Lipid Metabolism by Gut Microbes and Atherosclerosis

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A recent metabolomic study provides evidence that microbial metabolism of lipids in the gut yields product(s) that promote atherosclerosis. These results add another dimension—the microbiome—to the complex determinants of atherogenesis.

Atherosclerotic vascular disease is a complex pathophenotype that is governed by genetic and environmental determinants. The mechanistic interplay between specific genetic loci that predispose to atherosclerosis and predictive dietary factors is well known and serves as the basis for current preventive therapeutic strategies designed to limit expression of overt disease. For example, we routinely urge our patients at risk for atherosclerosis to reduce the intake of cholesterol-rich foods and, for those who are glucose intolerant, to limit the intake of carbohydrates. These exhortations are predicated on the view that dietary risk factors are directly absorbed from the gut to exert their adverse vascular effects. What has been neglected until recently, however, is the role of commensal intestinal microorganisms in the metabolism of foodstuffs and their potential consequences for host metabolism and disease pathogenesis.

A recent outcome of the application of contemporary genomics to human pathobiology is the ability to characterize the microbiome of these commensal organisms and to do so in conjunction with exploring the functional consequences of these unique microbiota for human biology and disease. Gut flora can significantly influence the bioavailability of dietary constituents and their metabolism in mammalian hosts. Susceptibility to obesity and insulin resistance are determined by the specific metabolic characteristics of gut microbiota. The ability of this unique microbial community to exert such dramatic effects on metabolism should not be surprising when one considers that nearly 99% of the genes in humans are microbial in origin. With the rapid evolution of metagenomic technologies, the genetic variation in intestinal microbiota and its stratification have recently been characterized, as well, with three specific enterotypes of gut flora defined and their relationships to host phenotypes (eg, body mass index) elucidated.

Armed with this growing knowledge of the influence of gut flora on metabolism and supportive of prior work showing that gut microbiota modulate host energy and lipid metabolism through coordinated regulation of the metabolome and lipidome, Wang and colleagues demonstrated a unique cluster of three phospholipid-associated molecules that appear to promote atherosclerosis. They first performed a rigorous metabolomic analysis of the plasma of patients with known but stable atherosclerotic disease, identified three metabolites of dietary phosphatidylcholine—choline, betaine, and, trimethylamine N-oxide (TMAO)—and showed that these phospholipid metabolites were independent predictors for the risk of a clinical vascular event. These simple epidemiologic associations were taken one major step further by studies in a murine model of atherosclerosis, the apoE−/− mouse. The authors demonstrated that plasma TMAO levels in apoE−/− mice positively correlated with atheroma burden. They then showed that hepatic gene expression levels of enzymes that convert trimethylamine to TMAO, flavin monoxygenases, in mice positively correlated with TMAO levels and that expression levels of flavin monooxygenase 3 in humans correlated with TMAO levels. They next suppressed gut flora with broad-spectrum antibiotic therapy in the apoE−/− mice and found that this treatment decreased TMAO levels significantly. In these experiments, TMAO levels positively correlate with the size of atheroma. Last, these investigators showed that a 1% choline diet given to apoE−/− mice increased foam cell formation with an accompanying increase in the scavenger receptor CD36 and SRA1 protein in murine macrophages and that this effect was prevented by broad-spectrum antibiotic treatment. These results are consistent with the view that trimethylamine, the precursor of TMAO, is produced by gut microbiota and confirms earlier observations in germ-free mice on the bacterial origin of this methylamine.

Four key questions follow from this interesting report. First, what is the specific bacterial source(s) of trimethylamine? Meat in the diet is a major source of phosphatidylcholine, and prior work showed that dietary meat increases TMAO levels in plasma. The conversion of choline, released from phosphatidylcholine by phospholipase D, to trimethylamine appears to be exclusively a function of bacterial metabolism in the colon. Many colonic bacterial species engage in this conversion, including Clostridia, Proteus, Shigella, and Aerobacter. In addition, one study suggested that the oral microbe, Streptococcus sanguis, is capable of metabolizing choline to trimethylamine, offering an intriguing potential mechanistic link between periodontal...
disease and vascular disease risk\textsuperscript{13,14} involving the trimethylamine pathway.

Second, what are the genetic determinants of flavin monooxygenase 3 expression, and are there variants of this gene that confer an increased risk of atherothrombosis in humans? Although there are many known genetic variants of this hepatic oxidoreductase, none has been analyzed for association with the risk of atherothrombotic disease. Hepatic flavin monooxygenase transcript levels were shown to correlate with the extent of atheroma in mice in the study by Wang and colleagues\textsuperscript{8} but no correlative data yet exist in human atherosclerosis.

Third, what is the mechanism by which TMAO levels promote atherosclerosis? TMAO limits protein denaturation in urea-rich cells\textsuperscript{15} and has been shown to limit renal ischemia-reperfusion injury likely by modulating osmolality in the medulla\textsuperscript{16}; however, neither of these actions can account for its adverse atherogenic effects. TMAO levels are elevated in plasma in juvenile inflammatory arthropathies\textsuperscript{17} and in hypertension,\textsuperscript{18} but no clear mechanism accounts for these associations. In some bacteria, growth under anaerobic conditions can depend on TMAO, which serves as a terminal electron acceptor in place of oxygen\textsuperscript{19}; perhaps more important from the perspective of atheromatous risk, TMAO has also been shown to inhibit electron transport (under aerobic conditions) in Staphylococcus aureus,\textsuperscript{20} an effect that could lead to oxidant stress via mitochondrial uncoupling and reactive oxygen species generation were it to occur in vascular cells. These theoretical adverse actions must be contrasted with the published evidence for the ability of TMAO to eliminate endoplasmic reticulum stress (via its chemical chaperone action) and oxidant stress in some cell systems.\textsuperscript{21} Clearly, exploring the potential adverse action of TMAO would be important in future studies. Yet, the possibility remains that TMAO is simply a correlate and not a mechanistic culprit in the atherogenic process.

Fourth, what is the effect of the related compounds, choline and betaine, on atherogenesis? Importantly, they are involved in the methylation cycle, particularly betaine (trimethylglycine), which serves as an alternate methyl source for remethylating homocysteine to methionine via betaine:homocysteine methyltransferase. In as much as methylation reactions govern epigenetic regulation of gene expression, increasing levels of betaine by increasing S-adenosylmethionine and the S-adenosylmethionine:S-adenosylhomocysteine ratio could increase methylation potential and suppress gene expression. The consequences of this altered genomic methylation in vascular cells would, of course, depend on the genes whose expression is affected by promoter methylation. Thus, exploring the methylated genome in the setting of elevated betaine in atherosclerosis-prone and -resistant mice would seem a reasonable approach by which to dissect this complex process.

If mechanistic links to any of these three lipid metabolites can be ascertained, this study offers new and exciting possibilities for therapeutic approaches to the prevention or treatment of atherosclerosis. Probiotic therapies have already been used to decrease TMAO levels,\textsuperscript{22} and the beneficial consequences of such modifications on atherogenesis are suggested by the study of Wang and colleagues.\textsuperscript{8} These remarkable observations demonstrate for the first time that gut microbiota engage in the metabolism of phospholipids to yield a product (trimethylamine) that is further metabolized (to TMAO) in the host and that can contribute to atherogenesis. These observations also clearly indicate the extraordinary complexity of the atherogenic process: not only does it depend on the host genome and environmental (dietary) factors, but it also depends critically on the commensal gut microbiota and their unique microbiome-determined metabolic capacity (Figure). Thus, the report by Wang and colleagues\textsuperscript{8} supports the notion that diet is, indeed, destiny, a destiny that is shaped not only by our individual genome but also by our personal microbial metagenome.

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References


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