

Eggs as a dietary source for gut microbial production of trimethylamine-*N*-oxide^{1–3}

Stanley L Hazen and J Mark Brown

Recent studies provide compelling evidence for a new nutritional pathway contributing to the development of atherosclerosis and incident risks of major adverse cardiovascular events such as heart attack, stroke, and death (reviewed in reference 1). The pathway was originally discovered on the basis of an unbiased metabolomics investigation searching for plasma analytes whose concentrations in subjects predict cardiovascular risks. Through studies that used an initial learning cohort, followed by multiple validation cohorts, a compound called trimethylamine-*N*-oxide (TMAO)⁴ was discovered whose concentrations in plasma reproducibly track with risk of prevalent cardiovascular disease (2). More recently, in a subsequent prospective expansion study of >4000 subjects, elevated plasma TMAO concentrations were shown to predict incident risk of major adverse cardiac events (myocardial infarction, stroke, or death) over the ensuing 3-y period after enrollment, independent of traditional cardiovascular risks factors, inflammation markers, and renal function (3). “Reverse engineering” of the origins of TMAO in both animal models and humans with the use of stable isotope–labeled precursors unequivocally showed that TMAO is formed by ingestion of specific dietary nutrients with a trimethylamine moiety, including choline (2), phosphatidylcholine (lecithin) (2, 3), and L-carnitine (4). In studies with either germ-free mice or the use of a cocktail of poorly absorbed broad-spectrum antibiotics, the pathway was shown to involve an obligatory role for gut microbiota (2–4). Specifically, gut microbes convert nutrients such as choline, phosphatidylcholine (lecithin), or L-carnitine into trimethylamine, and then a cluster of host hepatic enzymes called flavin monooxygenases (FMOs), particularly FMO3 (2, 5), generate TMAO.

Subsequent animal model studies showed that TMAO is more than simply an associated biomarker for cardiovascular disease risk; rather, TMAO appears to directly participate in the atherosclerotic process. For example, the addition of TMAO to the diet in atherosclerosis mouse models showed accelerated aortic root atherosclerosis (2). In addition, supplemental dietary precursors, choline (2) or L-carnitine (4), similarly fostered both TMAO generation and accelerated atherosclerosis in animal model studies. The mechanisms through which TMAO augments atherosclerosis, although not yet completely elucidated, appear to involve changes in cholesterol and sterol metabolism in multiple compartments, including TMAO-mediated upregulation of scavenger receptors leading to cholesterol deposition in macro-

phages (4). In addition, TMAO and its dietary precursors, choline and L-carnitine, were shown to promote alteration in bile acid synthesis, composition, and transport in the liver and changes in both bile acid and cholesterol transport in the intestines (4). In vivo model studies showed that TMAO, or the dietary nutrients choline or L-carnitine (but only in the presence of gut microbes where TMAO was formed), also significantly inhibited reverse cholesterol transport (4). In more recent clinical studies, TMAO elevations were associated with increased cardiovascular risks among subjects with heart failure, independent of cardiorenal indexes (6), and to account for the increased cardiovascular disease risks associated with elevated plasma concentrations of choline or betaine in subjects (7). Thus, a growing body of evidence is accruing that indicates a new nutritional contribution to the pathogenesis of cardiovascular disease involving specific dietary trimethylamine-containing nutrients and a meta-organismal pathway involving both gut microbial and host hepatic (eg, FMO3) enzymes.

With the discovery of this new pathway as a contributor to cardiovascular disease pathogenesis and risks, it becomes increasingly important to understand the dietary nutrients that potentially contribute to TMAO formation in vivo. Importantly, numerous dietary precursors carry a trimethylamine moiety and thus potentially can form TMAO. L-Carnitine, a nutrient abundant in red meat, is one important source, particularly among omnivores (4). We also reported that free choline and phosphatidylcholine, a major dietary source of total choline, are alternative nutrients that can promote TMAO generation via gut microbes (2, 3). However, the relative importance of phosphatidylcholine and, in particular, egg ingestion, for TMAO production was questioned. In the current issue of the *Journal*, Miller et al (8) report results from an elegantly designed study aimed at clarifying whether the ingestion of eggs can result in significant increases in TMAO concentrations in subjects.

¹ From the Department of Cellular and Molecular Medicine, Lerner Research Institute, Cleveland, OH.

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³ Address correspondence to SL Hazen, 9500 Euclid Avenue, NC-10, Cleveland Clinic, Cleveland, OH 44195. E-mail: hazens@ccf.org.

⁴ Abbreviations used: CRP, C-reactive protein; FMO, flavin monooxygenase; TMAO, trimethylamine-*N*-oxide.

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A small longitudinal, double-blind, randomized dietary intervention study was performed in which subjects ($n = 6$) were provided a single breakfast composed of 0, 1, 2, 4, or 6 egg yolks, with >2-wk washout periods between dietary challenges. In addition, on the day before and the day of each challenge, subjects received a standardized low choline menu. The authors observed, in general, a dose-dependent increase in TMAO concentrations in both plasma and 24-h urine collections across the range of egg ingestion examined, with ≥ 2 eggs resulting in significant increases in TMAO concentrations over baseline. These results confirmed and extended observations previously reported by Tang et al (3) showing both natural abundance and heavy isotope-labeled TMAO generation in subjects after the ingestion of 2 hard-boiled eggs and a capsule containing synthetic deuterium (d9-trimethyl)-labeled phosphatidylcholine. These latter studies also showed virtually complete elimination in detectable TMAO and d9-TMAO production, despite phosphatidylcholine ingestion (natural abundance and d9-isotopologue), after 1 wk of oral antibiotic treatment, thus showing a requirement for gut microbes in TMAO generation from phosphatidylcholine in humans. In the present study by Miller et al (8), a clear dose-response relation was established for TMAO production in response to a single meal of eggs, with ~ 11 – 15% of total choline ingested converted to TMAO. Furthermore, significant interindividual variability in TMAO production was noted, all subjects examined showed elevated TMAO production with the larger amounts of eggs consumed.

Given the previous reports linking TMAO to cardiovascular disease development in animal models (2, 4, 5) and adverse event risks in subjects (2–4, 6), the present study showing elevations in plasma and urinary TMAO concentrations after ingestion of ≥ 2 eggs (8) raises important new questions about appropriate amounts of dietary phosphatidylcholine and choline. Choline is an essential nutrient and is necessary in the diet. In fact, one of the authors, Steven Zeisel, is a world leader in the study of choline metabolism and has made numerous seminal contributions enumerating choline as an essential dietary nutrient critical for normal liver and muscle function and in fetal development (9, 10). However, although it is clear that a minimal essential amount of dietary choline is necessary to prevent deficiency, this does not exclude the possibility that long-term excess ingestion may, reciprocally, be linked to adverse health consequences, such as cardiovascular disease risk. It is not known whether humans, like the results observed in rodent models (2, 4), will develop accelerated atherosclerosis with excess ingestion of TMAO directly or nutrient precursors that generate TMAO including choline, phosphatidylcholine, and L-carnitine. To try and address cardiovascular risks, Miller et al (8) tested for and found no change in high-sensitivity C-reactive protein (CRP) and oxidized LDL concentrations in subjects acutely after exposure to a single meal containing eggs. However, one would not expect to see changes in these nonspecific biomarkers after the single meal exposure, and on the basis of known mechanisms through which TMAO appears to be linked to cardiovascular disease (2, 4). In fact, multiple prior studies have shown that circulating TMAO concentrations are not correlated with CRP concentrations and the prognostic value of TMAO is not significantly attenuated by inclusion of CRP into multilogistic regression models (2–4, 6, 7). Thus, among those at high risk, such as those with cardiovascular disease or multiple risk factors, it would seem prudent to avoid

excess ingestion of foods that contain high amounts of nutrients (choline, lecithin, L-carnitine) that give rise to TMAO formation. Indeed, simply adhering to current dietary recommendations for limiting cholesterol-rich foods such as red meat, egg yolks, and high-fat dairy products among those at risk of coronary artery disease would similarly reduce ingestion of the nutrient precursors for TMAO.

It is important to note that the present study only examined the effect of single-meal exposures to phosphatidylcholine (eggs), with at least a 2-wk washout period between dietary challenges. It is thus conceivable that the concentrations of TMAO produced might have been even higher had more chronic dietary exposures been examined. For example, previous studies in both animal models and humans examining an alternative dietary nutrient that serves as a precursor for gut microbe-dependent formation of trimethylamine and TMAO, L-carnitine, showed that chronic dietary exposure played a major role in the ultimate TMAO concentrations observed (4). Specifically, mice fed a diet chronically (6–7 wk) supplemented with L-carnitine showed a >10-fold increase in baseline plasma TMAO concentrations and a >10-fold increase in TMAO produced after a d3(methyl)-L-carnitine challenge (4). Similarly, in humans, subjects with minimal dietary L-carnitine (vegetarians and vegans) showed nominal capacity to form TMAO from defined L-carnitine dietary ingestion compared with subjects with chronic exposure to L-carnitine in their diet (omnivores) (4). Analysis of gut microbial composition in the rodent models, and the human studies, showed that chronic dietary exposure to L-carnitine was associated with substantial reorganizations in microbial community structure, with proportions of specific microbial genres being associated with TMAO concentrations (4). It is thus of interest whether a study design with more chronic dietary exposure to phosphatidylcholine would have permitted microbial community structural changes (ie, the microbes that prefer lecithin as food would have a selective advantage and their proportions would increase), resulting in both an increase in fasting plasma TMAO concentrations and an even greater increase in TMAO production after acute dietary exposure. Although fecal microbial compositions were examined in the study by Miller et al (8), the limited sample size precluded the ability to make meaningful conclusions.

The authors wisely caution against extrapolation of their findings with egg ingestion and TMAO production to changes in policy for the minimum choline intake recommendations for individuals. Neither the present studies nor the recent prior investigations into the TMAO meta-organismal pathway examined the issue of minimum daily choline requirements. Rather, they address what dietary nutrients generate TMAO, and in the case of prior investigations, the associations between elevated concentrations of TMAO and both atherosclerotic cardiovascular disease risks in subjects and accelerated atherosclerosis in animal models. It is also critical to recognize that choline and phosphatidylcholine are not the sole nutrient sources for the production of TMAO by gut microbes. In addition, L-carnitine appears to be a major source among many omnivores (4). Furthermore, recent studies of betaine (7), an oxidation product of choline, show that it, too, can generate TMAO, although to a substantially lower extent than free choline. Whether other trimethylamine-containing nutrient precursors such as short- or long-chain acylcarnitines, sphingomyelin, glycerophosphocholine, or phosphocholine can give rise to trimethylamine and TMAO, and affect atherosclerosis susceptibility, remains unknown.

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