A TECHNICAL REVIEW

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TMAO (Trimethylamine N-oxide) Test

A marker of the microbiome and cardiovascular risk

TMAO is a dietary metabolite produced by a pathway involving gut microbiota and high levels of TMAO that have been associated with an increased risk of heart disease.[1-9] TMAO concentrations increase in the blood after ingestion of dietary choline and L-carnitine, which are abundant in red meat, eggs, liver, wheat germ, and energy drinks. Choline and L-carnitine are metabolized in the gut by microbiota to form trimethylamine (TMA), which is subsequently oxidized in the liver into TMAO by flavin monooxygenases.[2-9] TMAO concentrations have been shown to be reduced in animals and humans treated with broad-spectrum oral antibiotics, confirming the requirement for gut bacteria in the formation of TMA and TMAO.[4] TMAO has been hypothesized to promote atherosclerosis by upregulating macrophage scavenger receptor activity and downregulating bile acid synthesis, which together reduce reverse cholesterol transport.[3]

The TMAO Test may be used:

1) As an aid in the assessment of risk for cardiovascular disease (CVD), independent of established risk factors[1-24];
2) As an aid in the determination of altered gut microbiome (gut dysbiosis) in individuals who may benefit from intensive dietary intervention;
3) To monitor therapy aimed at reducing TMAO concentrations.[1-24]

TMAO and Cardiovascular Disease

Several recent clinical studies have shown an association of high plasma TMAO levels with increased risk of CVD, independent of established risk factors.[2-9] A study of 4,007 adults undergoing cardiac catheterization (an intermediate risk population) revealed that subjects with high TMAO levels (highest quartile; defined as level >6.18 µM) had a 2.5-fold increased risk for a major adverse cardiovascular event (myocardial infarction (MI), stroke or death) compared to subjects in the lowest quartile (<2.43 µM) over a 3-year period.[4] In another study, subjects with underlying chronic kidney disease (estimated glomerular filtration rate, <60 mL/min per 1.73 m2, n=521) had higher fasting plasma TMAO levels and a higher risk of mortality over a 5-year follow-up than those with normal renal function (hazard ratio (HR), 1.93; 95% confidence interval (CI), 1.13-3.29; p<0.05).[10]

The relationship between plasma TMAO levels and prevalent CVD was shown in a randomly sampled cross-sectional study of multiethnic adults in Canada.[11] TMAO levels measured in 292 consecutive subjects (99 CVD cases and 193 unmatched controls) showed a significant, graded association with CVD (odds ratio, 3.17; 95% CI: 1.05-9.51; p trend=0.02) but not with measured carotid intimal media thickness. CVD was defined in subjects with angina, a self-reported admission for a MI, silent MI, percutaneous coronary angioplasty or coronary artery bypass graft surgery, or cerebrovascular disease.

High TMAO levels have also been observed in patients with heart failure.[12] In a cohort of 720 patients with a history of heart failure, elevated plasma TMAO was associated with increased risk of mortality over a 5-year period, independent of traditional risk factors and B-type natriuretic peptide (BNP) levels (HR, 2.2; 95% CI: 1.42-3.43; p<0.001).

TMAO and Gut Dysfunction

It has been shown that subjects with cardiometabolic diseases such as type 2 diabetes and non-alcoholic fatty liver disease (NAFLD) exhibit altered gut microbiome and higher circulating levels of TMAO compared to healthy subjects.[13-15] Current thought in the field dictates that the bacterial changes (and accompanying alterations in metabolic signaling) that are observed in the gut microbiota of subjects with metabolic diseases elicit increases in TMAO, which then contributes to increased CVD risk in these patients.[16-19] In support of this hypothesis, several studies have reported the use of dietary supplements that alter gut microbiota composition and reduce TMAO concentrations in animal models.[20] Dietary allicin, a potent antimicrobial compound found in garlic, reduced TMAO by impacting the gut microbiota in mice.[20] DMB or 3,3-dimethyl-1-butanol, a structural analog of choline that is prevalent in some wines, olive oils and grapeseed oils, inhibited gut production of TMA and led to a reduction in TMAO in mice fed a high-choline or L-carnitine diet.[21] These studies suggest that dietary interventions or addition of daily supplements to the diet in humans may also lead to reductions in TMAO and potentially CVD risk.

Treatments to Reduce TMAO

While few inhibitors of TMAO production have been reported to date, it has been shown that 3,3-dimethyl-1-butanol (DMB),

an inhibitor of TMA formation, prevents cardiac inflammation and fibrosis in mice on a western diet.[22] Treatment with probiotics and prebiotics, which are thought to improve the gut microbiome, however, have produced mixed results in clinical trials to date. Treatment with the probiotic LKM512 for 12 weeks reduced fecal TMA concentrations and body mass index in healthy adults.[23] However, the prebiotic inulin did not reduce plasma TMAO in individuals at risk for type 2 diabetes.[24] Taken together, these studies suggest that more clinical research needs to be conducted in order to gain a better understanding of which treatments will mediate a positive effect on this gut-dependent pathway and potentially lead to a reduction in CVD risk.

Laboratory Analysis of TMAO

Specimen Freshly drawn serum collected in plain red-top blood collection tubes is the preferred specimen. Freshly drawn serum collected in NMR LipoTubes (also known as Greiner serum separator tubes; manufactured by Greiner Bio-One, Inc. Part No. 456293P) and plasma collected in lavender-top EDTA or green-top sodium heparin tubes are also acceptable specimens. Serum or plasma specimens drawn in gel barrier collection tubes other than the NMR LipoTube are unsuitable for analysis and should not be used. Patient should refrain from consuming fish and other marine food items the day before the blood draw to avoid temporary elevation of TMAO. Fasting for 10 to 12 hours is recommended.

Volume 1 mL (minimum volume: 0.5 mL)

Stability Specimens are stable for 14 days at 2-8°C or -20°C and up to 14 days at controlled room temperature. Specimens can also be frozen at -80°C before testing. Specimens may be frozen and thawed up to 3 times.

TMAO Medical Decision Limits[4]

Low	<6.2 µM
Moderate	6.2-9.9 µM
High	≥10.0 µM

Relevant Assays*

Test Name	Test Number
TMAO Test	123413

*For the most current information regarding test options, including specimen requirements and CPT codes, please consult the online Test Menu at www.LabCorp.com.

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