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 MtqB, 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 molecules (3). However, no obvious single candidate enzyme could be found for potential use by other bacteria. This metabolic route for car nitine 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 shuts the methyl group into the Wood–Ljungdahl pathway (7). This pathway typically uses one-carbon units derived from CO2 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 B12), ultimately methylating tetrahydrofolate (THF) (7). The resulting methyl-THF is then combined with CO2 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 MtqB, 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 determining 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).
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 pyrolysine 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 range of trimethylammonium compounds metabolized by *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.

**Editors’ Pick Highlight: *E. limosum* carnitine demethylation**

 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.

**Acknowledgments**—We thank members of the Taga laboratory for critical reading of the manuscript.

**Funding and additional information**—This work was supported by Department of Energy Grant DE-SC0020155.

**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.

**References**

1. Lozano, R., Naghavi, M., Foreman, K., Lim, S., Shibuya, K., Aboyans, V., Abraham, J., Adair, T., Aggarwal, R., Ahn, S. Y., Alvarado, M., Anderson, H. R., Anderson, L. M., Andrews, K. G., Atkinson, C., *et al.* (2012) Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study. *Lancet* 380, 2095–2128 CrossRef Medline

2. Zeisel, S. H., and Warrier, M. (2017) Trimethylamine N-oxide, the microbiome, and heart and kidney disease. *Annu. Rev. Nutr.* 37, 157–181 CrossRef Medline

3. Picking, J. W., Behrmann, E. J., Zhang, L., and Krzycki, J. A. (2019) MtpB, a member of the MttB superfamily from the human intestinal acetogen *Eubacterium limosum*, catalyzes proline betaine demethylation. *J. Biol. Chem.* 294, 13697–13707 CrossRef Medline

4. Biagi, E., Nylund, L., Candela, M., Ostan, R., Bucc, L., Pini, E., Nikkia, J., Monti, D., Satokari, R., Franceschi, C., Brigidi, P., and De Vos, W. (2010) Through ageing, and beyond: gut microbiota and inflammatory status in seniors and centenarians. *PLoS One* 5, e10667 CrossRef Medline

5. Kountz, D. J., Behrmann, E. J., Zhang, L., and Krzycki, J. A. (2020) MtcB, a member of the MttB superfamily from the human gut acetogen *Eubacterium limosum*, is a cobalamin-dependent carnitine demethylase. *J. Biol. Chem.* 295, 11971–11981 CrossRef Medline

6. Kleber, H. P., Seim, H., Aurich, H., and Strack, E. (1978) Interrelationships between carnitine metabolism and fatty acid assimilation in *Pseudomonas putida*. *Arch. Microbiol.* 116, 213–220 CrossRef Medline

7. Ragsdale, S. W., and Pierce, E. (2008) Acetogenesis and the Wood-Ljungdahl pathway of CO₂ fixation. *Biochim. Biophys. Acta* 1784, 1873–1898 CrossRef Medline

8. Song, Y., Shin, J., Jin, S., Lee, J.-K., Kim, D. R., Kim, S. C., Cho, S., and Cho, B.-K. (2018) Genome-scale analysis of syngas fermenting acetogenic bacteria reveals the translational regulation for its autotrophic growth. *BMC Genomics* 19, 837 CrossRef Medline

9. Ticak, T., Kountz, D. J., Girsky, K. E., Krzycki, J. A., and Ferguson, D. J. J. (2014) A nonpyrrolysine member of the widely distributed trimethylamine methyltransferase family is a glycine betaine methyltransferase. *Proc. Natl. Acad. Sci. U.S.A.* 111, E4668–E4676 CrossRef Medline

10. Hao, R., Gong, W., Ferguson, T. K., James, C. M., Krzycki, J. A., and Chan, M. K. (2002) A New UAG-encoded residue in the structure of a methano- gen methyltransferase. *Science* 296, 1462–1466 CrossRef Medline