Relation of Circulating Trimethylamine N-Oxide With Coronary Atherosclerotic Burden in Patients With ST-segment Elevation Myocardial Infarction

Zhaoxue Sheng, MD[#], Yu Tan, MD[#], Chen Liu, MD, Peng Zhou, MD, Jiannan Li, MD, Jinying Zhou, MS, Runzhen Chen, MS, Yi Chen, MD, Li Song, MD, Hanjun Zhao, MD, and Hongbing Yan, MD, PhD*

The gut microbial metabolite trimethylamine N-oxide (TMAO) promotes atherosclerosis and cardiovascular diseases. TMAO levels are associated with the coronary atherosclerotic burden in patients with stable coronary artery disease. However, the relation between TMAO levels and the coronary atherosclerotic burden in patients with ST-segment elevation myocardial infarction (STEMI) is unknown. We prospectively enrolled 2 cohorts in this study, including 335 patients with STEMI and 53 healthy controls. The coronary atherosclerotic burden was quantified by the number of diseased coronary arteries and the SYNTAX score. The median TMAO levels in patients with STEMI and healthy controls were 2.18 (interquartile range [IQR]: 1.34 to 3.90) μM and 1.23 [IQR: 0.84 to 2.42] μM, respectively. Of the 335 patients with STEMI, TMAO levels were significantly higher in the multivessel disease group than in the single-vessel disease group ($p < 0.001$) and in the group with intermediate-high SYNTAX scores (SYNTAX score ≥23) than in the group with low SYNTAX scores (SYNTAX score ≤22; $p < 0.001$). Based on the ordinal logistic regression analysis adjusted for traditional risk factors, elevated TMAO levels predicted both a high SYNTAX score (adjusted odds ratio [OR]: 1.16; 95% confidence interval [CI] 1.06 to 1.29; $p = 0.001$) and the presence of multivessel disease (adjusted OR: 1.15; 95% CI 1.01 to 1.32; $p = 0.035$). In conclusion, plasma TMAO levels are associated with a high coronary atherosclerotic burden in patients with STEMI. **(Am J Cardiol 2019;123:894−898)**

Cardiovascular disease (CVD) remains the leading cause of mortality worldwide.[1] Despite sufficient attention to traditional risk factors and the widespread use of modern pharmacotherapies, CVD-associated mortality and morbidity have been reduced by only approximately 30%,[1] indicating an urgent demand for a deeper understanding of CVD pathogenesis and more effective preventative measures for CVD. According to recent metabolomics studies, the gut microbiota contribute to and/or are associated with cardiometabolic diseases.[2−5] Remarkably, trimethylamine N-oxide (TMAO), a gut microbial metabolite, exerts a proatherogenic effect[3,6] and is associated with cardiovascular risk.[3,6−11] Plasma TMAO levels were recently shown to be an independent predictor of a high atherosclerotic burden in patients with stable coronary artery disease (CAD).[7] However, the relation between plasma TMAO levels and the coronary atherosclerotic burden has not been investigated in patients with acute myocardial infarction. In this study, we aimed to examine the association of plasma TMAO levels with the extent of CAD, as quantified by the number of diseased coronary vessels, and with coronary atherosclerotic lesion complexity and burden, as quantified by the SYNTAX score, in patients with ST-segment elevation myocardial infarction (STEMI).

Methods

We prospectively enrolled 2 cohorts in this study. The first cohort comprised 416 consecutively enrolled patients with STEMI (age ≥18 years; symptom onset ≤12 hours before presentation) who underwent emergency coronary angiography and primary percutaneous coronary intervention (PCI) from March 2017 to April 2018 at Fuwai Hospital. STEMI was defined as continuous chest pain lasting >30 minutes, ST-segment elevation >0.1 mV in at least 2 contiguous leads or a new left bundle-branch block on an 18-lead electrocardiogram (ECG), and an elevated troponin I level.[12] Patients with a history of PCI, coronary artery bypass grafting, and end-stage renal disease were excluded. Finally, 335 patients with STEMI were included in our study. The second cohort examined was an independent set of 53 individuals (age ≥18 years) without known cardiovascular diseases who were prospectively recruited from health screens solely for the purpose of providing reference interval values for TMAO levels. This study was performed in accordance with the Declaration of Helsinki and was

Department of Cardiology, Fuwai Hospital, National Center for Cardiovascular Diseases, Peking Union Medical College & Chinese Academy of Medical Sciences, Beijing, China. Manuscript received October 3, 2018; revised manuscript received and accepted December 7, 2018.

Funding: This study was supported by the Chinese Academy of Medical Sciences Innovation Fund for Medical Sciences (2016-I2M-1-009), Beijing, China.

[#]These authors contributed equally to this manuscript.

*Corresponding author: Tel: 86-13701339287.

E-mail address: hbyanfuwai@aliyun.com (H. Yan).

0002-9149/
https://doi.org/10.1016/j.amjcard.2018.12.018

approved by the Ethics Committee of Fuwai Hospital. All subjects provided written informed consent.

All patients received standard-of-care therapy according to institutional guidelines, namely oral treatment with 300 mg of aspirin (followed by 75 to 100 mg daily) and 180 mg of ticagrelor (followed by 90 mg twice daily for ≥12 months) or 600 mg of clopidogrel (followed by 75 mg daily for ≥12 months), and intravascular infusions of 70 to 100 IU/kg of unfractionated heparin before PCI. Infusions of GP IIb/IIIa receptor inhibitors were administered if necessary. The SYNTAX score was separately evaluated by 2 experienced interventional cardiologists who were blinded to the TMAO levels and clinical data using the SYNTAX score calculator version 2.28 provided by the official SYNTAX score website (www.syntaxscore.com/calculator/syntaxscore/frameset.htm). When a discrepancy between the 2 results occurred, the opinion of a senior interventional cardiologist was accepted. All patients were stratified into 2 groups according to their SYNTAX score: the group with low SYNTAX scores (SYNTAX score ≤22) and the group with intermediate-high SYNTAX scores (SYNTAX score ≥23). Single-vessel disease (SVD) or multiple-vessel disease (MVD) was defined as one or more major coronary vessels exhibiting ≥50% stenosis on diagnostic coronary angiography, without considering left main artery disease.

Blood samples were collected using vacutainer tubes containing EDTA via radial or femoral access before heparinization and then immediately centrifuged and stored at −80°C until analysis. Plasma TMAO levels were measured using stable isotope dilution high-performance liquid chromatography with online tandem mass spectrometry on an API 3200 triple quadrupole mass spectrometer (AB SCIEX, Framingham, MA).[13] The estimated glomerular filtration rate (eGFR) was calculated using the modified Diet in Renal Disease study equation.[14]

Continuous data are presented as means ± standard deviations (SD) or medians (interquartile ranges [IQR]). Comparisons between 2 groups were performed using Student's *t* test or the Mann-Whitney *U* test. Categorical variables are presented as numbers and percentages. Comparisons of the frequency between 2 groups were performed using Pearson's Chi-square tests or Fisher's exact tests. Pearson's and Spearman's correlation analyses were used to examine the correlations between TMAO levels and SYNTAX scores and the numbers of diseased coronary vessels. Ordinal logistic regression analyses with adjustments for traditional risk factors (including age, gender, hypertension, diabetes mellitus, smoking, low-density lipoprotein cholesterol level, high-density lipoprotein cholesterol level, and triglyceride level), CRP level, eGFR, and body mass index (BMI) were used to determine the association of TMAO with an elevated SYNTAX score and the presence of MVD. The area under the receiver-operating characteristic curve (AUC) was calculated to evaluate the ability of TMAO to predict a high atherosclerotic burden. A two-tailed p value <0.05 was considered statistically significant.

Results

We first performed a cross-sectional comparison of TMAO concentrations between the study cohort of patients with STEMI and the independent set of 53 prospectively recruited healthy controls (Supplemental Table). Significantly higher median TMAO levels were observed in patients with STEMI than in healthy controls (Figure 1 and Supplemental Table).

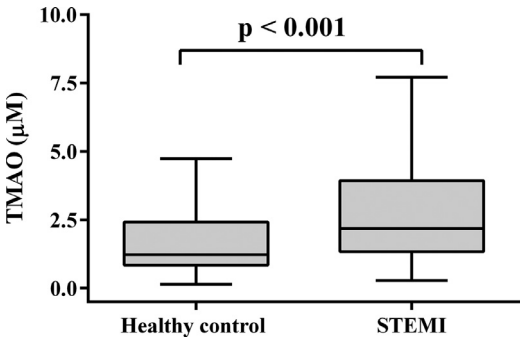

Figure 1. Comparison of TMAO levels between patients with STEMI and healthy controls. STEMI, ST-segment elevation myocardial infarction; TMAO, trimethylamine N-oxide.

Baseline characteristics of the study cohort of 335 patients with STEMI stratified by SYNTAX score are displayed in Table 1. The mean age was 58.7 years, and 80.6% of the patients were male; 58.2% had hypertension, and 27.5% had diabetes. Ninety-one patients (27.2%) had SVD and 244 (72.8%) patients had MVD. The median SYNTAX score was 18.0 (IQR: 11.0 to 23.5), and 242 (72.2%) and 93 (27.8%) patients had low and intermediate-high SYNTAX scores, respectively. A comparison of the baseline characteristics between the groups with low and intermediate-high SYNTAX scores showed that patients with intermediate-high SYNTAX scores were more likely to be older and have a lower eGFR, but higher glycosylated hemoglobin and CRP levels than patients with low SYNTAX scores. However, gender, histories of diabetes mellitus and hypertension, smoking, and BMI were similar between the 2 groups (Table 1). Remarkably, significantly higher TMAO levels were observed in the MVD group than in the SVD group (2.46 [IQR: 1.47 to 3.99] μM vs 1.65 [IQR: 1.01 to 3.09] μM, p <0.001) and in the group with intermediate-high SYNTAX scores than in the group with low SYNTAX scores (Table 1 and Figure 2). Moreover, TMAO levels were significantly higher with an increasing SYNTAX score tertiles (a low score defined as ≤22 vs an intermediate score as 23 to 32 vs a high score as ≥33; Supplemental Figure).

Plasma TMAO levels were significantly correlated with both the SYNTAX score (Pearson's correlation coefficient: r = 0.237, p <0.001) and the number of diseased vessels (Spearman's correlation coefficient: r = 0.192, p <0.001). According to the receiver operating characteristic curve (ROC) analysis, the AUC of TMAO levels in discriminating a high coronary atherosclerotic burden (SYNTAX score ≥23) was 0.656 (95% confidence interval [CI] 0.591 to 0.722, p <0.001; Figure 3). Based on the ordinal logistic regression analysis adjusted for traditional risk factors, BMI, CRP level, and eGFR, elevated TMAO levels were independently associated with intermediate-high SYNTAX scores and the presence of MVD (Table 2).

Table 1.
Baseline characteristics of subjects stratified by SYNTAX score

Variables	Total (n = 335)	SYNTAX Score		p Value
		≤22 (n = 242)	≥23 (n = 93)	
Age (years)	58.7 ± 12.1	57.5 ± 11.7	62.1 ± 12.4	0.001
Men	270 (80.6%)	192 (79.3%)	78 (83.9%)	0.361
Body mass index (kg/m^2)	26.0 ± 3.6	26.1 ± 3.5	25.7 ± 4.0	0.355
Diabetes mellitus	92 (27.5%)	61 (25.2%)	31 (33.3%)	0.171
Hypertension	195 (58.2%)	136 (56.2%)	59 (63.4%)	0.266
Hyperlipidemia	251 (74.9%)	181 (74.8%)	70 (75.3%)	1.000
Previous myocardial infarction	18 (5.4%)	12 (5.0%)	6 (6.5%)	0.593
Ischemic stroke	39 (11.6%)	24 (9.9%)	15 (16.1%)	0.129
Smoker	222 (66.3%)	162 (66.9%)	60 (64.5%)	0.700
White blood cells (10^6/L)	10.9 ± 3.6	10.8 ± 3.5	11.1 ± 4.1	0.497
Hemoglobin (g/L)	146 ± 18	147 ± 17	143 ± 19	0.043
Platelets (10^6/L)	235 ± 91	236 ± 98	231 ± 69	0.645
C-reactive protein (mg/L)	2.4 (1.0-5.1)	2.3 (1.0-4.4)	3.0 (1.3-7.1)	0.036
Erythrocyte sedimentation rate (mm/h)	8 (4-16)	7 (4-14)	16 (5-23)	0.044
Estimated glomerular filtration rate (mL/min/1.73 m^2)	94.94 (77.78-109.81)	96.76 (82.55-111.92)	83.40 (70.35-103.41)	<0.001
Glycosylated hemoglobin (%)	6.5 ± 1.5	6.4 ± 1.4	6.8 ± 1.7	0.035
Triglyceride (mg/dl)	122.19 (86.33-175.30)	124.40 (89.20-175.25)	115.99 (77.92-176.87)	0.642
Low-density lipoprotein cholesterol (mg/dl)	112.72 (90.10-133.02)	114.07 (91.26-130.80)	107.70 (87.68-134.67)	0.638
High-density lipoprotein cholesterol (mg/dl)	40.21 (34.80-46.40)	40.99 (35.19-47.18)	39.06 (34.03-44.08)	0.047
Lipoprotein (a) (mg/L)	148.5 (66.7-313.0)	137.6 (58.4-313.5)	198.5 (85.8-318.7)	0.088
Trimethylamine N-oxide (μM)	2.18 (1.34-3.90)	1.93 (1.27-3.34)	3.34 (1.87-4.58)	<0.001

Continuous data are presented as means ± SD or medians (interquartile ranges), and categorical variables are presented as counts (%). Hyperlipidemia was defined as a fasting total cholesterol level ≥200 mg/dl and/or triglyceride level ≥150 mg/dl.

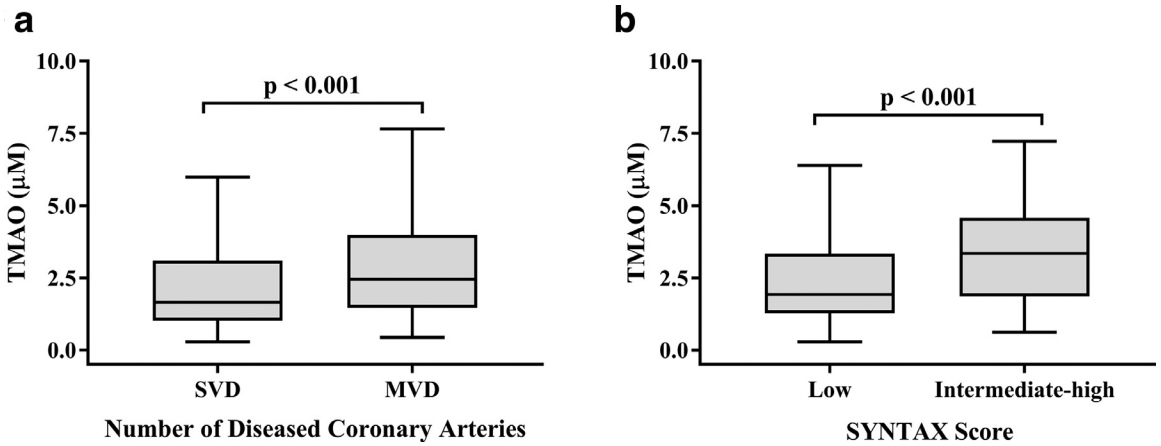

Figure 2. Relations between TMAO levels and the number of diseased vessels (a) and the SYNTAX score (b). MVD, multivessel disease; SVD, single-vessel disease; TMAO, trimethylamine N-oxide.

Discussion

Our study has 2 major findings: (1) plasma TMAO levels are significantly associated with the coronary atherosclerotic burden, as quantified by the number of diseased coronary vessels and the SYNTAX score, in patients with STEMI and (2) elevated TMAO levels serve as an independent predictor of a high SYNTAX score and the presence of MVD, even after adjusting for traditional risk factors.

The gut microbiota contribute to and/or are associated with many types of CVDs, such as atherosclerosis, heart failure, and hypertension.[15–17] Choline, a trimethylamine-containing compound and part of the head group of phosphatidylcholine, is metabolized by the gut microbiota to produce an intermediate compound known as trimethylamine (TMA), which is further rapidly oxidized by hepatic flavin monooxygenase 3 (FMO3) to form TMAO. Based on current evidence, dietary supplementation with TMAO in hyperlipidemic mice promotes atherosclerosis in a gut microbiota-dependent manner, suggesting that circulating TMAO levels are mechanistically involved in atherosclerosis and CVD pathogenesis.[2,6] Moreover, a study by Senthong et al confirmed that plasma TMAO levels were significantly associated with a high atherosclerotic burden, as determined by SYNTAX scores, in patients with stable CAD.[7] Our study extends these observations by showing

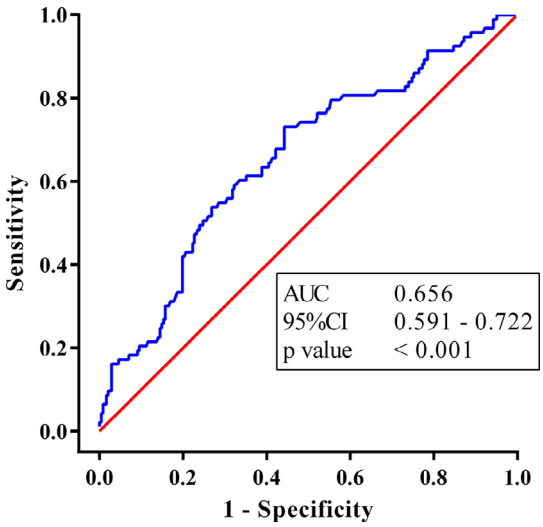

Figure 3. ROC curves of TMAO for predicting a high atherosclerotic burden. AUC, area under the receiver-operating characteristic curve; CI, confidence interval.

that circulating TMAO levels are an independent predictor of a high coronary atherosclerotic burden, as assessed by the presence of MVD and the SYNTAX score, in patients with STEMI. Notably, the median TMAO levels in patients with STEMI enrolled in our study were apparently lower than in patients with stable CAD reported in the study by Senthong (2.2 μM vs 5.5 μM).[7] However, a recent study[18] by Liu et al, in which all the subjects were Chinese and 66% of the patients had acute coronary syndrome, reported a comparable TMAO level to our study (1.53 μM vs 2.2 μM). This difference may be explained by different characteristics of the Eastern and Western populations, such as eating habits and basic metabolism, and further investigations are needed to clarify this issue. Moreover, the prevalence of MVD in our study was relatively higher than other STEMI cohorts (72.8% vs approximately 50%), which might be partially attributed to the higher proportion of patients with diabetes in our cohort than in the previous studies (27.5% vs approximately 16%).[19–21]

TMAO has historically been viewed as an osmolyte in the freeze-avoidance response of some species.[22] TMAO was recently shown to enhance atherosclerosis development in mice and contribute to an increased risk of major adverse cardiovascular events in humans.[2,6,8–11] According to Wang et al, dietary choline or TMAO supplementation increases the expression of scavenger receptors on macrophages and subsequently promotes foam cell formation.[6] Dietary sources of TMAO inhibit reverse cholesterol transport in macrophages.[2] Collectively, TMAO promotes an imbalance in cholesterol uptake and efflux from macrophages, leading to an abundance of proatherogenic foam cells migrating into the arterial wall. In addition, Warrier et al found that FMO3, by which the TMA produced by gut microbes is transformed to TMAO, is a master regulator of hepatic cholesterol and triacylglycerol metabolism, inflammation, and endoplasmic reticulum (ER) stress, implying that FMO3 also contributes to the development of atherosclerosis and the degree of atherosclerotic burden in patients with CAD.[23] Another potential mechanism involved in the relationship of TMAO with atherosclerotic burden is inflammation, which plays a pivotal role in the pathogenesis of atherosclerosis and its detrimental clinical ischemic complications.[24,25] TMAO is involved in increasing the expression of scavenger receptors on macrophages and in subsequently promoting foam cell formation.[6] Furthermore, TMAO induces vascular inflammation by activating the NLRP3 inflammasome and the mitogen-activated protein 14 kinase/nuclear factor-kappa B pathway.[26,27] TMAO is also associated with systemic inflammation by increasing the expression of various inflammatory factors, including TNF-α, sTNF-R p55, and sTNF-R p75.[28] Therefore, TMAO may promote focal and systemic inflammation, further contributing to the initiation and development of atherosclerosis.

Table 2.
Association of TMAO levels with the SYNTAX score and multivessel disease

	SYNTAX Score		Multivessel Disease	
	OR (95% CI)	p Value	OR (95% CI)	p Value
Unadjusted	1.17 (1.09-1.27)	<0.001	1.16 (1.04-1.30)	0.008
Adjusted*	1.16 (1.06-1.29)	0.001	1.15 (1.01-1.32)	0.035

* Adjusted for age, gender, triglyceride levels, high-density lipoprotein cholesterol levels, low-density lipoprotein cholesterol levels, diabetes mellitus, hypertension, smoking, body mass index, log C-reactive protein levels, and log eGFR.

This study has several limitations. First, it was a single-center study, and the number of subjects in our study, particularly the number of patients in the group with intermediate-high SYNTAX scores, was small. Therefore, selection bias cannot be excluded. Second, despite adjustments for traditional risk factors in our study to draw the final conclusions, other potential confounding factors, such as patients' nutritional status and recent diet before this attack, were not assessed and might also influence the results.

In conclusion, the plasma TMAO level is an independent predictor of a high atherosclerotic burden in patients with STEMI. Further investigations into the mechanisms by which elevated TMAO levels contribute to the coronary atherosclerotic burden and the value of TMAO levels in risk stratification of patients with CAD are warranted.

Conflicts of interest

The authors have no financial conflicts of interest.

Acknowledgment

The authors gratefully acknowledge all individuals who participated in this study and the editors at American Journal Experts for their help in editing the manuscript.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.1016/j.amjcard.2018.12.018.

1. Mozaffarian D, Benjamin EJ, Go AS, Arnett DK, Blaha MJ, Cushman M, Das SR, de Ferranti S, Despres JP, Fullerton HJ, Howard VJ, Huffman MD, Isasi CR, Jimenez MC, Judd SE, Kissela BM, Lichtman JH, Lisabeth LD, Liu S, Mackey RH, Magid DJ, McGuire DK, Mohler ER 3rd, Moy CS, Muntner P, Mussolino ME, Nasir K, Neumar RW, Nichol G, Palaniappan L, Pandey DK, Reeves MJ, Rodriguez CJ, Rosamond W, Sorlie PD, Stein J, Towfighi A, Turan TN, Virani SS, Woo D, Yeh RW, Turner MB. Heart Disease and Stroke Statistics-2016 Update: a report from the American Heart Association. *Circulation* 2016;133:e38–e360.
2. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, Britt EB, Fu X, Wu Y, Li L, Smith JD, DiDonato JA, Chen J, Li H, Wu GD, Lewis JD, Warrier M, Brown JM, Krauss RM, Tang WH, Bushman FD, Lusis AJ, Hazen SL. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med* 2013;19:576–585.
3. Li J, Zhao F, Wang Y, Chen J, Tao J, Tian G, Wu S, Liu W, Cui Q, Geng B, Zhang W, Weldon R, Auguste K, Yang L, Liu X, Chen L, Yang X, Zhu B, Cai J. Gut microbiota dysbiosis contributes to the development of hypertension. *Microbiome* 2017;5:14.
4. Tang WH, Wang Z, Li XS, Fan Y, Li DS, Wu Y, Hazen SL. Increased trimethylamine N-oxide portends high mortality risk independent of glycemic control in patients with type 2 diabetes mellitus. *Clin Chem* 2017;63:297–306.
5. Schugar RC, Shih DM, Warrier M, Helsley RN, Burrows A, Ferguson D, Brown AL, Gromovsky AD, Heine M, Chatterjee A, Li L, Li XS, Wang Z, Willard B, Meng Y, Kim H, Che N, Pan C, Lee RG, Crooke RM, Graham MJ, Morton RE, Langefeld CD, Das SK, Rudel LL, Zein N, McCullough AJ, Dasarathy S, Tang WHW, Erokwu BO, Flask CA, Laakso M, Civelek M, Naga Prasad SV, Heeren J, Lusis AJ, Hazen SL, Brown JM. The TMAO-producing enzyme flavin-containing monooxygenase 3 regulates obesity and the beiging of white adipose tissue. *Cell Rep* 2017;19:2451–2461.
6. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu X, Chung YM, Wu Y, Schauer P, Smith JD, Allayee H, Tang WH, DiDonato JA, Lusis AJ, Hazen SL. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* 2011;472:57–63.
7. Senthong V, Li XS, Hudec T, Coughlin J, Wu Y, Levison B, Wang Z, Hazen SL, Tang WH. Plasma trimethylamine N-oxide, a gut microbe-generated phosphatidylcholine metabolite, is associated with atherosclerotic burden. *J Am Coll Cardiol* 2016;67:2620–2628.
8. Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, Wu Y, Hazen SL. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *N Engl J Med* 2013;368:1575–1584.
9. Suzuki T, Heaney LM, Jones DJ, Ng LL. Trimethylamine N-oxide and risk stratification after acute myocardial infarction. *Clin Chem* 2017;63:420–428.
10. Li XS, Obeid S, Klingenberg R, Gencer B, Mach F, Raber L, Windecker S, Rodondi N, Nanchen D, Muller O, Miranda MX, Matter CM, Wu Y, Li L, Wang Z, Alamri HS, Gogonea V, Chung YM, Tang WH, Hazen SL, Luscher TF. Gut microbiota-dependent trimethylamine N-oxide in acute coronary syndromes: a prognostic marker for incident cardiovascular events beyond traditional risk factors. *Eur Heart J* 2017;38:814–824.
11. Senthong V, Wang Z, Li XS, Fan Y, Wu Y, Tang WH, Hazen SL. Intestinal microbiota-generated metabolite trimethylamine-N-oxide and 5-year mortality risk in stable coronary artery disease: the contributory role of intestinal microbiota in a COURAGE-like patient cohort. *J Am Heart Assoc* 2016;5:e002816.
12. Ibanez B, James S, Agewall S, Antunes MJ, Bucciarelli-Ducci C, Bueno H, Caforio ALP, Crea F, Goudevenos JA, Halvorsen S, Hindricks G, Kastrati A, Lenzen MJ, Prescott E, Roffi M, Valgimigli M, Varenhorst C, Vranckx P, Widimsky P. 2017 ESC guidelines for the management of acute myocardial infarction in patients presenting with ST-segment elevation: the Task Force for the management of acute myocardial infarction in patients presenting with ST-segment elevation of the European Society of Cardiology (ESC). *Eur Heart J* 2018;39:119–177.
13. Wang Z, Levison BS, Hazen JE, Donahue L, Li XM, Hazen SL. Measurement of trimethylamine-N-oxide by stable isotope dilution liquid chromatography tandem mass spectrometry. *Anal Biochem* 2014;455:35–40.
14. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. *Ann Intern Med* 1999;130:461–470.
15. Santisteban MM, Qi Y, Zubcevic J, Kim S, Yang T, Shenoy V, Cole-Jeffrey CT, Lobaton GO, Stewart DC, Rubiano A, Simmons CS, Garcia-Pereira F, Johnson RD, Pepine CJ, Raizada MK. Hypertension-linked pathophysiological alterations in the gut. *Circ Res* 2017;120:312–323.
16. Koren O, Spor A, Felin J, Fak F, Stombaugh J, Tremaroli V, Behre CJ, Knight R, Fagerberg B, Ley RE, Backhed F. Human oral, gut, and plaque microbiota in patients with atherosclerosis. *Proc Natl Acad Sci U S A* 2011;108(Suppl 1):4592–4598.
17. Sandek A, Bauditz J, Swidsinski A, Buhner S, Weber-Eibel J, von Haehling S, Schroedl W, Karhausen T, Doehner W, Rauchhaus M, Poole-Wilson P, Volk HD, Lochs H, Anker SD. Altered intestinal function in patients with chronic heart failure. *J Am Coll Cardiol* 2007;50:1561–1569.
18. Liu X, Xie Z, Sun M, Wang X, Li J, Cui J, Zhang F, Yin L, Huang D, Hou J, Tian J, Yu B. Plasma trimethylamine N-oxide is associated with vulnerable plaque characteristics in CAD patients as assessed by optical coherence tomography. *Int J Cardiol* 2018;265:18–23.
19. Park DW, Clare RM, Schulte PJ, Pieper KS, Shaw LK, Califf RM, Ohman EM, Van de Werf F, Hirji S, Harrington RA, Armstrong PW, Granger CB, Jeong MH, Patel MR. Extent, location, and clinical significance of non-infarct-related coronary artery disease among patients with ST-elevation myocardial infarction. *JAMA* 2014;312:2019–2027.
20. Dziewierz A, Siudak Z, Rakowski T, Zasada W, Dubiel JS, Dudek D. Impact of multivessel coronary artery disease and noninfarct-related artery revascularization on outcome of patients with ST-elevation myocardial infarction transferred for primary percutaneous coronary intervention (from the EUROTRANSFER Registry). *Am J Cardiol* 2010;106:342–347.
21. Sorajja P, Gersh BJ, Cox DA, McLaughlin MG, Zimetbaum P, Costantini C, Stuckey T, Tcheng JE, Mehran R, Lansky AJ, Grines CL, Stone GW. Impact of multivessel disease on reperfusion success and clinical outcomes in patients undergoing primary percutaneous coronary intervention for acute myocardial infarction. *Eur Heart J* 2007;28:1709–1716.
22. Treberg JR, Wilson CE, Richards RC, Ewart KV, Driedzic WR. The freeze-avoidance response of smelt Osmerus mordax: initiation and subsequent suppression of glycerol, trimethylamine oxide and urea accumulation. *J Exp Biol* 2002;205:1419–1427.
23. Warrier M, Shih DM, Burrows AC, Ferguson D, Gromovsky AD, Brown AL, Marshall S, McDaniel A, Schugar RC, Wang Z, Sacks J, Rong X, Vallim TA, Chou J, Ivanova PT, Myers DS, Brown HA, Lee RG, Crooke RM, Graham MJ, Liu X, Parini P, Tontonoz P, Lusis AJ, Hazen SL, Temel RE, Brown JM. The TMAO-generating enzyme flavin monooxygenase 3 is a central regulator of cholesterol balance. *Cell Rep* 2015;10:326–338.
24. Raggi P, Genest J, Giles JT, Rayner KJ, Dwivedi G, Beanlands RS, Gupta M. Role of inflammation in the pathogenesis of atherosclerosis and therapeutic interventions. *Atherosclerosis* 2018;276:98–108.
25. Crea F, Libby P. Acute coronary syndromes: the way forward from mechanisms to precision treatment. *Circulation* 2017;136:1155–1166.
26. Chen ML, Zhu XH, Ran L, Lang HD, Yi L, Mi MT. Trimethylamine-N-oxide induces vascular inflammation by activating the NLRP3 Inflammasome through the SIRT3-SOD2-mtROS signaling pathway. *J Am Heart Assoc* 2017;6:e006347.
27. Seldin MM, Meng Y, Qi H, Zhu W, Wang Z, Hazen SL, Lusis AJ, Shih DM. Trimethylamine N-oxide promotes vascular inflammation through signaling of mitogen-activated protein kinase and nuclear factor-kappaB. *J Am Heart Assoc* 2016;5:e002767.
28. Rohrmann S, Linseisen J, Allenspach M, von Eckardstein A, Muller D. Plasma concentrations of trimethylamine-N-oxide are directly associated with dairy food consumption and low-grade inflammation in a German adult population. *J Nutr* 2016;146:283–289.