

Intestinal microbial metabolism of phosphatidylcholine: a novel insight in the cardiovascular risk scenario

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Abstract: Intestinal microbiota is a “dynamic organ” influencing host metabolism, nutrition, physiology and immune system. Among its several interactions, the role of a phosphatidylcholine metabolite derived by gut flora activity, i.e., trimethylamine-N-oxide (TMAO), allows perceiving a novel insight in the cardiovascular risk scenario, being a strong predictor of this condition. Based on current reports, including the paper of Tang *et al.*, we describe here: the possible role of intestinal microbiota in cardiovascular risk as well as potential interventions to reduce gut flora TMAO production by diet, probiotics and antibiotics. Finally, we highlight the possibility of evaluating, monitoring and modulating TMAO in order to use its serum levels as a marker of cardiovascular risk in the next future, when the need of controlled studies on large series will be satisfied.

Keywords: Intestinal microbiota; cardiovascular risk; trimethylamine-N-oxide (TMAO); choline; probiotics; antibiotics

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Basic information

The complex of bacterial populations inhabiting the human intestinal tract (called “intestinal microbiota”) modulates host immune system, preventing pathogen microorganism colonization (1) as well as it contributes to digestion, metabolism and absorption of nutrients. Genome-sequencing and culture-based studies have shown that healthy gut microbiota has interpersonal differences in composition and functions, depending on age, diet, hygiene and environmental exposures (2). Furthermore, changes in intestinal microbiota may play a role in gastrointestinal disorders such as infections, inflammatory bowel diseases and colo-rectal cancer, as well as in extra-intestinal conditions such as atopy, arthritis, cardiovascular and metabolic diseases (i.e., type 2 diabetes, obesity and metabolic syndrome) (3-8). Therefore, the awareness of gut dysbiosis impact on human health would allow developing preventive strategies, targeted therapies and personalized diets. A topic raising an appealing

interest is represented by the paper of Tang *et al.* (9) which analyses the influence of phosphatidylcholine metabolism products by gut flora on cardiovascular impairment.

Atherosclerosis and phosphatidylcholine-trimethylamine-N-oxide (TMAO) progression

As known, atherosclerosis is the main precursor of cardiovascular diseases, which are the leading causes of human deaths worldwide. Current metabolomics and model animal studies provide evidence of a relationship between atherosclerosis and trimethylamine-N-oxide (TMAO), a gut flora-dependent metabolite resulting from the oxidation of trimethylamine (TMA). The intestinal microbiota produces TMA from dietary quaternary amines, like choline and L-carnitine, as well as from phosphatidylcholine; then hepatic flavin monooxygenase (especially FMO3 and FMO1) convert TMA in TMAO (10,11). Furthermore, some types of seafood (cods and other teleosts, sharks, rays, molluscs

and crustaceans) are important direct sources of TMAO (12). Interestingly, a study in atherosclerosis-prone C57BL/6J-Apoe^{-/-} mice showed that a diet rich in choline extended atherosclerotic aortic lesions and enhanced cholesterol accumulation in macrophages. When broad-spectrum antibiotics were administered during choline-enriched diet, these effects were eliminated because of gut flora suppression (10). TMAO can play a role in atherogenesis by inhibiting reverse cholesterol transport and inducing foam cell formation by increasing the expression on macrophage cell surface of pro-atherogenic scavenger receptors CD36 (cluster of differentiation 36) and scavenger receptor A (SRA) (10). TMAO may also modify sterol metabolism reducing mRNA expression of hepatic enzymes Cyp7a1 and Cyp27a1, which catalyse bile acid synthesis (13).

Plasma levels of choline, betaine and TMAO-related cardiovascular risk

Choline is a constituent of phosphatidylcholine and it is essential for the structural integrity of cell membranes, acetylcholine synthesis, cell-membrane signalling, methyl-group metabolism; this last function occurs by means of betaine, which is a choline metabolite. Danne *et al.* have asserted that elevated whole blood and plasma levels of choline may be predictive for acute coronary syndrome linked to plaque instability (14). Studies performed in cardiac patients, undergoing elective coronary angiography, showed that elevated plasma levels of both choline and betaine were related to an increased risk of major adverse cardiovascular events only in the presence of high plasma TMAO levels (9,14). Therefore TMAO seems to have the major role in cardiovascular impairment compared to its precursors choline and betaine. Moreover, an association between elevated plasma levels of TMAO and high mortality risk among patients with chronic heart failure was found regardless conventional risk factors and cardio-renal indices (15).

Diet, probiotics, antibiotics and TMAO modulation through changes in microbiota composition

Alimentary habits may be of relevance to support the production of TMAO by intestinal microbiota. In this regard, choline is a semi-essential nutrient, so its complete exclusion from diet is not feasible. Food containing choline are mostly represented by meats, eggs, wheat germ, and some seafood (salmon, tuna, cod, scallop) and therefore,

they may induce gut flora production of TMA.

Intestinal bacterial species responsible of phosphatidylcholine metabolism are not completely defined. Sitaraman (16) supported that some species of *Bacteroidetes* (particularly *B. thetaiotaomicro* and *B. fragilis*) may be involved in TMA formation, since they exhibit phospholipases hydrolysing dietary phosphatidylcholine to choline. Moreover, it is reported that bacteria from the class of *Erysipelotrichia* (phylum *Firmicutes*) can produce TMA from choline (8). These evidences may be of great interest if we consider that *Bacteroidetes* and *Firmicutes* are the two main bacterial phyla among intestinal flora. Hence, probiotics may be used to control TMA concentration in gut lumen. Brugère *et al.* demonstrated that the “archaeal methanogen strains” such as *Methanomassiliicoccus luminyensis* B10, produce methane by reducing TMA through hydrogen. Based on these results, the authors proposed the use of *Archaeobiotics* in humans with the aim of depleting TMA (17). A recent study performed in mice has shown that cecal microbial transplantation can transmit a choline-diet dependent ability for TMA and TMAO production as well as atherosclerosis susceptibility (18). Therefore, changes of gut flora composition with microbial faecal transplantation may be an approach to prevent or treat atherosclerosis dependent diseases.

For the same purpose, the use of oral broad-spectrum antibiotics in mice and humans has been investigated in some studies (9-11). Plasma TMAO levels were suppressed for a short time, recurring after the suspension of antimicrobials. Moreover, the above mentioned study of Tang *et al.* (9) reports that the long term use of ciprofloxacin has a transient suppressive effect probably due to the development of intestinal flora resistant strains.

Trimethylaminuria (defined also as “fish odour syndrome”) is a metabolic disorder due to inactivating mutations in *FMO3* gene, characterized by the presence of TMA in sweat, urine and breath. In patients affected by trimethylaminuria, antimicrobials such as neomycin and metronidazole represent a therapeutic option and the cyclic assumption of low doses is suggested to avoid antimicrobial resistances (19). Based on these evidences, a short term treatment with non-systemic antibiotics (brief courses of neomycin and metronidazole) may be useful to reduce cardiovascular risk.

Conclusive remarks

The novelty of this review concerns the relationship between

intestinal microbiota (a dynamic “organ” influencing host metabolism, nutrition, physiology and immune system) and cardiovascular risk. The main studies about this topic come from the Department of Cellular and Molecular Medicine at Lerner Research Institute of Cleveland Clinic and offer a novel insight in the cardiovascular risk scenario by exploring the role of gut flora phosphatidylcholine metabolite TMAO in atherogenesis. A possible role of intestinal disorders in cardiovascular risk has been suggested also by a recent investigation of our group (20) which showed an endothelial dysfunction in subjects with inflammatory bowel diseases in an active phase, a group of patients with strong alterations in intestinal microbiota (3).

However, these experimental evidences have not been supported yet by studies on large samples in order to establish the usefulness of plasma TMAO as cardiovascular risk marker as well as the benefits of modulating TMAO production through probiotics, antibiotics and diet. In this regard, we believe that a crucial role of diet needs to be investigated. Indeed, a reduction in the intake of choline and/or L-carnitine sources (red meat and eggs) may be of benefit on cardiovascular health through a fall in TMAO production. Additionally, such foods also are inclined to increase cholesterol serum levels. On the other hand, some types of seafood (rays, molluscs and crustaceans) are important direct sources of TMAO as well as of cholesterol.

It is well-known the protective effect of Mediterranean diet on the risk of cardiovascular disorders so that on December 2013, UNESCO recognized, during its meeting, in Baku this diet pattern as an intangible cultural heritage of Italy, Portugal, Spain, Morocco, Greece, Cyprus and Croatia. The main topic of this review could support the importance of a Mediterranean style diet, including pulses, vegetables, fruit and simple proteins (fish, white meat) to maintain cardiovascular health even by reducing direct and indirect sources of TMAO.

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Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

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