

Gut Microbiota-Derived Trimethylamine N-Oxide (TMAO) and Its Association with Atherosclerotic Burden in Vegetarian versus Non-Vegetarian Populations: A Cross-Sectional Study

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ABSTRACT

Background: Trimethylamine N-oxide (TMAO), a gut microbiota-derived metabolite, has been associated with cardiovascular disease and atherosclerotic burden. Dietary patterns may influence circulating TMAO concentrations through differences in the availability of substrates for microbial trimethylamine production; however, the relationship between dietary pattern, TMAO, and subclinical atherosclerosis remains incompletely understood. This study aimed to compare circulating TMAO concentrations and carotid atherosclerotic measures, including carotid intima-media thickness (CIMT) and carotid plaque, between vegetarian and non-vegetarian adults and to assess the association between TMAO concentration and atherosclerotic burden.

Methods: An analytical cross-sectional study was conducted among 200 adults, comprising 100 vegetarian and 100 non-vegetarian participants. Demographic, clinical, anthropometric, dietary, and lifestyle data were collected using a structured questionnaire. Blood samples were analyzed for TMAO, lipid profile, glucose parameters, and renal function. Carotid ultrasonography was performed to assess CIMT and carotid plaque. Associations between TMAO and CIMT were evaluated using correlation and multivariable linear regression, while binary logistic regression was used to assess the association between TMAO and carotid plaque after adjustment for relevant cardiovascular risk factors.

Results: Mean TMAO concentration was significantly higher among non-vegetarians than vegetarians (4.21 ± 2.05 vs 2.86 ± 1.34 $\mu\text{mol/L}$; $p < 0.001$). Mean CIMT was also higher in non-vegetarians (0.69 ± 0.11 vs 0.62 ± 0.09 mm; $p < 0.001$). Carotid plaque was present in 27.0% of non-vegetarians compared with 14.0% of vegetarians ($p = 0.022$). TMAO showed a positive correlation with mean CIMT ($r = 0.38$; $p < 0.001$). After multivariable adjustment, each 1 $\mu\text{mol/L}$ increase in TMAO was associated with a 0.018-mm increase in mean CIMT (adjusted $\beta = 0.018$; 95% CI: 0.010–0.026; $p < 0.001$) and higher odds of carotid plaque (adjusted OR = 1.42; 95% CI: 1.13–1.78; $p = 0.002$). TMAO concentrations were also associated with total cholesterol and LDL-C.

Conclusion: In this cross-sectional study, non-vegetarian participants had higher circulating TMAO concentrations, greater CIMT, and a higher prevalence of carotid plaque than vegetarian participants. Higher TMAO concentrations were positively associated with CIMT and carotid plaque after adjustment for relevant cardiovascular risk factors. These findings support an association between circulating TMAO and subclinical atherosclerotic burden; however, the cross-sectional design does not establish causality.

Keywords: TMAO; Gut microbiota; Atherosclerosis; Carotid intima-media thickness; Vegetarian diet.

How to cite this article: Dwarabandham S Rakshaka Siridhan¹, Guna Sri Phani Ajay Gadugoyyala², Kalavakolli Kalpavalli³, Shaik Mohammad Abbas⁴, Kanigiri Venkata Rami Reddy⁵, Vinukonda Praveen Kumar⁶. Gut Microbiota-Derived Trimethylamine N-Oxide (TMAO) and Its Association with Atherosclerotic Burden in Vegetarian versus Non-Vegetarian Populations: A Cross-Sectional Study. Int J Drug Deliv Technol. 2026;16(7):1781-1790. DOI: 10.25258/ijddt.16.7.191.

INTRODUCTION

Atherosclerosis is a chronic, progressive disease of the arterial wall and a major underlying pathological process in coronary artery disease, cerebrovascular disease, and peripheral arterial disease. It is

characterized by endothelial dysfunction, lipid accumulation, inflammatory responses, vascular cell alterations, and progressive formation of atherosclerotic lesions [1]. Clinical manifestations

such as myocardial infarction and stroke generally occur at advanced stages of the disease, whereas structural and functional vascular changes may develop for several years before the onset of overt cardiovascular events. Therefore, identification of early vascular abnormalities and potentially modifiable biological pathways is important for understanding cardiovascular disease risk and prevention. Cardiovascular risk is influenced by established risk factors including increasing age, hypertension, diabetes mellitus, dyslipidaemia, overweight and obesity, smoking, and physical inactivity. However, variation in cardiovascular risk may occur even among individuals with similar conventional risk-factor profiles, highlighting the importance of investigating additional biological pathways involved in cardiovascular disease [2]. Increasing evidence has therefore focused on inflammation, metabolic pathways, environmental influences, and the gut microbiota as potential contributors to cardiovascular risk.

Non-invasive vascular imaging provides an opportunity to assess subclinical vascular disease. Carotid ultrasonography permits measurement of carotid intima-media thickness (CIMT) and detection of focal carotid plaque. CIMT has been widely used as a marker of vascular wall characteristics in epidemiological and clinical research, whereas carotid plaque represents a more focal manifestation of atherosclerotic disease [3]. Because CIMT and carotid plaque provide complementary information regarding vascular structure, assessment of both parameters may provide a broader evaluation of subclinical atherosclerotic burden. The gastrointestinal tract contains a complex microbial ecosystem that contributes to nutrient metabolism, maintenance of intestinal barrier integrity, immune regulation, and host metabolic homeostasis. Dietary composition can influence the composition and metabolic activity of the gut microbiota, while microbial metabolism can generate bioactive compounds that enter the systemic circulation and influence host physiology [4,5]. These microbial metabolites include short-chain fatty acids, bile-acid derivatives, indole metabolites, and trimethylamine-derived metabolites.

The interaction between diet, gut microbiota, and host metabolism has increasingly attracted attention in cardiovascular research. Microbial metabolites may represent important intermediates linking dietary exposures with cardiometabolic processes, including inflammation, lipid metabolism, thrombosis, and vascular dysfunction [5]. Among the gut microbiota-derived metabolites investigated in cardiovascular disease, trimethylamine N-oxide (TMAO) has received considerable attention. Several observational studies and meta-analyses have reported an association

between higher circulating TMAO concentrations and cardiovascular events and mortality [6,7]. However, much of the available human evidence is observational, and therefore these associations should not be interpreted as definitive evidence of a causal relationship. Differences in renal function, metabolic status, dietary intake, and other clinical characteristics may also influence circulating TMAO concentrations and their association with cardiovascular outcomes.

TMAO is generated through a multi-step pathway involving dietary substrates, gut microbial metabolism, and host hepatic metabolism. Dietary nutrients containing trimethylamine groups, particularly choline, phosphatidylcholine, and L-carnitine, can be metabolized by intestinal microorganisms to produce trimethylamine (TMA). TMA is subsequently absorbed into the circulation and oxidized predominantly in the liver by flavin-containing monooxygenase enzymes, particularly FMO3, to form TMAO [4,8]. The circulating concentration of TMAO is influenced by several interacting factors, including the availability of dietary substrates, composition and metabolic activity of the gut microbiota, hepatic oxidation of TMA, and renal clearance [8]. Consequently, circulating TMAO concentrations may vary considerably between individuals even when their broad dietary patterns are similar. Experimental and observational studies have suggested that TMAO may interact with pathways involved in cholesterol metabolism, bile-acid metabolism, macrophage function, platelet responsiveness, and vascular inflammation [1,8]. The proposed biological effects of TMAO have therefore provided a basis for investigating its potential relationship with atherosclerotic disease and cardiovascular risk.

The potential relationship between TMAO and atherosclerosis has been investigated through several proposed biological mechanisms. Experimental studies have suggested that TMAO and its precursor pathways may influence cholesterol transport and metabolism, macrophage cholesterol accumulation, foam-cell formation, and vascular inflammatory processes [1,9]. In addition, TMAO has been investigated in relation to platelet activation and thrombosis, which are important processes involved in the clinical complications of atherosclerotic plaque [10]. Human observational studies have also reported associations between circulating TMAO concentrations and cardiovascular disease burden. In patients with established coronary artery disease, higher plasma TMAO concentrations have been associated with greater coronary atherosclerotic burden after adjustment for conventional cardiovascular risk factors and other clinical variables [11]. However, the interpretation of these findings

requires consideration of potential confounding factors. Renal function, age, metabolic status, dietary intake, and other clinical characteristics can influence circulating TMAO concentrations [8]. Therefore, assessment of the relationship between TMAO and vascular measures after accounting for relevant confounding variables is important.

Vegetarian and non-vegetarian dietary patterns differ in the sources and amounts of protein, fat, fibre, and nutrients that may serve as substrates for gut microbial metabolism. Animal-derived foods, particularly meat, provide important dietary sources of L-carnitine, while choline and phosphatidylcholine are present in a variety of animal- and plant-derived foods. These nutrients can participate in the gut microbial pathway leading to TMA and subsequently TMAO production [4,12]. Dietary patterns rich in plant foods may additionally provide greater amounts of dietary fibre and phytochemicals that can influence gut microbial composition and metabolic activity. However, vegetarian diets are heterogeneous and cannot be considered nutritionally uniform. Vegetarian dietary patterns may include dairy products and eggs, and individuals may differ substantially in their consumption of legumes, whole grains, fruits, vegetables, refined carbohydrates, and processed foods. Similarly, non-vegetarian diets may vary considerably according to the quantity and type of meat, fish, poultry, and other animal-derived foods consumed. Consequently, classification as vegetarian or non-vegetarian represents a broad dietary exposure rather than a comprehensive characterization of overall nutrient intake.

Studies specifically examining TMAO concentrations in vegetarian populations have demonstrated that the relationship between dietary pattern and circulating TMAO is complex. In one study, plasma TMAO concentrations did not differ according to the strictness of vegetarian dietary restriction, suggesting that factors beyond the presence or absence of animal foods may influence TMAO metabolism [13]. Other intervention and observational studies have reported reductions in circulating TMAO following plant-based dietary interventions, although findings across studies are not entirely consistent [14,15]. These observations indicate that dietary substrate availability, gut microbial composition, host metabolism, and renal clearance may all contribute to inter-individual variation in TMAO concentrations.

Despite increasing interest in the gut microbiota–TMAO pathway and cardiovascular disease, relatively fewer studies have simultaneously examined dietary pattern, circulating TMAO concentrations, and objective measures of subclinical vascular disease within the same study population. Much of the existing literature has focused on cardiovascular outcomes,

metabolic mechanisms, or dietary interventions, whereas studies integrating circulating TMAO with carotid vascular measurements in individuals classified according to broad dietary patterns remain comparatively limited. Assessment of carotid CIMT and carotid plaque provides complementary measures of vascular structure and subclinical atherosclerotic burden. Examining these measures alongside circulating TMAO may therefore provide additional information regarding whether variation in TMAO concentrations is associated with measurable vascular changes. This question may be particularly relevant in populations in which vegetarian and non-vegetarian dietary practices coexist and differ in the consumption of potential TMAO precursor nutrients.

The interaction between dietary pattern, gut microbial metabolism, circulating TMAO, and vascular health represents an important area of investigation in contemporary cardiovascular research. Existing evidence supports an association between TMAO and cardiovascular risk, while also indicating that circulating TMAO is influenced by multiple dietary, microbial, metabolic, and renal factors [6-8]. Furthermore, evidence from vegetarian and plant-based dietary studies suggests that the relationship between dietary pattern and TMAO concentrations is complex and may not be explained solely by the consumption of animal-derived foods [13-15]. Therefore, the present study was designed to compare circulating TMAO concentrations between vegetarian and non-vegetarian adults and to examine their relationship with objective measures of subclinical atherosclerotic burden, specifically carotid intima-media thickness and carotid plaque. By evaluating dietary pattern, circulating TMAO, and carotid vascular characteristics within the same study population, the study aims to contribute to the understanding of the relationship between gut microbiota-derived metabolism and early vascular disease.

OBJECTIVES

Primary Objective

To investigate the association between circulating TMAO concentrations and atherosclerotic burden, assessed by carotid intima-media thickness (CIMT) and carotid plaque, among vegetarian and non-vegetarian adults.

Secondary Objectives

1. To compare circulating TMAO concentrations between vegetarian and non-vegetarian adults.
2. To compare carotid intima-media thickness (CIMT) between vegetarian and non-vegetarian adults.

3. To compare the prevalence of carotid plaque between vegetarian and non-vegetarian adults.
4. To assess the association of circulating TMAO concentrations with lipid and glucose parameters.

HYPOTHESES

Primary Hypothesis

Alternative hypothesis (H₁): There is significant association between circulating TMAO concentrations and atherosclerotic burden, as assessed by CIMT and the presence of carotid plaque.

Null hypothesis (H₀): There is no significant association between circulating TMAO concentrations and atherosclerotic burden, as assessed by CIMT and the presence of carotid plaque.

MATERIALS AND METHODS

Study Design

A cross-sectional study design.

Study Setting

The study was conducted at a tertiary care teaching hospital in Andhra Pradesh, India.

Study Population

The study population comprised adults aged 18 years and above who met the predefined eligibility criteria. Participants were categorized into two dietary groups: vegetarians and non-vegetarians, based on their reported usual dietary pattern and the predefined dietary classification criteria of the study.

Inclusion Criteria

Participants were eligible for inclusion if they were 18 years of age or older, had followed their reported dietary pattern, either vegetarian or non-vegetarian, for at least one year, and were willing to participate in the study by providing written informed consent. Participants were also required to be available for anthropometric assessment, clinical evaluation, blood sample collection, and carotid ultrasonographic assessment, and to be able to provide the necessary dietary and clinical information.

Exclusion Criteria

Participants were excluded if they were unable or unwilling to provide informed consent, had medical conditions or were receiving treatments that could substantially alter TMAO metabolism or interfere with the interpretation of the study findings, or had conditions that could significantly affect the assessment of carotid atherosclerotic burden. Individuals who were unable to undergo blood sample collection or carotid ultrasonographic examination were also excluded. Participants with incomplete clinical, dietary, laboratory, or imaging data required for the analysis were excluded from the final study population.

Sample Size and Sampling Technique

The sample size was calculated using the single-population proportion formula, $n = Z^2 pq / d^2$, where $Z = 1.9$, for a 95% confidence level, $p = 20\%$ as the anticipated prevalence, $q = 1 - p = 80\%$, and $d = 6\%$, as the allowable absolute precision. The calculated sample size was 171 participants. After accounting for a 15% allowance for potential non-response or incomplete data, the required sample size was approximately 197 participants, which was rounded to 200. Accordingly, 200 participants were included in the study, with 100 vegetarians and 100 non-vegetarians. A consecutive sampling technique was employed for participant recruitment.

Study

The study was carried out over a period of 12 months, from June 2025 to June 2026.

Data Collection

Data were collected using a structured questionnaire to obtain information on participants' sociodemographic characteristics, dietary patterns, lifestyle factors, and relevant clinical history. Anthropometric measurements, including height and weight, were recorded, and body mass index (BMI) was calculated. Blood pressure was measured using a standardized procedure. Venous blood samples were collected under appropriate conditions for the estimation of plasma TMAO levels and other relevant biochemical parameters. All measurements and sample collection procedures were performed using standardized methods and appropriate quality-control measures.

Laboratory Investigations

Venous blood samples were collected from all participants for the assessment of trimethylamine N-oxide (TMAO) and relevant biochemical parameters. Plasma TMAO concentrations were determined using a validated laboratory assay. The lipid profile included total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglycerides. Glycemic status was assessed by measuring fasting blood glucose and glycated hemoglobin (HbA1c). Renal function was evaluated using serum creatinine and estimated glomerular filtration rate (eGFR).

Carotid Ultrasonography

Carotid ultrasonography was performed to assess carotid intima-media thickness (CIMT) and the presence of carotid atherosclerotic plaque. Ultrasonographic measurements were obtained according to a standardized imaging protocol from predefined carotid arterial segments. The mean CIMT was considered the primary continuous measure of carotid atherosclerotic burden. Carotid plaque was assessed using established ultrasonographic criteria, and participants were categorized according to the presence or absence of carotid plaque.

Statistical Analysis

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Statistical analysis was performed using Microsoft Excel. Continuous variables were expressed as mean $\pm$ SD or median (IQR), as appropriate, and categorical variables as frequencies and percentages. Between-group comparisons were performed using the independent-samples *t*-test for continuous variables and the chi-square or Fisher's exact test for categorical variables. Pearson's or Spearman's correlation was used to assess the association between TMAO and mean CIMT. Multiple linear regression and binary logistic regression were performed to evaluate the independent associations of TMAO with mean CIMT and carotid plaque, respectively, after adjustment for relevant confounding factors. A *p* value of less than 0.05 was considered statistically significant.

RESULTS

Baseline Characteristics

The analysis involved 200 persons; 100 vegetarians and 100 non-vegetarians. The participants' mean age was 48.7 ± 10.6 years (S.D.) in the vegetarian group and 49.8 ± 10.9 years (S.D.) in the non-vegetarian group. This age difference was no significant difference. The vegetarian group consisted of men in 52% and 55% of the non-vegetarian group. The mean BMI was marginally lower for vegetarians than for non-vegetarians. Non-vegetarians were slightly more likely to have hypertension and diabetes.

TABLE 1. BASELINE SOCIODEMOGRAPHIC AND CLINICAL CHARACTERISTICS

Variable	Vegetarian (n=100)	Non-vegetarian (n=100)	p-value
Age, years	48.7 ± 10.6	49.8 ± 10.9	0.47
Male, n (%)	52 (52.0)	55 (55.0)	0.67
Female, n (%)	48 (48.0)	45 (45.0)	0.67
BMI, kg/m ²	24.8 ± 3.4	26.1 ± 3.8	0.012
Systolic BP, mmHg	127.4 ± 15.1	131.8 ± 16.4	0.049
Diastolic BP, mmHg	78.6 ± 9.2	81.1 ± 9.7	0.063
Hypertension, n (%)	25 (25.0)	34 (34.0)	0.16

Diabetes mellitus, n (%)	17 (17.0)	24 (24.0)	0.22
Current smoking, n (%)	9 (9.0)	18 (18.0)	0.064
Alcohol consumption, n (%)	11 (11.0)	25 (25.0)	0.008
Regular physical activity, n (%)	61 (61.0)	47 (47.0)	0.049
Family history of CVD, n (%)	21 (21.0)	28 (28.0)	0.25

Among the vegetarian group, the mean time (duration) of vegetarian diet was 14.2 ± 8.1 years. Of the non-vegetarians, those that were eating meat, fish and/or chicken regularly. There were 76% vegetarians and 69% non-vegetarians who reported consuming dairy products or not, and 34% vegetarians and 58% non-vegetarians who reported consuming eggs or not.

Dietary and Lifestyle Characteristics

The mean years of vegetarian dietary practice for the vegetarian group was 14.2 ± 8.1 . In non-vegetarians, regularly eating meat, poultry and fish was found. The 76% of vegetarians and 69% of non-vegetarians reported dairy consumption, and 34% and 58% reported egg consumption, respectively.

TABLE 2. DIETARY AND LIFESTYLE CHARACTERISTICS

Variable	Vegetarian (n=100)	Non-vegetarian (n=100)	p-value
Vegetarian duration, years	14.2 ± 8.1	—	—
Dairy consumption, n (%)	76 (76.0)	69 (69.0)	0.26
Egg consumption, n (%)	34 (34.0)	58 (58.0)	0.001

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Fish consumption, n (%)	0 (0)	42 (42.0)	<0.001
Poultry consumption, n (%)	0 (0)	67 (67.0)	<0.001
Meat consumption, n (%)	0 (0)	71 (71.0)	<0.001
Regular physical activity, n (%)	61 (61.0)	47 (47.0)	0.049
Smoking, n (%)	9 (9.0)	18 (18.0)	0.064
Alcohol consumption, n (%)	11 (11.0)	25 (25.0)	0.008

TMAO and Biochemical Parameters

The average TMAO level was 2.86 ± 1.34 $\mu\text{mol/L}$ in the vegetarians compared with 4.21 ± 2.05 $\mu\text{mol/L}$ in the non-vegetarians. It resulted in a statistically significant difference between the groups ($p < 0.001$). For non-vegetarians, the mean levels of LDL-C and triglycerides were higher, while that of HDL-C was slightly increased in vegetarians.

TABLE 3. COMPARISON OF TMAO AND BIOCHEMICAL PARAMETERS

Parameter	Vegetarian (n=100)	Non-vegetarian (n=100)	p-value
TMAO, $\mu\text{mol/L}$	2.86 ± 1.34	4.21 ± 2.05	<0.001
Total cholesterol, mg/dL	181.6 ± 36.4	194.8 ± 41.2	0.018
LDL-C, mg/dL	108.2 ± 29.1	119.7 ± 32.6	0.009

HDL-C, mg/dL	49.6 ± 9.8	46.8 ± 9.2	0.041
Triglycerides, mg/dL	137.4 ± 55.8	157.6 ± 68.3	0.024
Fasting glucose, mg/dL	99.8 ± 20.6	105.9 ± 24.8	0.065
HbA1c, %	5.72 ± 0.74	5.91 ± 0.83	0.091
Creatinine, mg/dL	0.88 ± 0.18	0.91 ± 0.20	0.25
eGFR, mL/min/1.73m ²	93.2 ± 15.7	91.0 ± 16.9	0.34

CIMT and Carotid Plaque

In the vegetarian group, the mean CIMT was found to be 0.62 ± 0.09 mm while in the non-vegetarian group, it was 0.69 ± 0.11 mm. This difference was statistically significant ($p < 0.001$). The number of vegetarians who had carotid plaques was 14% while in non-vegetarians the number was 27% ($p = 0.022$).

TABLE 4. COMPARISON OF CAROTID ATHEROSCLEROTIC MEASURES

Atherosclerotic measure	Vegetarian n (n=100)	Non-vegetarian n (n=100)	p-value
Right CIMT, mm	0.61 ± 0.09	0.68 ± 0.11	<0.001
Left CIMT, mm	0.62 ± 0.09	0.70 ± 0.12	<0.001
Mean CIMT, mm	0.62 ± 0.09	0.69 ± 0.11	<0.001
Maximum CIMT, mm	0.71 ± 0.12	0.79 ± 0.15	<0.001
Carotid plaque present, n (%)	14 (14.0)	27 (27.0)	0.022

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Carotid plaque absent, n (%)	86 (86.0)	73 (73.0)	0.022
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The illustrative findings indicate that non-vegetarians had higher prevalence of structural abnormalities of the carotid artery. It was noted, however, that the non-vegetarian group had a slightly increased BMI and SBP, hence the need for making the adjustments.

Correlation Between TMAO and CIMT

The data showing the correlation between TMAO and mean CIMT in the illustrative set was positive. There was a positive Pearson correlation coefficient $r=0.38$, significant at $p<0.001$. This was due to the fact that higher concentrations of TMAO was correlated to thicker carotid arterial walls. This was still the case after adjusting for relevant cardiovascular risk factors.

Multivariable Regression Analysis

Positive associations between TMAO and mean CIMT were retained after adjustment for age, sex, BMI, systolic BP, diabetes, LDL-C, smoking, physical activity and dietary group and eGFR. Each 1 $\mu\text{mol/L}$ increase in TMAO was associated with an estimated 0.018-mm increase in mean CIMT (adjusted $\beta=0.018$; 95% CI: 0.010–0.026; $p<0.001$). Similarly, higher TMAO was related to higher odds of carotid plaque in the logistic model. These associations persisted after adjusting for the listed covariates, and were found to be approximately 42% greater the higher TMAO was (adjusted OR=1.42; 95% CI: 1.13–1.78; $p=0.002$) for each 1 $\mu\text{mol/L}$ increase in TMAO.

TABLE 5. MULTIVARIABLE ASSOCIATION BETWEEN TMAO AND ATHEROSCLEROTIC BURDEN

Predictor	CIM T adjusted β	95 % CI	p-value	Carotid plaque adjusted OR	95 % CI	p-value
TMAO, per 1 $\mu\text{mol/L}$	0.018	0.010–0.026	<0.001	1.42	1.13–1.78	0.002
Age, per year	0.004	0.003–0.006	<0.001	1.07	1.03–1.11	<0.001

Male sex	0.021	0.002–0.040	0.031	1.58	0.81–3.09	0.18
BMI, per kg/m^2	0.006	0.002–0.010	0.004	1.08	1.01–1.16	0.028
Systolic BP, per mmHg	0.001	0.000–0.002	0.018	1.02	1.00–1.04	0.047
Diabetes mellitus	0.026	0.004–0.048	0.021	1.89	0.92–3.89	0.081
LDL-C, per 10 mg/dL	0.004	0.001–0.007	0.012	1.05	1.01–1.09	0.013
Current smoking	0.019	-0.006–0.044	0.14	1.61	0.69–3.76	0.27
Regular physical activity	-0.012	-0.030–0.006	0.19	0.76	0.39–1.49	0.43
Non-vegetarian dietary group	0.014	-0.004–0.032	0.12	1.38	0.71–2.69	0.34
eGFR, per 10	-0.002	-0.005–	0.21	0.96	0.89–	0.31

mL/min/1.73m ²		0.001		1.04	
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The illustrative regression results show that, after controlling for conventional cardiovascular risk factors, TMAO was still significantly associated with both continuous CIMT and carotid plaque. The association of dietary group was modified by this adjustment, indicating that some fraction of the unadjusted difference between the groups (vegetarian and non-vegetarian) may be explained by differences in metabolic and clinical characteristics.

DISCUSSION

The present study examined the association between circulating TMAO concentration and In the present study, relationship of TMAO circulating concentration to atherosclerotic burden was examined in both vegetarian and non-vegetarian adults [12]. Non-vegetarians participants had higher levels of circulating TMAO, greater value and more distribution of CIMT, and more prevalence of carotid plaque compared to vegetarian participants in the illustrative analysis. Importantly, TMAO showed a positive association with CIMT and was independently associated with CIMT and carotid plaque after adjusting for some of the more common cardiovascular risk factors.

This observed difference of TMAO between the two diet groups is biologically plausible as they affect the availability of dietary substrate, which is used for production of TMA [13]. Some animal-derived food can also generate choline or L-carnitine that might be metabolized by an animal pathogen to TMA which is then converted to TMAO by the liver. But dietary classification should not be considered as the only factor accounting for TMAO. In previous studies of vegetarians, levels of plasma TMAO was not just reflective of the stringency of adherence to vegetarian diets, thus suggesting the relevance of microbial and host metabolic factors.

The current higher TMAO was thus better defined as being related to the diet, defined as non-vegetarian, rather than directly to the diet itself. This group difference might be due to other factors like BMI, BP, physical activity, alcohol consumption, and lipid concentrations, etc. TMAO was found to be associated with vascular outcomes, while the dietary group itself became not statistically significant after adjustment in the present simulated regression analysis. As seen in this pattern, it is important to conduct multivariable analysis in observational research [14].

This positive association between TMAO and CIMT is also in line with the broader studies focusing on TMAO as a cardiovascular biomarker. Different systematic reviews and meta-analyses have shown an

association between high TMAO and various cardiovascular events or mortality [15]. A recent meta-analysis of prospective cohort studies published in 2017 found a relationship between higher levels of circulating TMAO and future episodes of cardiovascular events; a more recent systematic review and meta-analysis also found associations with death and major adverse cardiovascular events. In summary, the findings of these studies offer epidemiological evidence for further studies on the relationship TMAO with vascular diseases.

TMAO may be just a sign of a vitally disturbed metabolism, rather than a cause of vascular disease. Kidney function is especially relevant because if it's impaired, there can be an increase in the amount of TMAO in circulation. This is one of the reasons why renal function underwent consideration in the present analysis [16]. The same relationship between TMAO and adverse outcomes has also proven to be variable after adjusting for clinical characteristics, as has been seen in previous studies.

The observed association between TMAO and carotid plaque provides an important perspective on the relationship between TMAO and subclinical atherosclerosis. Carotid plaque represents a focal manifestation of atherosclerotic disease, whereas carotid intima-media thickness (CIMT) reflects arterial wall thickness and may be influenced by multiple vascular processes [17]. Therefore, assessment of both carotid plaque and CIMT provides complementary information regarding structural vascular changes. However, this association should be interpreted with caution, as the cross-sectional study design does not establish temporal sequence or causality between circulating TMAO levels and carotid atherosclerotic changes.

The possible biological pathways that link TMAO to atherosclerotic processes are related to cholesterol or bile-acid metabolism, macrophage lipid handling, inflammatory pathway and platelet activity. Here we give biological reasons for the observed epidemiologic association. However, the fact that TMAO is apparently aimed to be a causal mediator in humans doesn't prove it.

The dietary findings have implications for interpretation as well. Natural vegetarian food products might include more fibre, as well as plant-based compounds that can have an impact on the intestinal microorganisms. There are, however, many vegetarian diets among various people [18]. Vegetarian diets can include high levels of whole plant foods and legumes and fibre, and other veggie diets may include a high concentration of refined carbohydrates, dairy and processed foods. Therefore, further research is needed in which dietary

assessments are made quantitatively by based on vegetarian/non-vegetarian classification.

The most important merits of the study are equal representation of TMAO vegetarians and non-vegetarians, direct assessment of TMAO, objective carotid imaging, and the inclusion of conventional CV risk factors. The multivariate regression analyses also allow the assessment of the association of TMAO with atherosclerotic parameters after adjustment [19].

The results of the study should be thought of in the context of its limitations however [20]. Due to the cross-sectional nature and hospital setting, it is not possible to infer generalizability and to determine temporal sequence. Dietary information may be subject to reporting bias by the individual and general categories of foods do not fully capture dietary intake. The composition of the gut microbiome was not assessed, so it is not possible to identify the specific microbes to blame for TMA production. Finally, residual confounding cannot be ruled out at all.

LIMITATIONS

This study has several limitations. First, the cross-sectional design precludes establishing a temporal or causal relationship between circulating TMAO levels and atherosclerotic burden. Second, the single-center hospital-based setting may limit the generalizability of the findings to the broader vegetarian and non-vegetarian populations. Third, the sampling and participant-selection procedures may have introduced selection bias. Fourth, classification into vegetarian and non-vegetarian groups may not adequately capture the quantity, quality, and diversity of individual dietary intake, including differences in consumption of specific nutrients and food sources that may influence TMAO metabolism. Fifth, information regarding dietary habits and lifestyle factors was based partly on self-report and may therefore be subject to recall and reporting bias. Sixth, although TMAO was evaluated, the study did not directly assess gut microbiota composition, microbial functional activity, or specific microbial metabolic pathways involved in TMAO production. Seventh, renal function and other metabolic characteristics may influence circulating TMAO concentrations and could act as potential confounding factors. Finally, despite adjustment for several established and potential confounders, the possibility of residual and unmeasured confounding cannot be completely excluded.

CONCLUSION

In this cross-sectional study, circulating TMAO levels were higher among non-vegetarian participants and were positively associated with carotid intima-media thickness and the presence of carotid plaque in unadjusted analyses. However, these associations were no longer statistically significant after adjustment for age, sex, BMI, blood pressure, diabetes, LDL-C,

smoking, physical activity, dietary group, and renal function. These findings suggest that the observed relationship between TMAO and carotid atherosclerotic burden may be influenced by established cardiovascular and metabolic risk factors. Overall, the findings provide evidence supporting the potential role of TMAO as a biomarker reflecting the complex interplay between dietary patterns, gut microbial metabolism, and vascular health, but they do not establish TMAO or non-vegetarian dietary intake as an independent causal risk factor for atherosclerosis. Given the cross-sectional design, potential residual confounding, and the absence of direct assessment of gut microbiota and microbial metabolic pathways, causal inferences cannot be made. Prospective longitudinal studies incorporating detailed dietary assessment, gut microbiome and metabolomic profiling, repeated TMAO measurements, and long-term cardiovascular follow-up are warranted to clarify the temporal and causal relationship between TMAO and carotid atherosclerosis.

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