New metabolic markers of heart disease

By Tim Fulmer, Senior Writer

A **Cleveland Clinic** team has identified three phospholipid-based metabolites derived from gut bacteria that are associated with risk of cardiovascular disease in humans.[1] The team plans to develop a serum diagnostic based on the metabolites, which could help identify patients at risk for atherosclerosis and other heart diseases.

The wide variety of bacteria that populate the gut play an essential role in helping humans digest and absorb nutrients. However, recent studies in mice suggest those bacteria also can contribute to disease, in particular to obesity and diabetes.[2–4]

Based on those findings, a Cleveland Clinic team hypothesized that metabolites produced by intestinal bacteria also might play a role in cardiovascular diseases.

To test that hypothesis, the researchers initially did a small molecule screen using plasma from 50 stable cardiac patients who subsequently progressed to myocardial infarction (MI), stroke and/or death over three years and 50 control cardiac patients who underwent the same evaluation but did not show such progression.

The mass spectrometry–based screen, which looked at the levels of more than 2,000 metabolites, identified 40 molecules as significantly associated with cardiovascular disease ($p < 0.05$). A cohort of 25 additional patients and 25 controls confirmed 18 of the original hits as significantly associated with cardiovascular disease ($p < 0.05$).

Of the 18 confirmed hits, 3 stood out with similar p values ($p < 0.001$), suggesting they shared a common biochemical pathway. A combination of NMR, mass spectrometry and liquid and gas chromatography revealed the three molecules as choline, trimethylamine N-oxide (TMAO) and betaine, which all result from the breakdown of phosphatidylcholine by intestinal bacteria and the liver.

Phosphatidylcholine is a dietary phospholipid. Compared with serum levels of the two other main types of dietary lipids, cholesterol and triglyceride, relatively little is known about the relationship between levels of serum phospholipid metabolites and cardiovascular disease.

The team next set out to confirm that the three phospholipid metabolites are derived from the activity of gut bacteria. In mice treated with broad-spectrum antibiotics to suppress intestinal bacteria, feeding of phosphatidylcholine failed to generate serum TMAO, whereas untreated control mice that had intact intestinal bacteria showed high TMAO levels in response to eating phosphatidylcholine.
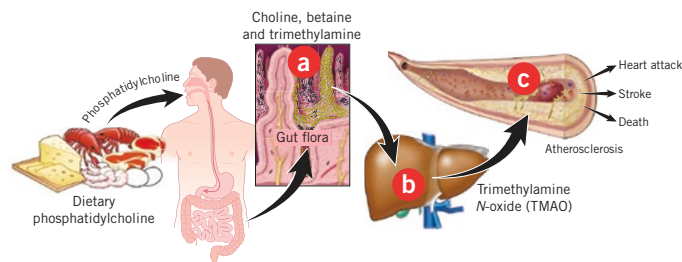

Figure 1. Metabolites derived from gut bacteria may drive formation of atherosclerotic plaques.

Finally, the researchers asked whether the increased serum levels of phospholipid metabolites were associated with atherosclerosis.

In *apolipoprotein E* (*Apoe*)$^{-/-}$ mice, which are an established model of atherosclerosis, a diet enriched with the metabolites choline or TMAO significantly increased aortic plaque size compared with that in mice fed normal chow ($p = 0.01$). Suppression of intestinal bacteria by antibiotics blocked choline-mediated formation of aortic plaques compared with that in control mice with intact intestinal flora.

In sum, the findings published in *Nature* suggest that gut bacteria can drive progression of atherosclerosis (*see* **Figure 1, "Metabolites derived from gut bacteria may drive formation of atherosclerotic plaques."**). Phosphatidylcholine in foods such as eggs, milk and meat initially is broken down by intestinal bacteria into multiple trimethylamine-derived compounds, including choline, betaine and trimethylamine (*see* **Figure 1[a]**). Those compounds are then absorbed by the intestine, enter systemic circulation and are further metabolized by enzymes in the liver, generating serum TMAO (*see* **Figure 1[b]**), which helps drive formation of arterial atherosclerotic plaques by an as yet unknown mechanism (*see* **Figure 1[c]**).

Corresponding author Stanley Hazen, director of the Center for Cardiovascular Diagnostics & Prevention and section head of Preventive Cardiology & Rehabilitation at Cleveland Clinic, told *SciBX* that his team now is focusing on developing a serum diagnostic based on the three phosphatidylcholine metabolites.

The test will help "identify individuals at risk of cardiovascular disease who otherwise are not identified by traditional risk factors and blood tests currently used in clinical practice," such as those that look at serum cholesterol and triglycerides, he said.

Thus, the potential value of the three new markers is that they "should help better tailor information for a patient on whether their diet is appropriate for them and indicate how aggressively they might have to cut back on meat, cheese, dairy, liver and eggs," said Hazen.

According to Hazen, the findings are covered by patents and the Cleveland Clinic is "seeking a licensing partner to help commercialize the IP and bring it to patient care."

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