Editorial

Dietary choline derived TMAO: new role in thrombosis

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Increasing pieces of evidence link the intestinal gut microbiota and its produced metabolites to many chronic diseases, including cardiovascular and atherosclerotic diseases (1). Signatures of gut microbial composition and functions are associated with symptomatic atherosclerosis in the carotid artery (2). Atherosclerotic plaques also contain bacterial DNA, which is also associated with known cardiovascular risk factors (CVDRF) (3). The importance of gut microbial gene richness in these diseases is highlighted by the finding that increased bacterial richness associates with an improved lifetime CVD risk even after adjustment for known CVDRF (4). This result is reminiscent of previous reports in the metabolic field describing associations between low microbial gene richness, obesity and dyslipidemia, insulin resistance and low-grade systemic inflammation, and all CVDRF (5,6). Causal links between the gut microbiota and atheroma lesions severity come from preclinical studies. A proof of concept study demonstrates that mice treated with a poorly absorbed large spectrum antibiotic (i.e., vancomycin), which induces significant depletion of gut microbiota composition, display smaller myocardial infarcts as compared to controls (7). Interestingly, protective effects are also observed after probiotic supplementation in mice prone to develop myocardial infarcts. Likewise, a study using western diet-fed ApoE (–/–) mice, which develop atherosclerotic lesions in the aorta, demonstrates lesion reversal after oral supplementation with *Akkermansia muciniphila*, used as a probiotic surrogate (8). Increased *Akkermansia muciniphila*, a commensal mucin-degrading bacterium, is associated with several beneficial metabolic health outcomes, such as improved insulin sensitivity both in mice (9,10) and in humans (11). The mechanisms by which *Akkermansia muciniphila* could reduce the severity of atherosclerotic lesions, include reduced metabolic endotoxemia (8) and improved artery endothelial dysfunction, the latter of which was recently shown in mice study supplemented with prebiotic inulin-type fructans. In this context, prebiotic treatment leads to an increase in *Akkermansia muciniphila* and stimulates NO-producing bacteria, concomitantly with changes in bile acid composition and GLP-1 production, both of which act on the NO pathway (12).

Given dietary components are substrates for the gut microbiota, ingested food which is subsequently processed by the gut microbiota is also key in cardiovascular disease development, progression, and mortality (1). In this context, Hazen's team has highlighted the contribution of TMAO, a liver-produced circulating metabolite derived from the processing of dietary phosphatidylcholine or L-carnitine by gut microbiota (13), to cardiovascular disease development or progression (14-16). In their studies, the authors elegantly show the obligatory role of gut microbiota in the production of TMA (trimethylamine), a gas precursor of TMAO, by using antibiotic treatment after an oral load of TMAO, choline, L-carnitine in humans or mice (14,15). TMAO levels were markedly increased in patients with significant atherosclerosis that further developed acute cardiac events during the follow-up as compared to event-free atherosclerotic patients (16,17). Increased circulating TMAO was predictive of cardiovascular death or major adverse cardiac events even after adjustment for

known CVDRF (14). Increased TMAO levels were also dependent on consumption of phosphatidylcholine (14) or L-carnitine (15). The importance of TMAO was causally substantiated by dietary challenge experiments in a murine model prone to develop atherosclerotic lesions. To this end, adding choline to the diet increased TMAO circulating concentrations as well as aortic lesions and increased infiltration of cholesterol-laden foam cells in these lesions as compared to animal controls (16).

A myriad of factors contributes to the progression and severity of atherosclerotic lesions and among these factors, those that predispose to thrombus formation are of major importance in atherothrombotic diseases. As such, an increased potential for platelet activation (platelet hyperreactivity) and aggregation is involved in the extent and severity of cardiac events.

In this context, Zhu *et al.* recently extended the involvement of TMAO to platelet hyperreactivity (18). They first reported that TMAO levels were significantly increased in patients with increasing thrombosis event incidence. Their *in vitro* experiments revealed that platelet aggregation and adhesion to collagen were enhanced in the presence of TMAO. Similarly, TMAO injections in mice induced carotid thrombus with shortened occlusion time. Furthermore, the authors used different murine models to demonstrate the obligatory role of gut microbiota. As such, chow-fed mice supplemented with TMAO or choline displayed increased platelet aggregation and shortened occlusion time, and these effects were prevented during antibiotic administration for the choline group only. Moreover, germ free mice supplemented with choline were protected from increased thrombosis and shortened occlusion time as compared to conventional mice fed a similar diet. However, both germ free and conventional mice fed a TMAO-supplemented diet displayed an adverse phenotype (18), which is explained because TMAO is a by-product of dietary choline processed by the gut microbiota (14,15). Finally, they performed microbiota transfer in germ free mice using cecal contents from mice with different genetic backgrounds: one group with high TMAO levels and shortened occlusion time and one with the opposite phenotype (19). Cecal microbiota transfer reproduced the phenotype of the donor in recipient germ free mice. This was further aggravated when the germ free mice received the microbiota of mice supplemented with choline (18). Overall, this interesting study is a further demonstration of the role of TMAO in cardiovascular disease and extends its contribution to platelet hyperresponsiveness and the essential role of gut microbiota in the pathophysiology of arterial thrombosis.

The same team also recently published results translating these observations to humans using a dietary interventional study (20). The authors found that supplementing vegan/vegetarian or omnivorous patients with oral choline induced differential elevation of circulating TMAO, which was markedly increased in omnivores. Choline supplementation also induced an increase in platelet aggregation, which was dose-dependently associated with circulating TMAO concentrations. Finally, aspirin treatment given to omnivores supplemented with choline was able to reduce both TMAO concentrations as well as platelet hyperresponsiveness (20).

The complete understanding of how aspirin reduces TMAO level remains to be elucidated. Indeed, the authors observed a smaller increase of TMAO after choline supplementation when patients were taking aspirin compared to patients not taking aspirin. Yet the increase in circulating TMAO was not completely blunted, which suggests that aspirin-independent mechanisms are also involved in TMAO production and platelet aggregation. The authors hypothesize that aspirin is able to modify gut microbiota composition that could potentially affect its function and produced metabolites. Indeed, a recent study demonstrates that non-steroidal anti-inflammatory drugs induce a specific microbial signature, and in this study, some patients were taking aspirin only (21). However, the hypothesis from Zhu *et al.* (20) remains unanswered as they have not analyzed the microbiome composition of their volunteers before and after aspirin intervention. In particular, it will be interesting to examine whether these changes in microbial profile indeed impact bacteria involved in TMA/TMAO production. Other drugs used in secondary care prevention such as statin, β-blockers, and other anti-hypertensive drugs may also impact microbial composition and thus TMAO production.

Despite the above accumulating evidence on the contribution of TMAO in CVDs, its role remains debated. Several teams did not replicate the increased risk of major adverse cardiac events along with increased TMAO circulating levels. These discrepancies could be due to the differences in the studied cohorts' geographic locations or food intake habits. For example, Chinese patients with large artery atherosclerotic stroke or transient ischemic attack, if anything, display reduced TMAO levels as compared to asymptomatic atherosclerotic patients (22). Likewise, in a Norway study, TMAO levels were not different in patients with carotid atheromas and healthy controls (23).

Yet another metabolite [i.e., γ-butyrobetaine (γBB)] of the TMAO pathway was significantly increased in CVD patients, which is in line with previous findings from Hazen's team (24). In another mice model prone to develop atherosclerosis, increased circulating TMAO concentration appear to be associated with slow atherosclerotic lesion development (25) providing some agreements with the results obtained by Yin *et al.* in humans (22). These diverging results warrant the necessity to confirm the newfound role of TMAO in thrombosis using multiple cohorts from different countries. The role of the other identified metabolites from the TMAO pathway also needs to be authenticated. One can anticipate that future studies might highlight other microbial metabolites that may also interact with TMAO and modulate its effects on CDV pathophysiology as well as thrombus formation.

Despite some of the limits discussed above, the most recent human study from Zhu *et al.* (20) enables the translation of previous mechanistic studies found both *in vitro* or in mice models to humans (18). These results point to a direct role of dietary choline intake in increasing TMAO concentrations and subsequently, platelet hyperresponsiveness. This phenotype can be partially prevented by oral doses of aspirin (20). Altogether, these recent results enable us to better understand the complete pathophysiopathology of atheroma formation and subsequent increased thrombus formation, responsible for major adverse cardiac events previously observed after choline or L-carnitine ingestion and subsequent increase in TMAO concentrations (14,15). As pointed out by the authors themselves, more work remains to be performed to understand how TMAO signals platelet hyperresponsiveness (18). Nevertheless, this work enables us to anticipate the emergence of CVD patient care management involving gut microbial composition or eventually metabolite production. For example, Wang et al nicely demonstrated that using a nonlethal inhibitor of gut microbiota TMAO production, reduces atherosclerotic lesions in mice models (26,27). However, this still remains to be translated to human studies.

Overall, human observations and preclinical investigations paved the way toward the implication of intestinal microbiota and derived metabolites in CVD physiopathology, atheroma formation and its severity. In addition to TMA/TMAO pathway, more microbial functions and pathways remain to be uncovered, which should lead to the discovery of further relevant gut microbiota derived metabolites that could impact on the progression of these common and chronic disorders.

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