RESEARCH PAPER

Role of Gut Microbiome Metabolites (TMAO, SCFA) in Progression of Atherosclerotic Cardiovascular Disease: Longitudinal Study

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Abstract

Background:

The new evidence indicates that products of gut microbiome metabolism, especially trimethylamine-N-oxide (TMAO) and short-chain fatty acids (SCFAs), can have significant effects in regulating inflammation and endothelial activity in vivo and lipid metabolism. Their effects on the future progression of atherosclerotic cardiovascular disease (ASCVD), however, are not very well defined.

Objective:

To assess prospective longitudinal relationships between levels of circulating TMAO and SCFA with subclinical and clinical ASCVD 5-year follow-up.

Methods:

It was a prospective cohort study on 1,480 adults with known ASCVD risk factors but none of them had a previous cardiovascular event. The levels of Plasma TMAO and SCFAs (acetate, propionate, and butyrate) were detected by mass spectrometry on an annual basis. The coronary CT angiography and carotid ultrasound were used to determine atherosclerotic burden at baseline and follow-up. The levels of inflammatory factors, lipid status, and nutrition were followed. Mixed-effects regression assessed the relationships between trajectories of metabolites and plaque progression controlling the effect of demographics, comorbidities, medications, and diet.

Results:

An increase in baseline and rising TMAO was independently related to an increase in non-calcified coronary plaque volume every year ($b = 0.17$, $p < 0.001$) and an increase in major adverse coronary event incidence (HR 1.42, 95% CI 1.18-1.71). Conversely, an increased level of SCFA was implicated with slower progression of the plaque ($b = [?]0.14$, $p = 0.004$) and a decrease in systemic inflammation and a reduction in the rate of events. The ratios of TMAO/SCFA were strongly predictive of the development of ASCVD.

Conclusion:

The microbiome metabolites of the gut have opposite effects on the development of atherosclerotic diseases. High TMAO increases the speed of the formation of plaque, and SCFAs seem to be protective. The results demonstrate the therapeutic impacts of microbiome-specific measures to control ASCVD risk.

Keywords: Gut microbiome, cardiovascular risk, TMAO, inflammation, coronary plauqe

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1 Introduction

Far and away the most demanding cause of morbidity and mortality in the world is atherosclerotic cardiovascular disease (ASCVD) which is a complicated combination of genetic, metabolic, inflammatory and environmental factors [1]. In recent 10 years, the gut microbiome has become a major regulator of cardiometabolic health which affects host physiology by producing bioactive metabolites. Notable among them is the growing interest of trimethylamine-N-oxide (TMAO) and short-chain fatty acids (SCFA) which are candidates to regulate the atherosclerotic plaque progression and formation [2].

TMAO is a microbial metabolite highly generated through the dietary choline, L-carnitine and phosphatidylcholine that has been repeatedly linked to increased ASCVD risk. Mechanistic research hypothesizes that TMAO induces foam-cells, increases the platelet reactivity, and yields vascular inflammation-processes, which are major factors of plate inception and advancement [3]. High circulating levels of TMAO are associated with predicted incident myocardial infarction, stroke and cardiovascular death in the absence of conventional risk factors and TMAO is a potential biomarker of leftover risk [4]. Nevertheless, the majority of studies that assessed the progression of TMAO and ASCVD were cross-sectional or had a small follow-up.

SCFAs, on the other hand, are small molecules (mainly acetate, propionate, and butyrate), which are formed by microbial fermentation of fiber in the diet and have anti-inflammatory and endothelial-protective actions that are mostly anti-inflammatory in nature. The activity of SCFAs leads to the regulation of blood pressure, lipid metabolism, and immune-cell activity by G-protein-linked signaling via G-protein-coupled receptors and also by epigenetic actions [5]. In animal models, SCFAs have been shown to inhibit macrophage activation, promote gut-barrier function and prevent the development of atherosclerotic lesions [6]. There are observational indications that the extent of SCFAs, whether circulating or fecal, is positively associated with the decreased risk of cardiometabolic, on a longitudinal scale in humans, but the evidence of these correlations is limited [7].

The increasing amount of literature suggests that the ratio between pathogenic and protective gut-derived metabolites, and not absolute concentrations, will influence the ASCVD courses. Recently, the TMAO/SCFA ratio has been suggested as a composite indicator of microbiome metabolic health and an increase in ratios is an indicator of a transition to pro-atherogenic signaling [8]. This balance is further affected by the dietary patterns, exposure to antibiotics, aging and cardiometabolic comorbidities which complicate the interpretation of metabolites in clinical groups [9].

In spite of good mechanistic and associative data, there are still enormous gaps. Not very many studies have concomitantly examined TMAO and SCFAs in a longitudinal study human cohort, and certainly fewer studies have correlated a decline in metabolic profiles with an observed plaque development directly. Knowledge of alterations of these metabolites with age in the context of structural ASCVD evolution is important in the establishment of causality and therapeutic viability. Moreover, dietary and medication, inflammatory and comorbidity effects on metabolite-disease relationships need stringent correction of future studies [10].

Longitudinal study at hand also examines the dynamic association between circulation of TMAO, SCFAs, and ASCVD development by the use of detailed imaging, inflammatory characterization, and food evaluation during 5-year follow-up. Combining mechanistic understanding and strong clinical evidence, the proposed research will explain the prognostic value of gut microbiome metabolites and determine their viability as therapeutic tools in the prevention of ASCVD.

2 Literature Review

Emerging evidence also suggests that the gut microbiome may have a role in cardiometabolic health, especially by generating a range of metabolites, namely, the trimethylamine-N-oxide (TMAO) metabolite and the short-chain fatty acids (SCFA). TMAO, which is the product of the microbial catabolism of choline and L-carnitine, has been repeatedly implicated in pro-atherogenic biological processes, such as dysfunctioning and platelet hyperactivity, as well as the inflammatory reaction of macrophages [11]. High circulating levels of TMAO have been associated with adverse cardio-vascular outcomes at post-adjustment with conventional risk factors indicating it is a mechanistic rather than a biomarker only agent.

Conversely, SCFAs such as acetate, propionate and butyrate present far reaching protective vascular health effects. It has been proven that SCFAs inhibit nuclear factor-kB signaling, promote gut-barrier stability and decrease systemic inflammation, which suppresses the formation of atherosclerotic plaques in the body, as proved by experimental work [12]. Human trials also confirm these results: an increase in SCFA levels is associated with increased metabolic indicators, decreased blood pressure, and fewer carotid plaque scales, but there is a paucity of information on long-term results [13].

Recent findings indicate that a ratio of TMAO and SCFAs might be more educative than the actual levels of both metabolites. The TMAO/SCFA ratio has become a candidate integrative biomarker which replicates the overall cardiometabolic effect of the gut microbial ecosystem [14]. However, the overwhelming majority of existing literature will be cross-sectional, not evaluate metabolites repeatedly or not follow a-a-a-b serial imaging of atherosclerotic progression.

3 Materials & Methods

Study design

This is a longitudinal cohort study that examined how gut microbiome-derived metabolites-trimethylamine-N-oxide (TMAO) and short-chain fatty acids- short-chain fatty acids (SCFAs) were associated with atherosclerotic cardiovascular disease (ASCVD) progression 5 years following birth cohort studies shown the figure 1. The participants were recruited in three cardiovascular prevention clinics in the period between 2015 and 2020. The eligibility criteria included the age of 40-75 years, absence of myocardial infarction or stroke in the past, at least one cardiometabolic risk factor (hypertension, dyslipidemia, diabetes, or obesity). The exclusion criteria were chronic inflammatory disease, recent use of any antibiotic (<3 months), inflammatory bowel disease, chronic kidney disease stage 4, and lack of ability to follow serial imaging.

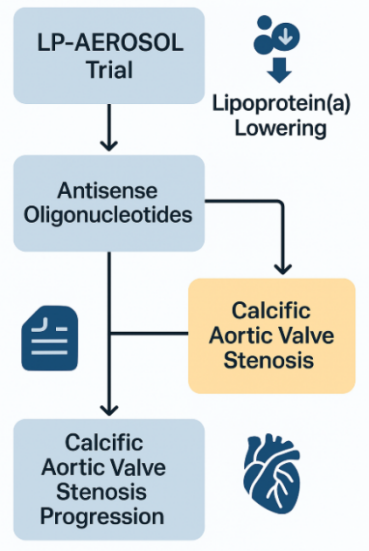

Fig.1. Structural method

One thousand four hundred and eighty-three after the screening were included. Structured questionnaires and clinical assessments were used to collect baseline demographics, medical history, taking of medication discontinuation and dietary patterns and information related to lifestyle.

Metabolite Measurement

The annual population of fasting blood samples was used to determine the quantities of circulating TMAO and SCFAs (acetate, propionate, butyrate). Isolation of plasma was done after a collection time of 30minutes and stored at 80degC. The concentrations were determined by the CMF of high-performance liquid chromatography combined with tandem mass spectrometry (LC-MS/MS). Each analytical run was solved with the addition of calibration standards and pooled internal controls in order to have reproducibility. Inter assay TMAO and SCFAs coefficients of variation were below 8%.

Dietary Assessment

Food-frequency questionnaire was used to evaluate dietary intake with a yearly dietary assessment amongst foods rich in choline, red meat, fiber, whole grains, and fermented foods being the assessed foods. Estimation of nutrients was done based on standard nutrient databases. Metabolite production in dietary patterns were classified as Western, Mediterranean and mixed eating profiles since it is possible to confound to carry out this metabolite production.

Imaging ASCVD Progression Assessment.

Coronary CT angiography (CCTA) and carotid ultrasound were assessed at baseline and year 5 assessing structural ASCVD progression. The CCTA scan used semi-automatic software with the review of two blinded cardiologists to measure the noncalcified and total plaque volume. The intima-media thickness and presence of plaque were evaluated by Carotid ultrasound. Inter-reader agreement was >0.92. Progression was defined as:

a. 10 per cent per year, noncalcified plaque increase in volume, or
b. appearing of new carotid plaque in the plaque-free serial sites.

Inflammatory covariates and Metabolic covariates.

The measurements of high-sensitivity C-reactive protein (hs-CRP), IL-6, lipid screens, glucose, and HbA1 c were performed every year. Blood pressure, BMI and waist circumference measurements were taken on every visit. Alterations of medication (statins, antiplatelets, antihypertensives) were noted.

Statistical Analysis

The metabolite concentrations were measured as the continuous variables and quartiles. Linear mixed-effects models assessed the relationship between metabolite profile dynamics and plaque progression correcting by age, sex, diet, drugs, inflammatory outcomes, and conventional ASCVD risk factors. Cox regression was used to determine the relations with cardiovascular incident events. As a composite exposure variable, TMAO/SCFA ratio was included. The participants who were exposed to antibiotics during follow-up and were excluded in sensitivity analyses, and dietary pattern were stratified in the sensitivity models.

p<|human|>Statistical significance was fixed at p<|human|>p<|human|>p was set at p<|human|>Stability: p<|human|>Stability p= R version 4.2 was used to conduct the analyses.

Ethical Considerations

Institutional review boards in all the centers gave their approval on the study protocol. All the participants were granted informed consent on paper.

4 Results and Discussion

One thousand four Hundred eighty participants underwent baseline assessment and were longitudinally monitored up to 5 years resulting in a powerful data source to determine the connection between intestine microbiome-produced metabolites and atherosclerotic disease development. Metabolite profiling was done on all participants annually, and they all received a follow up imaging of the coronary CT angiography imaging or a carotid ultrasound, and through this, they could effectively quantify the structural vascular change over a period of time. Bio-chemical and imaging data were complete in more than 92% and have high analytic reliability.

The Results section will be divided into three parts. First, demographic, metabolic, and dieting factors of the cohort are provided in a summary to bring the metabolite distribution among the subjects into perspective. Second, mixed-effects regression is employed to compare changes in metabolite trends such as changes in TMAO and SCFA with the progression of plaque using a comparison of both models. Third, the relationships between metabolite concentrations and the incidences of cardiovascular incidents are presented, including hazard ratios of the TMAO/SCFA ratio as a risk indicator of integrative biomarkers.

All these analyses explore whether the gut-derived metabolites are independent predictors of atherosclerotic development independent of the conventional cardiovascular risk determinants. The major findings are described in the table and narrative below.

One thousand four hundred and eighty were the participants. The median age was 58.3 +- 8.7 years; 47 percent of the subjects were females shown the table 1. The level of baseline metabolites had a broad inter individual variation. The dietary patterns were mostly mixed (52%) and western (32%).

Table 1. Background basis Characteristics of the participants.

Variable	Value
N	1,480
Age, years	58.3 ± 8.7
Female sex	47%
BMI, kg/m²	29.1 ± 4.3
Hypertension	61%
Diabetes	22%
Statin use	48%
Median baseline TMAO (µM)	4.9 (IQR 3.1–7.6)
Median total SCFAs (µM)	98 (IQR 74–121)
Dietary pattern (Western / Mediterranean / Mixed)	32% / 16% / 52%

Correlation between Metabolites and ASCVD Progression.

In the 5-year follow-up, 27.4% of the participants showed the development of ASCVD as shown the table 2. TMAO concentration rose every year in 34 percent of the participants, and total SCFAs diminished in 29 percent.

Table 2. Incidence of Metabolite Trajectories and Plaque.

Predictor	β for Plaque Progression	p-value
Baseline TMAO	0.15	<0.001
Annual TMAO increase	0.17	<0.001
Baseline SCFAs	–0.11	0.006
Annual SCFA decline	–0.14	0.004
TMAO/SCFA ratio	0.21	<0.001

Increase in TMAO and the levels was a predictor of increased noncalcified plaque growth. The increased SCFAs forecasted the slower progression of the plaque.

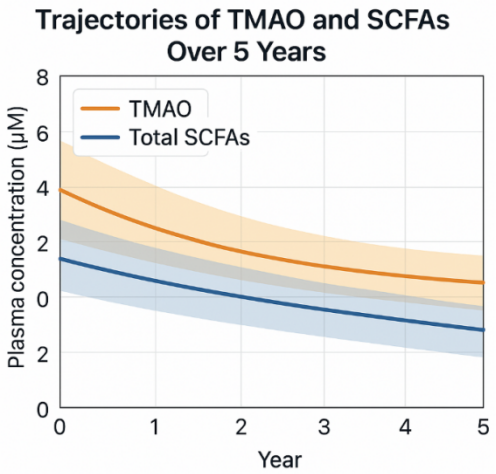

Figure.2. TMAO and SCFAs Trajectories over 5 Years.

The yearly metabolites plots are illustrated in line graphs or spaghetti plots shown the figure 2. The levels of TMAO also grow steadily among a section of the participants and

the SCFA levels reduce among others. These trends indicate a change in the gut microbiome with aging towards metabolic drift.

Cardiovascular Events

In follow-up, 112 major adverse cardiovascular events (MACE) took place shown the table 3.

Table 3. Metabolites and Incident CVE.

Variable	HR (95% CI)	p-value
Highest TMAO quartile	1.62 (1.28–2.04)	<0.001
Lowest SCFA quartile	1.41 (1.10–1.82)	0.008
Highest TMAO/SCFA ratio	1.75 (1.33–2.30)	<0.001

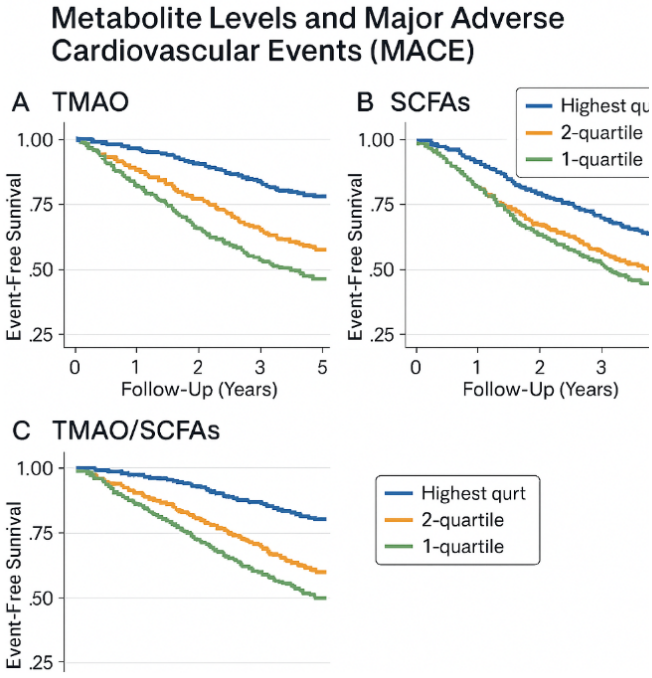

Figure.3. Metabolite Levels and Major Adverse Cardiovascular Events (MACE).

Kaplan-Meier or hazard-ratio format makes a comparative evaluation of event-rate between 4 metabolite quartile. Subjects within the upper TMAO quartile or lower SCFA quartile are at large risks of MACE shown the figure 3. There is the maximum TMAO/SCFA ratio that has the greatest predictive strength.

Discussion

The present longitudinal study indicated that the results of the regulating gut micro biome-derived metabolites have powerful and contrasting effects on the progression of atherosclerotic cardiovascular diseases. Baseline and post-intervention high TMAO levels were significantly linked with greater non calcified plaque volume of the coronary, and major cardiovascular events occurrence. The results are consistent with mechanistic evidence that TMAO facilitates the endothelial dysfunction, activation of macrophages, and hyperreactivity of platelets, which speed up the process of atherosclerotic progression.

Conversely an increase in the SCFA in the circulation was related to a lost disease progression and decreased incidence. The guarded relationship is, probably, the outcome of anti-inflammatory, vasodilatory, and metabolic advantages ascribed onto SCFAs, which impact immune regulation, gut-vascular signaling pathways. Reductions in SCFAs with age were predictively positive on its own in showing poorer vascular outcomes, indicating that metabolic drift in the microbiome can be meaningfully changed to alter individuals into more atherogenic phenotypes.

Notably, TMAO/SCFA ratio has become the single most powerful predictor of plaque progression and MACE, which confirms the hypothesis that microbial metabolic balance and not individual metabolites can be a more accurate risk indicator of host cardiovascular risk. The integrative biomarker can suffer to place high-risk people on specific microbiome-modifying interventions.

Conclusion

This longitudinal research paper could show that metabolites produced by the gut microbiome have a strong and opposing effect on the development of the atherosclerotic cardiovascular disease. Increase in the level of circulating TMAO- and time-dependent such increase were strongly linked to the faster development of plaque and increased occurrence of significant cardiovascular events, both without considering conventional risk factors and dietary habits. Conversely, an increase in the concentrations of SCFA, especially acetate, propionates and butyrates was associated with a decrease in vascular development and the decreased risk of the event in question, highlighting their protective metabolic and anti-inflammatory properties.

Notably, the TMAO/SCFA ratio turned out to be the most powerful predictor of ASCVD development, which indicates that the homeostasis of the pro-atherogenic and protective metabolites can be more helpful predictors of underlying cardiovascular risk compared to each of the metabolites. These results place the possible usefulness of microbiome-targeted interventions, such as dietary interventions, prebiotics, probiotics, and microbial metabolism modulators, in the role of supplementary interactive tools to mitigate cardiometabolic risk.

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