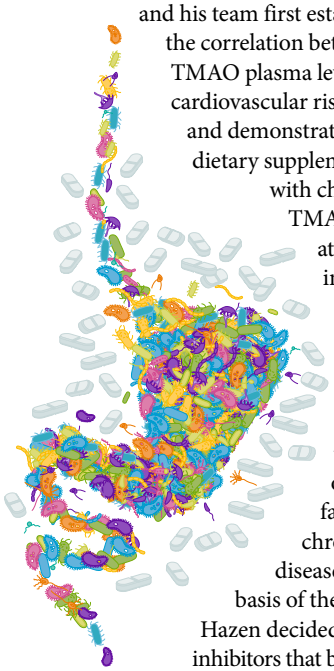
THROMBOSIS

Targeting the gut to protect the heart

> the full extent of the therapeutic potential of TMAO inhibition in CVD still needs to be investigated

The gut is a niche for a variety of microorganisms that can influence cardiometabolic health and disease by modulating the plasma levels of bioactive metabolites. In a new study, Stanley Hazen and colleagues report the discovery of two new inhibitors that reduce the susceptibility to thrombosis — a critical adverse complication in heart disease — by blocking the generation of the gut-microbiota-derived metabolite trimethylamine *N*-oxide (TMAO).

"It is remarkable that we are giving a drug that impacts a single pathway in the microorganisms in our gut and yet it is having a profound measurable impact on host functions," says Hazen. TMAO is generated through a two-step pathway that begins with the conversion of dietary nutrients, such as phosphatidylcholine, choline, and carnitine, into trimethylamine (TMA) by the gut microbiota; TMA then circulates to the liver, where it is oxidized into TMAO. Hazen and his team first established the correlation between TMAO plasma levels and cardiovascular risk in 2011 and demonstrated that dietary supplementation with choline or TMAO promotes atherosclerosis in mice. Since then, many studies have linked the TMAO pathway to the risks of heart failure and chronic kidney disease. On the basis of these results, Hazen decided to develop inhibitors that block TMAO production as a therapeutic strategy to reduce the risk of cardiovascular disease (CVD). In 2015, the group reported that a natural product found in cold-pressed extra-virgin olive oil, 3,3-dimethyl-1-butanol (DMB), inhibits microbiotal choline TMA lyase activity, leading to reductions in plasma TMAO level and in atherosclerotic lesion development in mice.



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In the past 3 years, additional studies have demonstrated that TMAO can also increase platelet hyperactivity and the risk of thrombosis; Hazen and his group therefore thought to investigate the effects of DMB on platelet responsiveness in mice fed with a choline-supplemented diet. Exposure to DMB significantly reduced TMAO plasma level and stimulus-dependent platelet aggregation, but despite the high doses of DMB administered, TMAO plasma level could not be reduced to the level of TMAO observed in chow-fed mice. In an effort to develop a generation of more potent choline TMA lyase inhibitors, a new class of suicide substrates, including choline analogues iodomethylcholine (IMC) and fluoromethylcholine (FMC), were designed to irreversibly inactivate choline TMA lyase activity. In vitro, the potency of IMC was 10,000-times greater than that of DMB. In mice receiving a choline-supplemented diet, a single dose of IMC or FMC resulted in >95% reduction in plasma TMAO levels ($P<0.0001$) after 24 h compared with no treatment. Functionally, the inhibitors completely suppressed the effects of choline supplementation on platelet responsiveness and, in a model of carotid artery injury, reduced the time to thrombus formation in choline-fed mice beyond that observed in chow-fed mice. According to this new study, IMC and FMC have additional therapeutic advantages, such as being nontoxic for the host and nonlethal for the microbiome. "We felt it was important that the drug does not kill the microorganism as antibiotics do; this would prevent problems like antibiotic resistance from becoming an issue," explains Hazen. In addition, IMC and FMC selectively accumulated in the gut microbiome, limiting systemic exposure of the drug in the host. In contrast to several antiplatelet agents, the inhibitors had no effect on bleeding potential. "Inhibiting this pathway is like turning down a rheostat on platelets, inhibiting hyper-responsiveness, but without reducing platelet functions to below normal."

Inhibition of microbial choline lyase activity with IMC also reversed the changes in microbial composition induced by a high-choline diet, restoring microbial communities that are associated with metabolic health. Therefore, the full extent of the therapeutic potential of TMAO inhibition in CVD still needs to be investigated. "With the current therapeutic strategies to treat CVD and prevent its progression, a substantial residual risk remains. The present discovery — the role of the TMAO pathway — could therefore be a major missing piece to that puzzle," concludes Hazen.

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ORIGINAL ARTICLE Roberts, A. B. et al. Development of a gut microbe-targeted nonlethal therapeutic to inhibit thrombosis potential. *Nat. Med.* https://doi.org/10.1038/s41591-018-0128-1 (2018)

FURTHER READING Lindskog Jonsson, A. et al. Role of gut microbiota in atherosclerosis. *Nat. Rev. Cardiol.* **14**, 79–87 (2017)