Meat-Loving Microbes

Do Steak-Eating Bacteria Promote Atherosclerosis?

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1. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, et al. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med*. 2013;19:576–585.

Study Hypothesis

The importance of the interaction among diet, gut microbiota, and host genetics in modulating disease risk in humans is being increasingly recognized and studied. In this study, Koeth et al examined the interaction between dietary L-carnitine and gut microbiota in promoting atherosclerosis. Previous work from this group identified the metabolite trimethylamine N-oxide (TMAO) as being potentially proatherogenic, with increased plasma TMAO predicting cardiovascular disease (CVD) risk. TMAO is present in some dietary sources, predominately seafood, but is mainly produced by hepatic oxidation of trimethylamine by flavin monooxygenases. Trimethylamine is derived in the gut by the action of bacteria on multiple diet–derived nutrients, including choline, phosphatidylcholine (lecithin), betaine, creatinine, and carnitine, and diffuses into the bloodstream through the intestinal wall. In this study, the authors hypothesized that dietary L-carnitine, found in high concentrations in red meat, promotes atherosclerosis through gut-mediated generation of TMAO.

How Was the Hypothesis Tested?

Koeth et al studied both humans and mice to test the relationship between dietary intake of L-carnitine and gut bacteria–mediated production of TMAO, as well as the development of atherosclerosis. Five volunteer omnivores were given an L-carnitine–rich meal (steak, containing ≈180 mg L-carnitine) combined with 250 mg isotope-labeled d3-L-carnitine supplementation. Plasma and urine samples were taken to measure the conversion of L-carnitine to TMAO. This L-carnitine challenge was repeated after 1 week of oral antibiotics, designed to eliminate gut microbiota, and repeated a third time ≈1 month later when the gut microbiome was assumed to have recovered. To assess the impact of habitual meat consumption, the researchers then administered the steak and d3-L-carnitine challenge to a vegan. In a larger group of vegetarians/vegans (n=23) and omnivores (n=51), stool samples were obtained, and plasma TMAO was measured. The researchers administered d3-L-carnitine supplementation without steak to a subset of these individuals (n=5 vegans and n=5 omnivores) to assess plasma d3-L-carnitine. Finally, they analyzed the association between plasma L-carnitine and CVD in a large independent sample. The authors extended their work into mouse models using both germ-free mice and antibiotic treatment to study the role of gut bacteria on TMAO production from L-carnitine and investigating L-carnitine effects on the gut biome and on the development of atherosclerosis.

Principal Findings

In the analysis of metabolomics data in a discovery sample, L-carnitine was identified as a plasma metabolite modestly associated with CVD risk. In an independent sample of 2595 individuals undergoing elective cardiac evaluation, plasma L-carnitine was significantly associated with increased CVD, although this association was no longer significant after adjusting for plasma TMAO. A postprandial increase in plasma TMAO was observed in omnivorous subjects consuming dietary L-carnitine (in the form of steak) and supplemental d3-L-carnitine; this increase was absent in the same subjects after broad-spectrum antibiotic treatment, with the postprandial effect reappearing after the gut was allowed to recolonize for several weeks. A postprandial increase in TMAO was not observed in a vegan subject, presumably as a result of the absence of selection for carnitine-metabolizing gut bacteria in this subject. In additional vegans/vegetarians (n=5), TMAO production after d3-L-carnitine supplementation was reduced compared with omnivores (n=5). The researchers analyzed stool samples (n=23 vegetarians/vegans and n=30 omnivores) and found significant differences in microbial composition by meat consumption, significantly higher plasma TMAO in subjects characterized by a microbial enterotype enriched with the genus *Prevotella*, as well as lower baseline TMAO in the vegans/vegetarians compared with omnivores. The essential role of gut bacteria in the generation of TMAO from L-carnitine was demonstrated in germ-free mice before and after microbial colonization and in *Apoe*$^{-/-}$ mice after antibiotic treatment and subsequent reacquisition of gut microbiota. The gut microbial composition was shown to differ in mice supplemented with dietary L-carnitine versus chow alone. *Apoe*$^{-/-}$ mice supplemented with high-dose L-carnitine for 15 weeks developed significantly more atherosclerotic plaque compared with chow-fed mice, and this proatherosclerotic effect was removed by antibiotic treatment. In mechanistic studies, the

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authors showed that reverse cholesterol transport was reduced in mice consuming L-carnitine, choline, or TMAO-rich diets and that TMAO was associated with reduced expression of genes involved in hepatic bile acid synthesis.

Implications

Koeth et al highlight the key role of gut microbiota in metabolism and outline an intriguing new mechanistic link between diet and CVD risk, whereby red meat consumption may increase atherosclerosis. However, given the complexity of the interactions among host genetics, host diet, and microbiota and our still-limited understanding of specific mechanisms of L-carnitine effects on atherogenesis, it is unclear whether this should be translated into specific dietary recommendations. Mice supplemented with L-carnitine developed increased atherosclerosis compared with nonsupplemented chow-fed mice; however, the dose of L-carnitine given was relatively high, perhaps several orders of magnitude higher than what the most dedicated steak eater would typically consume. In humans, there seemed to be differences in the efficiency of conversion of L-carnitine to TMAO depending on whether the L-carnitine supplement was accompanied by steak. Thus, there may be important differences between the physiological effects of L-carnitine given alone versus intake in the context of a food. Urinary levels of TMAO are known to increase after consumption of a variety of different foods, most notably fish, and thus further studies are required to understand the relative impacts of TMAO precursors found in different foods. The *Prevotella*-enriched enterotype associated with higher TMAO in this study has previously been found to be associated with higher carbohydrate intake; it is likely that specific species within these enterotypes have different effects, and understanding the complexity of the microbiome will provide research fodder for many years to come. Combining nutrigenomic and metagenomic studies may be crucial for understanding the complex interactions between diet and health and has important therapeutic implications. Greater understanding of microbial action on specific dietary components may allow atherosclerotic risk to be mediated through dietary or pharmacological manipulation of the gut microbiota.

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Disclosures

None.

KEY WORDS: atherosclerosis ■ cardiovascular disease ■ carnitine ■ diet

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