent studies have shown that other components in meat such as phosphatidylcholine (PC), choline and L-carnitine can promote atherosclerotic CVD in animal models through a novel meta-organismal metabolic pathway. The concept of the meta-organism was first introduced by Karl Möbius over a century ago when he noted that the health of European oyster populations was dependent of the presence of other species in the surrounding ecosystem [5]. In the context of humans, the meta-organismal theory postulates that cross species interaction between microbial communities and the human host that they colonize can serve as the basis of health or disease [6-9]. Within the last decade there has been an explosion of reports linking meta-organismal pathways to diverse human diseases such as obesity [10-17], diabetes [18,19], hepatic steatosis [18-21], cancer [22,23], osteoporosis [24], and most recently CVD [25-28]. Collectively, these reports have bolstered the concept that diet-microbe-host interactions can contribute to the development of many chronic diseases. However, our understanding of the complex interactions between microbes and the hosts they inhabit is only in its infancy. The purpose of this review is to highlight recent reports linking meta-organismal metabolism of choline and L-carnitine to CVD [25-28]. Here we discuss the current state of knowledge of this newly described metabolic pathway responsible for the generation of the proatherogenic compound trimethylamine-N-oxide (TMAO), and discuss potential therapeutic intervention strategies targeting this pathway for protection against CVD.

DISCOVERY OF A META-ORGANISMAL PATHWAY CONTRIBUTING TO ACCELERATED CARDIOVASCULAR DISEASE

Metabolomics has emerged as a powerful platform for the discovery of both small molecules and biochemical pathways that are associated with human disease [28,29]. Using an unbiased metabolomic approach to identify small molecule metabolites associated with CVD risk in human plasma, Wang and colleagues [25] recently uncovered a meta-organismal nutrient metabolism pathway driving CVD. In two small and one large independent clinical cohort of subjects it was found that plasma levels of three metabolites (choline, TMAO, and betaine) of the dietary lipid phosphatidylcholine (PC) were highly predictive of CVD risk [25]. In agreement, feeding atherosclerosis prone mice diets enriched in either choline or TMAO enhanced atherosclerosis development [25]. Strikingly, both TMAO production and the enhanced atherosclerosis seen with dietary choline supplementation was dependent on gut microbiota, given that antibiotic treatment prevented choline-induced atherosclerosis and studies in germ-free mice demonstrated that blood TMAO levels are only detected in mice following conventionalization (placement in conventional cages and colonization with gut microbiota) [25]. Collectively, these original studies uncovered a novel meta-organismal metabolic pathway linking oral intake of PC, the major dietary source of choline, and CVD risk. This pathway is highlighted in Figure 1, and involves the gut microbiota-dependent metabolism of dietary PC to generate the gas

trimethylamine (TMA), which is subsequently metabolized by enzymes of the flavin monooxygenase (FMO) family in the host's liver to generate the circulating proatherogenic compound TMAO. Although major steps in this meta-organismal metabolic pathway have been recently defined (Fig. 1), much additional work is needed to determine how environmental and genetic factors impact plasma levels of proatherogenic TMAO.

ANOTHER DIETARY LINK: META-ORGANISMAL METABOLISM OF L-CARNITINE PROMOTES ATHEROSCLEROSIS

L-carnitine is a particularly abundant nutrient in red meat, and contains a trimethylamine structure very similar to choline. Although L-carnitine has long been claimed to provide health benefit by the dietary supplement industry, the effects of chronic L-carnitine supplementation need to be carefully reconsidered given that L-carnitine has recently been described as a key substrate for meta-organismal promotion of atherosclerosis [26]. Much like dietary choline [25], the tertiary amine structure of L-carnitine is readily converted to TMA by gut microflora [26]. In fact, bacterial metabolism of L-carnitine can provide an abundant source of TMA that ultimately produces proatherogenic TMAO in both mice and man [26]. For instance, feeding L-carnitine to hyperlipidemic mice alters gut microbe composition, increases blood TMAO levels, and promotes atherosclerosis in a gut microbiota-dependent fashion [26]. In agreement, plasma L-carnitine levels, which previous studies show are loosely correlated with carnitine intake, predict increased risks for both prevalent CVD and incident major adverse cardiac events (myocardial infarction, stroke, or death) in a large clinical cohort (n=2,595), but only in subjects where TMAO levels were also elevated [26]. Also, omnivorous subjects were found to produce substantially more TMAO than vegans or vegetarians when challenged with comparable amounts of deuterium labeled L-carnitine, and clinical studies in subjects before versus following oral cocktail of antibiotics showed that dietary L-carnitine conversion into TMAO has an obligatory requirement on intact gut microbiota in humans [26]. Intriguingly, proportions of microbial taxa belonging to the Clostridiaceae and Peptostreptococcaceae families within feces were observed to be positively associated with both omnivorous dietary patterns and blood levels of TMAO in humans, making it tempting to speculate that these taxa may be involved in the initial metabolism of L-carnitine to TMA in the gut [26]. Collectively, these results suggest that in both mice and humans chronic exposure to dietary L-carnitine promotes the production of the proatherogenic compound TMAO, and may in part explain why high red meat consumption is associated with increased CVD risk. Of note, further investigation into the mechanisms through which TMAO may promote a pro-atherogenic effect revealed that dietary TMAO, and precursors like choline and carnitine that ultimately lead to TMAO formation in the presence of intact gut microbiota, potentially alter cholesterol and sterol metabolism in multiple different compartments *in vivo*, including up regulation of macrophage scavenger receptors, and alternations in bile acid metabolism and sterol transporters both within the liver and intestine [25,26]. Thus, the atherogenic effects of TMAO appear to be acting through changes in cholesterol and sterol metabolism. These studies have important implications for the dietary supplement industry, given that supplementation of L-carnitine is pervasive, and long-term safety of supplementation has not been explored. These recent studies question whether chronic exposure to carnitine rich diet

from a pill, an energy drink or red meat consumption may alter the gut microbiota composition in a way that enhances the generation of proatherogenic TMAO and advances CVD risk [26].

CLINICAL RELEVANCE: META-ORGANISMAL PRODUCTION OF TMAO IS LINKED TO CVD RISK IN MAN

It is important to point out that the association between blood levels of TMAO and CVD risk was originally discovered using a metabolomics screening approach in two independent clinical cohorts, and then subsequently by a large non-overlapping clinical cohort [25], giving this pathway clear relevance to human disease. The clinical relevance of the TMAO pathway was recently expanded on by Tang and colleagues [27], who demonstrated that the production of TMAO from dietary PC is dependent on gut microbial metabolism, and that elevated TMAO levels associate with increased risk of incident major adverse cardiovascular events in an even larger independent clinical cohort (n=4007). In the first series of studies in this recent report, healthy subjects received stable isotope-labeled PC in a capsule along with two hard boiled eggs before versus following a week of broad-spectrum antibiotics. These studies showed that several PC metabolites including TMAO were elevated following PC challenge, as expected in subjects not receiving antibiotic treatment. In contrast, antibiotic treatment abolished TMAO (and TMA) production from dietary PC [27], indicating an obligatory role of gut microbes in initiating this pathway in man. Furthermore, when the relationship between fasting plasma levels of TMAO and incident major adverse cardiovascular events (myocardial infarction, stroke, or death) over the ensuing three-year period was examined in 4007 patients undergoing elective coronary angiography, a striking association with adverse events was observed [27]. In fact, the hazard ratio for the highest versus lowest TMAO quartile in this cohort was 2.54, which was significantly higher than traditional risk factors such as LDL-cholesterol [30]. Elevated TMAO levels retained strong prognostic value for predicting incident adverse cardiovascular events after adjustment for traditional risk factors, markers of inflammation, and estimated renal function [27]. In agreement, an independent study recently showed that circulating TMAO levels can predict acute clinical event in patients with myocardial infarction [31]. Collectively, these new data suggest that the meta-organismal pathway responsible for the production of TMAO is an important contributor to increased CVD risk in humans, and provides a strong rationale for continued study of how TMAO mechanistically promotes atherosclerosis and associated CVD.

MECHANISMS OF ACTION: HOW DOES TMAO PROMOTE ATHEROSCLEROSIS?

Given that blood levels of TMAO are linked to CVD risk in man, the next obvious question is whether circulating TMAO is simply a biomarker of disease or whether TMAO is mechanistically involved in CVD pathogenesis. Current evidence suggests the latter, given that dietary supplementation of TMAO in hyperlipidemic mice promotes atherosclerosis in a gut microbiota-dependent manner [25,26]. However, the mechanism(s) by which circulating TMAO promotes CVD is currently unclear and under active investigation. TMAO has

historically been thought of as a key osmolyte, serving as a chaperone to stabilize proteins under denaturing conditions such as high urea or elevated water pressure [32,33]. More recently, TMAO has been shown to regulate distinct steps in cholesterol and sterol metabolism and macrophage foam cell formation that have clear impact on CVD pathogenesis [25,26]. Wang and colleagues recently demonstrated that dietary choline or TMAO supplementation results in increased expression of scavenger receptors (CD36 and SR-A1) on macrophages and subsequently promotes foam cell formation [25]. In addition, TMAO feeding reduces macrophage reverse cholesterol transport [26], which would be expected to advance atherosclerosis. Although, TMAO feeding clearly impacts multiple steps of both forward and reverse cholesterol transport [25,26] the true mechanism behind these phenomena are unclear. There are several key unresolved questions surrounding how circulating TMAO levels are sensed to elicit pathological responses, and additional research is needed to elucidate mechanisms by which TMAO promotes CVD.

PROJECTING FORWARD: CONSIDERATIONS FOR THERAPIES DESIGNED TO LOWER CIRCULATING TMAO LEVELS FOR ATHEROPROTECTION

The meta-organismal pathway responsible for balancing blood levels of TMAO is clearly associated with CVD risk in humans [25-27], and TMAO feeding worsens atherosclerosis in mice [25,26]. Therefore, it now becomes imperative to examine the pathway as a whole to determine potential sites of therapeutic intervention for either the prevention or treatment of CVD. The most straight-forward point of intervention is to limit the dietary consumption of tertiary amines that can serve as substrates for TMA production. Currently, we know that choline, PC [25,27] and L-carnitine [26] can all serve as dietary substrates to initiate this pathway. However, there are likely other abundant amines in the food supply that can also enter into microbiota-dependent TMA production. Hence, it will become extremely important to identify all major dietary amines that can enter into the TMAO pathway. Simply limiting the consumption of foods rich in TMA-producing amines will likely prove to be an effective strategy to limit circulating TMAO; however, based on current studies, the duration of how long reduced dietary exposure needs to be maintained to favorably alter intestinal microbial composition to one less poised to produce TMA is unknown [25-27]. It is relevant that people suffering from the condition trimethylaminuria, who often have mutations in the hepatic enzyme(s) that convert TMA to TMAO, can dramatically reduce circulating TMA levels by eating a low-fat and low-choline diet [34].

The next potential point of intervention lies at the level of modulating gut microbiota-dependent generation of TMA. In theory, this could be done using a probiotic approach to limit gut microbiota-dependent TMA production. In support of a probiotic approach, introduction of a several distinct “humanized” gut microbiomes into mice differentially altered the production of TMAO [35], supporting the idea that specific microbial taxa may preferentially foster (or hinder) direct formation of TMA or the microbial environment that favors TMA production and hepatic conversion to TMAO. Although probiotic treatments in theory may hold promise, demonstration of an anti-atherosclerosis effect via this mechanism has not yet been reported, and additional studies are needed.

Another potential site of therapeutic intervention lies at the level of the mammalian enzymes present in the liver that convert bacterially-derived TMA to TMAO. The enzymes responsible for this conversion belong to the flavin mono-oxygenase (FMO) enzyme family, with at least 5 paralogues present in human liver (FMO1, FMO2, FMO3, FMO4, and FMO5) [36-40]. The FMO enzyme family is responsible for the oxygenation of a plethora of nitrogen- and sulfur-containing compounds present in xenobiotics as well as endogenous substrates [36-40]. Recently, it was shown that FMO3 and FMO1 are the major human FMO enzymes that can efficiently oxidize TMA to form TMAO, and FMO3 exhibits a greater than 10-fold higher specific activity than FMO1 making it the predominant source of TMAO in humans [40]. Interestingly, it is reported that loss-of-function mutations of the FMO3 gene results in the inherited disorder trimethylaminuria [36-39]. This disease is also known as fish (mal)odor syndrome, which arises from the inability of those affected to convert TMA, which smells like rotten fish, to TMAO [36-39]. The FMO3 gene is under complex transcriptional control, where expression is dynamically regulated by inflammation, sex hormones, and the bile acid-activated nuclear receptor FXR [40-42]. Further, the impact of specific mutations on adjacent FMOs within the gene cluster and the potential for coordinated regulation amongst FMO makes this a complex target for inhibition [36-42]. Moving forward it will be important to better understand how hepatic FMO activity is regulated under physiological and pathophysiological conditions. In addition, it will be informative to determine whether patients with extremely low levels of circulating TMAO due to FMO3 mutations have a lower incidence of CVD. Likewise, FMO3 inhibitor or genetic knockout studies in animal models of atherosclerosis are warranted. Given that the link between the meta-organismal TMAO pathway and CVD has only been established in the last two years, many additional studies are required to gain insights into where therapeutic interventions should be targeted.

CONCLUSION

Recent discovery of the meta-organismal TMAO pathway has broad implications for CVD drug discovery, and in the future may factor into dietary recommendations for the prevention of CVD. Foods abundant in trimethylamines like PC, choline and carnitine include red meat, egg yolks, and high fat milk products – foods known to also be a rich source of cholesterol and saturated fats. However, it is notable that certain fish also contain a high level of TMAO, making dietary recommendations regarding this pathway probably best at present to remain based on the large-scale epidemiology studies and CVD risks. The recognition of this meta-organismal pathway in CVD pathogenesis suggests that a better understanding of intestinal bacterial metabolism of PC, choline, L-carnitine and other trimethylamine nutrients requires further examination. Elevated plasma TMAO levels are strongly associated with increased CVD risk in humans, and feeding PC, L-carnitine, or TMAO to mice promotes atherosclerosis. Collectively, these new data suggest that targeting the pathway responsible for maintaining circulating TMAO levels may provide therapeutic benefit for those suffering from CVD. To effectively target this new meta-organismal pathway, we need to understand: 1) the dietary substrates that can enter into microbial metabolic pathways, 2) the microbes and their enzymes that generate TMA from dietary sources, 3) host enzymes that convert TMA to TMAO, and 4) molecular mechanisms by

which TMAO alters cholesterol and sterol metabolism, and promotes CVD. Advancement in these areas has strong potential to lead to novel therapies for the prevention or treatment of CVD. These recent findings also bolster the concept of the meta-organismal basis of metabolism, where cross talk between microbial communities and the host in which they reside plays a central role in human disease.

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Abbreviations Used

CD36	cluster of differentiation 36
CVD	cardiovascular disease
FMO3	flavin monooxygenase 3
FXR	farnesoid X receptor
HDL	high density lipoprotein
LDL	low density lipoprotein
PC	phosphatidylcholine
RCT	reverse cholesterol transport
SR-A1	scavenger receptor A1
TMA	trimethylamine
TMAO	trimethylamineoxide

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Key Points

- Resident microbial communities and host collectively create a meta-organism that promote metabolism of nutrients that influence susceptibility for development of cardiovascular disease.
- Intestinal microbiota-dependent metabolism of PC and L-carnitine produces TMA, which is further metabolized by host enzymes to the proatherogenic compound TMAO.
- Elevated plasma TMAO levels are associated with an increased risk of incident major adverse cardiovascular events in man.
- To effectively target this new meta-organismal pathway, we need to understand: the dietary substrates that can enter into microbial metabolic pathways, the microbial enzymes that generate TMA from dietary sources, host enzymes that convert TMA to TMAO, and molecular mechanisms by which TMAO promotes CVD.
- Therapies that target TMAO forming pathways, such as intervening at the level of gut microbial communities, may provide a new therapeutic strategy for CVD prevention.

FIGURE 1. Meta-organismal pathways linking dietary phosphatidylcholine or choline and L-carnitine and cardiovascular disease

Once ingested by the host, both the choline component of phosphatidylcholine (lecithin) and L-carnitine can be metabolized by gut microbiota to generate trimethylamine (TMA). The resulting bacterial metabolite TMA can then be converted to trimethylamine-N-oxide (TMAO) by the flavin monooxygenase family of enzymes (FMOs) in the host's liver. Elevated circulating TMAO levels are associated with increased risk of incident major adverse cardiovascular events including heart attack, stroke, and death in humans. Studies in mice have demonstrated that TMAO promotes atherosclerosis by dually increasing foam cell formation in the artery wall and decreasing reverse cholesterol transport. Collectively, this

sequential nutrient metabolism pathway represents a new pathway for cardiovascular disease drug discovery efforts.