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Taking the "Me" out of meat: A new demethylation pathway dismantles a toxin's precursor

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Carnitine, a molecule found in red meat, is metabolized to trimethylamine (TMA) by the gut microbiota. TMA is then converted in the liver to trimethylamine oxide, a causative agent for atherosclerosis. Kountz *et al.* have discovered an alternative pathway for carnitine metabolism in the gut bacterium *Eubacterium limosum*. Instead of forming TMA, carnitine is demethylated by the newly discovered methyltransferase MtcB, sending one-carbon units into production of short-chain fatty acids. These results suggest that bacterial metabolic activities could promote cardiovascular health by preventing the buildup of toxin precursors.

Cardiovascular disease is the leading cause of death in the world, contributing to nearly 1 in 4 deaths per year in the past decade (1). One marker of chronic cardiovascular disease is high levels of trimethylamine oxide (TMAO), a chemical produced during the digestion of red meat (2). TMAO is produced in the liver from the precursor trimethylamine (TMA), which is in turn produced by bacteria that reside in the large intestine—the gut microbiota (2) (Fig. 1). TMA is derived from trimethylammonium compounds such as carnitine, which is abundant in red meat because it is critical for generating energy via fatty acid oxidation in muscle tissue.

But does all carnitine metabolism in the gut promote heart disease? Some bacteria seem to be working quietly behind the scenes to transform TMA precursors into less harmful compounds. One potential alternative pathway for detoxifying trimethylammonium compounds could be demethylation of the trimethylammonium moiety: the resulting dimethylamine group would no longer be a functional TMA precursor. It was recently shown that *Eubacterium limosum* could demethylate the quaternary amine proline betaine (3), suggesting that this organism might serve as a starting point for identifying other demethylases in the gut microbiome. Intriguingly, centenarians tend to have more *E. limosum* in their gut, suggesting a possible association between *E. limosum* and health (4). *E. limosum* is known for eliciting beneficial effects including decreasing intestinal inflammation, as well as processing isoflavones to phytoestrogens with potent anticancer and cardioprotective effects (4). Recent work by Kountz *et al.* (5) adds another potential role that *E. limosum*–rich microbiota might play in promoting positive health outcomes: reducing TMA levels.

To determine whether *E. limosum* might be able to redirect TMA precursors to less toxic products, Kountz *et al.* set out to determine how *E. limosum* metabolizes carnitine (5). The authors found that metabolites produced by *E. limosum* during growth on carnitine include short-chain fatty acids, which are common end products in gut bacterial growth processes, as well as norcarnitine, a singly demethylated derivative of carnitine. This marks the first time that norcarnitine has been observed as a naturally occurring metabolite, previously only being used as a synthetic chemical to probe biological systems (6). Thus, it appears that *E. limosum* uses a single methyl group from carnitine for growth, discarding the product norcarnitine for potential use by other bacteria. This metabolic route for carnitine degradation is one way by which *E. limosum* might reduce TMA levels in the gut, because norcarnitine cannot be degraded to TMA (Fig. 1).

Having discovered a new metabolic process—demethylation of carnitine—and a new metabolic byproduct, norcarnitine, the authors sought to identify the enzymes involved in this transformation. Stoichiometric analysis of the SCFA products suggested that *E. limosum* shuttles the methyl group into the Wood–Ljungdahl pathway (7). This pathway typically uses one-carbon units derived from CO_2 to generate energy and build biomass. However, many bacteria can feed methyl groups from other sources into the pathway through a relay of proteins bound to corrinoid cofactors (such as vitamin B_{12}), ultimately methylating tetrahydrofolate (THF) (7). The resulting methyl-THF is then combined with CO_2 in a multistep process to generate acetyl CoA, a metabolite used in gluconeogenesis and the TCA cycle to generate energy and as a precursor for many biomolecules (8).

With this clue in hand, the authors searched for enzymes that participate in a corrinoid-dependent methyl relay that starts with carnitine. In other bacteria and archaea, betaine, TMA, and other trimethylammonium compounds are demethylated by corrinoid-dependent enzymes belonging to the MttB superfamily (9), leading the researchers to hypothesize that a dedicated carnitine demethylase exists in *E. limosum*. However, no obvious single candidate enzyme could be found because *E. limosum* encodes 42 members of the MttB superfamily, of which the substrates of only one were known. The authors therefore turned to proteomics and found one MttB superfamily member, which they named MtcB, that was induced in carnitine-grown cells. Also enriched in *E. limosum* grown on carnitine were homologs of two other proteins predicted to bind corrinoid cofactors that could be involved in the methyl relay, which they named MtqC and MtqA. Led by these proteomics clues, Kountz *et al.* reconstituted the entire methyl relay pathway from carnitine to THF *in vitro* by expressing and

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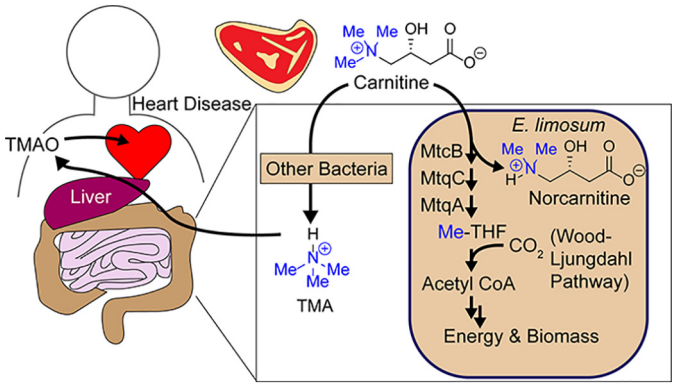

Figure 1. Meat contains carnitine, which is typically degraded to TMA in the gut by intestinal bacteria. TMA is oxidized in the liver to TMAO, which is implicated in atherosclerosis. In this issue, Kountz *et al.* discover and characterize a carnitine degradation pathway in *E. limosum* that produces norcarnitine, preventing further conversion of carnitine to TMAO. This alternative pathway supports bacterial growth by funneling one of the methyl groups through a protein relay into the Wood–Ljungdahl pathway.

purifying each protein. Their results support a model in which MtcB removes the methyl group from carnitine and passes it to the corrinoid bound to MtqC. MtqC then shuttles the methyl group to a corrinoid bound to MtqA, which methylates THF (Fig. 1).

The MttB superfamily has a handful of known enzymatic functions focused around demethylation of alkylated amines (9). However, most of the genes in this superfamily are annotated as TMA methyltransferases, despite the fact that their predicted products do not contain the conserved nonstandard pyrrolysine amino acid required for TMA methyltransferase activity (10). The unusually high number of MttB-like enzymes in the *E. limosum* genome raises the question: what are the metabolic functions of these proteins? Determining the expression patterns of these enzymes and their substrate specificities, using the methods employed by Kountz *et al.*, would establish the range of trimethylammonium compounds metabolized by *E. limosum* and other bacteria and could lead to a more complete understanding of the metabolic capacity of the human gut microbiota.

Kountz *et al.* demonstrate that carnitine can be degraded by *E. limosum* to products that do not elevate TMA (and TMAO) levels, leading the authors to speculate that this metabolic activity in the gut could promote cardiovascular health by reducing TMAO levels. To explore this intriguing possibility further, several questions need to be addressed in the future. Is the MtcB pathway functional in the human gut? What is the *E. limosum* niche in the gut, and how does it compete with bacteria that utilize other carnitine-degrading pathways? Have microbes evolved other degradation pathways for TMA precursors, and how will scientists pinpoint them? Looking beyond TMA, the rich chemical buffet served to the human gut microbiome by our highly diverse diets could give rise to thousands of unseen metabolisms—each of which has the potential to impact host health. As bacterial nutritional preferences and the networks underlying gut microbial communities becomes clearer, these insights could open up new modes for targeted perturbation of these communities, potentially allowing scientists to design interventions to select for "healthy" carnivore guts that demethylate carnitine, decrease TMA levels, and improve cardiovascular health.

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Conflict of interest—The authors declare that they have no conflicts of interest with the contents of this article.

Abbreviations—The abbreviations used are: TMAO, trimethylamine oxide; TMA, trimethylamine.

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