See corresponding article on page 1503.

Trimethylamine *N*-oxide variation in humans: the product of a diet–microbiota interaction?

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It has been a decade since trimethylamine *N*-oxide (TMAO), a metabolite generated by interconnected exogenous (e.g., diet and gut microbiota) and endogenous (e.g., hepatic enzymes) processes, was first linked to cardiovascular disease (CVD) (1). Since then, at least 2 meta-analyses (2, 3) of >25 studies concluded that higher circulating TMAO concentration is associated with greater risk of incident CVD events and death in humans. Regardless of whether this association is causal (4), understanding the factors that explain TMAO variation is necessary to determine if and how this metabolite can be modified and leveraged for CVD prevention.

TMAO can be absorbed directly from dietary sources, such as fish (5, 6), or produced through an intricate diet-microbe-liver pathway. In the latter, experimental models implicate intestinal microbes with the bioconversion of nutrients choline, carnitine, and betaine into trimethylamine (TMA) (1, 7), after which TMA is absorbed and oxidized to TMAO by hepatic enzymes (8). Still, our understanding of dietary influences on circulating TMAO remains unclear, because there is conflicting epidemiological evidence for the relative importance of specific foods with TMAO variation (9–12). Part of this heterogeneity could be due to the complex interplay between diet and gut microbiota, which has not yet been rigorously examined.

In this issue of the Journal, Mei et al. (13) investigated associations of serum TMAO and its precursors with diet, CVD, and gut microbiota in the Hispanic Community Health Study/Study of Latinos (HCHS/SOL), a community-based cohort study of US Hispanic/Latino adults. Serum TMAO and its precursor metabolites (i.e., choline, carnitine, and betaine) were measured using untargeted metabolomics. Dietary intake was assessed with a single 24-h recall per participant. Consistent with prospective studies (2), both TMAO and betaine were associated with higher odds of prevalent CVD in the overall analytic sample ($n = 3827$); yet, incongruent with prior literature, carnitine and choline were not associated with CVD. In participants with a 24-h recall ($n = 3466$), intakes of red meat, fish, and eggs were positively and significantly associated with serum TMAO levels independent of other foods. These 3 foods were also differentially associated with TMAO precursors. Red meat intake was positively associated with carnitine, whereas consumption of eggs was positively associated with choline and betaine; fish was not associated with any TMAO precursor. Interestingly, model adjustment for red meat, fish, and egg intake did not attenuate the TMAO-CVD association, suggesting that these associations are either independent of diet, or that diet was imprecisely measured and thus adjustment did not eliminate its confounding effect.

Perhaps most illuminating were the analyses conducted in a subset of individuals ($n = 626$) with intestinal microbiota data (collected 7 y after assessment of diet and TMAO), in which the authors identified 9 unique bacterial taxa associated with TMAO. There were 4 species, all within the *Clostridiales* order, that had positive associations with TMAO. Among those, *Oscillobacter* spp. are of particular interest because they can bioconvert TMA precursors to TMA (14) and because these bacteria were previously associated with stroke (15). Intriguingly, an investigation of diet–microbe interactions found that TMAO was elevated only in red meat consumers who had a high abundance of any of these 4 *Clostridiales* species; there were no interactions between these bacteria and egg or fish intake on TMAO levels. The fact that these bacteria lacked genes necessary for converting choline to TMA could explain why they did not modify associations of eggs with TMAO; still, that none of the bacterial taxa positively associated with TMAO levels had an interaction with egg intake was somewhat surprising, given prior research indicating that bacteria are necessary to convert phosphatidylcholine from eggs to TMAO (1).

These findings should be interpreted in light of study limitations. First, all analyses were essentially cross-sectional, with the caveat that gut microbiota was estimated from stool samples collected ∼7 y after assessment of diet, TMAO, and prevalent CVD. This study design hindered the ability to establish temporality, and it raises concerns about reverse causation, because diagnosis of CVD or other cardiometabolic diseases can lead to changes in diet and, consequently, the microbiome. Another limitation is that the authors used a single 24-h

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dietary recall for the primary analyses. This might have led to misclassification of diet and thus spurious false positive and false negative associations. Use of additional 24-h recalls, or of alternative dietary assessment methods, would have improved precision of diet estimates. In addition to these limitations, there were seemingly missed analytic opportunities that could have shed additional light on the roles of microbiota and diet in CVD. For example, the authors did not report on associations of the identified TMAO-associated *Clostridiales* species and prevalent CVD, which would lend more support to a role for these bacteria in TMAO-mediated CVD. And although authors did adjust their CVD analyses for red meat, egg, and fish intakes, they did not report whether these foods are themselves independently associated with CVD, nor if the TMAO-associated bacteria modify these associations.

Withstanding these limitations, Mei and colleagues should be applauded for an important contribution to the literature. In examining diet–microbe interactions underlying the interindividual variation in TMAO, they highlight that not all paths to TMAO are equal, which has potential implications for the role that TMAO plays in CVD etiology. Emerging from this work are several outstanding questions that merit follow-up investigation. For example, might the TMAO-associated bacteria herein identified modify the recently reported effect of meat compared with plant-based meat alternatives on TMAO (16)? Another question is what factors determine differential abundance of TMAO bacteria, particularly in red meat eaters? Might the bacteria be under selective pressure from other dietary factors, such as microbiota-accessible carbohydrates, or the processing or preparation of meat, which could also influence TMAO (12)? And ultimately, we are still left to wonder whether the reported associations are causal and whether intervening on TMAO-associated bacteria, or TMAO itself, reduces CVD risk? Some of these questions could be addressable using HCHS/SOL data, whereas others will require new observational studies or feeding studies, such as those proposed by the NIH precision nutrition initiative (17). We look forward to developments in this exciting research arena over the coming years, with optimism that the expansion in research at the nexus of nutrition and microbiome can lead to improved health.

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