Human Nutrition & Metabolism 21 (2020) 200112

Contents lists available at ScienceDirect

Human Nutrition & Metabolism

journal homepage: www.journals.elsevier.com/human-nutrition-and-metabolism

# No effect of plasma trimethylamine N-Oxide (TMAO) and plasma trimethyllysine (TML) on the association between choline intake and acute myocardial infarction risk in patients with stable angina pectoris

Anthea Van Parys[a,*], Vegard Lysne[a,e,f], Jannike Øyen[c], Jutta Dierkes[b,d,f], Ottar Nygård[a,e,f]

[a] Centre for Nutrition, Department of Clinical Science, University of Bergen, Haukelandsbakken 15, 5009, Bergen, Norway
[b] Centre for Nutrition, Department of Clinical Medicine, University of Bergen, Haukelandsbakken 15, 5009, Bergen, Norway
[c] Institute of Marine Research, Nordnesgate 50, 5005, Bergen, Norway
[d] Department of Laboratory Medicine and Pathology, Haukeland University Hospital, Jonas Lies Vei 65, 5021, Bergen, Norway
[e] Department of Heart Disease, Haukeland University Hospital, Jonas Lies Vei 65, 5021, Bergen, Norway
[f] Mohn Nutrition Research Laboratory, University of Bergen, Haukelandsbakken 15, 5009, Bergen, Norway

## HIGHLIGHTS

- No effect modification by trimethylamine-N-oxide on the association between choline intake and acute myocardial infarction.
- No effect modification by trimethyllysine on the association between choline intake and acute myocardial infarction.
- No indirect effect of trimethylamine-N-oxide on the association between choline intake and acute myocardial infarction.

## ARTICLE INFO

*Keywords:*
Trimethylamine-N-oxide (TMAO)
Trimethyllysine (TML)
Choline
Myocardial infarction
Effect modification

## ABSTRACT

Plasma concentrations of trimethylamine N-oxide (TMAO) have been linked to cardiovascular disease (CVD) risk and mortality. TMAO is formed through the bacterial conversion of trimethylamine which is obtained either directly from food, generated from dietary precursors (e.g. choline) or derived from endogenous trimethyllysine (TML) production. In a previous article, we reported an increased risk of acute myocardial infarction with increased total choline intake in patients with stable angina pectoris. Due to the close link between TMAO, TML, choline metabolism and possibly CVD, we investigated whether plasma TMAO and TML modified the effect of total choline intake on acute myocardial infarction (AMI) risk in a post-hoc analysis. We found plasma TMAO and TML do not modify the association between higher dietary choline intake and increased AMI risk. Additionally, this association is not mediated via TMAO.

## 1. Introduction

Trimethylamine N-oxide (TMAO) is formed through bacterial conversion of trimethylamine (TMA) by flavin-monooxygenases in the liver. TMA is obtained directly from food (e.g. fish, which is also rich in TMAO) or generated from dietary precursors such as choline, choline-containing compounds, betaine and L-carnitine or generated from the L-carnitine metabolite gamma-butyrobetaine (γ-BB) [1,2]. Additionally, γ-BB can be formed endogenously from trimethyllysine (TML) then afterwards converted to carnitine and potentially to TMA and TMAO [3]. An overview of the pathways contributing to TMAO synthesis is provided in Fig. 1. TMAO and its precursors are mainly found in food items of animal origin such as fish, meat, eggs, poultry and milk [1].

In 2011, a link was reported between plasma TMAO and cardiovascular disease (CVD) risk [4]. Since then more evidence has accumulated supporting this association [1]. In animal models, TMAO seems to be proatherogenic [4]; in humans, increased plasma concentrations are associated with elevated risk of CVD and other diseases [1,2]. However, inconsistencies remain and whether or not increased plasma TMAO is causally related to CVD remains unclear [1,2].

We recently reported that higher dietary choline intake is associated with increased risk of incident acute myocardial infarction (AMI) in patients with stable angina pectoris (SAP) [5]. This increase could be attributed only to the lipid-soluble forms PC and sphingomyelin. Additionally, plasma TMAO was positively associated with higher dietary choline intake, while no association between choline intake and plasma

* Corresponding author. Centre for Nutrition, Haukelandsbakken 15, 5021, Bergen, Norway.
*E-mail address:* anthea.parys@uib.no (A. Van Parys).

https://doi.org/10.1016/j.hnm.2020.200112
Received 18 February 2020; Received in revised form 3 June 2020; Accepted 18 July 2020
Available online 29 August 2020

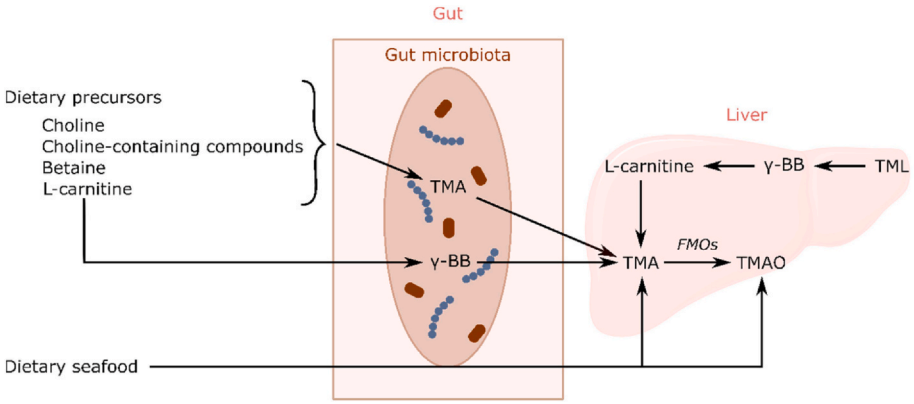


**Fig. 1. A general overview of various pathways contributing to trimethylamine N-oxide (TMAO) synthesis**. Trimethylamine (TMA) is formed in the intestinal lumen from metabolization of dietary precursors by gut microbiota. Additionally, γ-butyrobetaine (γ -BB) is formed by metabolization of dietary L-carnitine. Both TMA and TMAO can be directly obtained from seafood. After absorption in the liver, TMA is converted to TMAO by flavin monooxygenases (FMOs). Additionally, TMA can be formed endogenously via the formation of γ-BB from trimethyllysine (TML).

TML was observed. In the current post-hoc analysis, we wanted to investigate whether there was any effect modification by plasma TMAO or TML. We based this hypothesis on i) the recently discovered link between plasma TMAO and CVD risk and ii) recent reporting of carnitine synthesis from TML being an endogenous source of TMA, and thus TMAO.

## 2. Methods

Information on the collection of baseline data, follow-up, and study endpoints can be found in our original article [5].

### 2.1. Study cohort

In total 3090 adult patients from the Western Norway B-Vitamin Intervention Trial (WENBIT, NCT00354081) were included. This was a prospective, double-blind, placebo-controlled, secondary prevention study were participants were randomized to receive total homocysteine-lowering-B-vitamins. The study protocol has been described in detail elsewhere [6]. Only patients diagnosed with SAP were included (n = 2573). Patients with missing dietary data, including choline intake (n = 565), extreme energy intake (i.e. < 3000 kJ or > 15 000 kJ for women and < 3300 kJ or > 17 500 kJ for men) (n = 27), > 10 E% from alcohol (n = 48) and missing plasma TMAO and TML data (n = 17) were excluded, resulting in 1916 patients eligible for analyses.

The study was carried out according to the Declaration of Helsinki and approved by the Norwegian Data Inspectorate as well as the Regional Committee for Medical Health Research Ethics. All participants provided written informed consent.

### 2.2. Dietary assessment

A 169-item food frequency questionnaire (FFQ) was used to obtain information on dietary intake. The administered FFQ was an adaptation of an FFQ developed at the Department of Nutrition, University of Oslo designed to obtain information on habitual food intake of the Norwegian population over the past year. The FFQ has not been validated for choline intake and, therefore, we cannot assess how well it captures true choline intake, which is a limitation in the current analysis.

### 2.3. Biochemical analyses

Plasma TMAO and TML were measured using liquid chromatography-tandem mass spectrometry at Bevital AS (www.bevital.no).

### 2.4. Statistical analyses

Effect modification by TMAO or TML (continuous scale) was investigated by adding interaction product terms to a Cox regression model which estimated the association between total choline intake and risk of AMI. The model was adjusted for energy intake, age, sex, and smoking. Subgroup analyses were performed, stratifying for the original B vitamin intervention.

Mediation analyses quantify the proportion of an exposure-outcome association working via a mediator (a variable on the causal path between exposure and outcome) [7]. In this study, we applied a mediation analysis on the effect of dietary choline on AMI risk, considering TMAO as a mediator.

Statistical analyses were performed using R version 3.6.1 (The R Foundation for Statistical Computing, Vienna, Austria), the packages within the *tidyverse* and the *survival* and *medflex* package for survival and mediation analyses respectively. The mediation analyses were based on logistic regression analyses because survival models are currently not implemented in *medflex*. The estimates are expected to be similar, however, less powerful [7].

## 3. Results

The mean total energy-adjusted dietary choline intake was 287 mg/d. Detailed results are available in our original article [5]. Relevant baseline characteristics of the study population included in the current post-hoc analysis are provided in Supplementary Table 1. We previously reported a 10% increased risk of AMI per increment of 50 mg/d in energy-adjusted total choline intake during a median follow-up time (25th, 75th percentile) of 7.5 (6.3, 8.8) years [5]. We did not observe any effect modification according to baseline plasma TMAO and TML concentrations (Fig. 2). The interaction coefficient was 0.002 (standard error = 0.004, p = 0.567) for TMAO and −0.06 (standard error = −0.08, p = 0.417) for TML. Stratifying the analyses for the original B-vitamin intervention yielded generally identical results as in the full population (data not shown).

AMI indicates acute myocardial infarction; TMAO, trimethylamine N-oxide; TML, trimethyllysine.

Results from the mediation analyses show no indication that the choline effect was mediated via plasma TMAO (Table 1).

## 4. Discussion

In this population, no interaction effect was found between dietary choline and plasma TMAO or plasma TML regarding AMI risk. Further, we did not observe any indication of the choline effect being mediated through plasma TMAO.

Several observational and experimental studies suggested a positive correlation between plasma TMAO and CVD risk and mortality [1]. However, a clear mechanistic link has yet to be proven. Additionally, a recent study [8] failed to reproduce the results from the original paper [4] where TMAO increased atherosclerotic lesion size in mice. Hence,

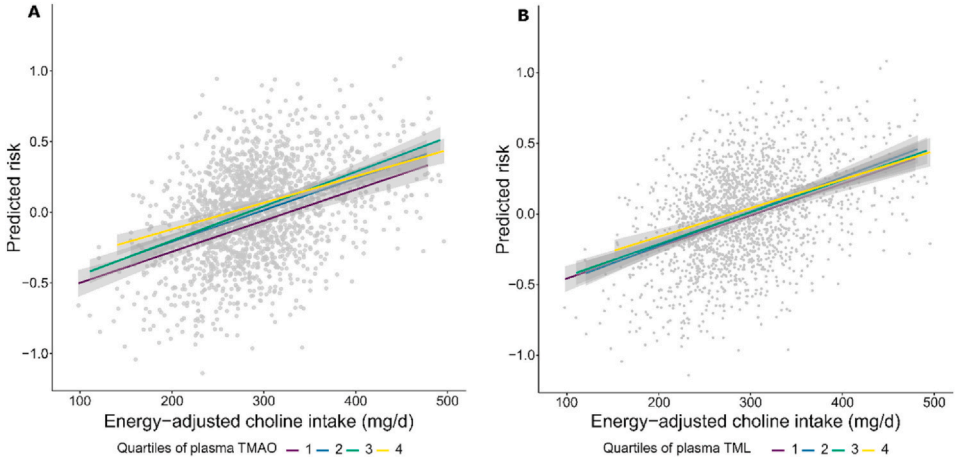


**Fig. 2.** Predicted AMI risk with increasing energy-adjusted total choline intake across quartiles of plasma TMAO (panel A) and TML (panel B).

**Table 1**
Summary of mediation analysis via plasma TMAO.

| | OR (95% CI) | *p* |
|---|---|---|
| Natural direct effect | 1.11 (1.01–1.21) | 0.027 |
| Natural indirect effect | 1.00 (1.00–1.01) | 0.608 |
| Total effect | 1.11 (1.02–1.21) | 0.022 |

CI indicates confidence interval; OR, odds ratio; TMAO, trimethylamine N-oxide.

the causal relation between plasma TMAO and CVD remains a point of discussion [1,2].

The results of our analyses indicate that the mechanism through which dietary choline is associated with increased AMI risk does not involve TMAO or TML. The hepatic choline metabolism suggests a possible interaction between endogenous choline synthesis and intake from the diet [9]. A choline-deficient diet increases the endogenous synthesis and distribution of choline from other tissues to the liver and the brain [10]. Whether the opposite takes place in the case of a choline excess, to the best of our knowledge, remains unclear. We can therefore only speculate that the balance between dietary choline and endogenous synthesis could be involved in the association between dietary choline and increased AMI risk. Additionally, extensive enterohepatic choline traffic further complicates the determination of the fate of the choline forms after absorption [10]. Thus far, the major part of research on choline and human health has focused on total choline intake [9]. More research is needed on absorption, digestion and metabolism of all choline forms to investigate possible mechanisms by which their intake could increase CVD risk.

## 5. Conclusion

Plasma TMAO and plasma TML levels do not modify the association between higher dietary choline intake and increased AMI risk. Additionally, this association is not mediated via TMAO.

## Funding

This project did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The work was conducted under the responsibility of the Department of Clinical Science, University of Bergen, Bergen, Norway.

## Author statement – Van Parys et al.

Anthea Van Parys: Formal analyses, writing – original draft.

Vegard Lysne: Formal analyses.

Vegard Lysne, Jutta Dierkes, Jannike Øyen, Ottar Nygård: writing – review & editing.

Jutta Dierkes, Jannike Øyen, Ottar Nygård: Supervision.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

AVP and VL analyzed data; AVP wrote the paper. All authors provided feedback and approved the final manuscript.

We wish to thank all WENBIT study personnel and participants.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.hnm.2020.200112.

## References

[1] S.H. Zeisel, M. Warrier, Trimethylamine N -oxide, the microbiome, and heart and kidney disease, Annu. Rev. Nutr. 37 (2017) 157–181, https://doi.org/10.1146/annurev-nutr-071816-064732.

[2] C.E. Cho, M.A. Caudill, Trimethylamine- N -Oxide : friend, foe, or simply caught in the cross-fire ? Trends Endocrinol. Metabol. 28 (2017) 121–130, https://doi.org/10.1016/j.tem.2016.10.005.

[3] K. Skagen, M. Trøseid, T. Ueland, S. Holm, A. Abbas, I. Gregersen, M. Kummen, V. Bjerkeli, F. Reier-Nilsen, D. Russell, A. Svardal, T.H. Karlsen, P. Aukrust, R.K. Berge, J.E.R. Hov, B. Halvorsen, M. Skjelland, The Carnitine-butyrobetaine-trimethylamine-N-oxide pathway and its association with cardiovascular mortality in patients with carotid atherosclerosis, Atherosclerosis 247 (2016) 64–69, https://doi.org/10.1016/j.atherosclerosis.2016.01.033.

[4] Z. Wang, E. Klipfell, B.J. Bennett, R. Koeth, B.S. Levison, B. Dugar, A.E. Feldstein, E.B. Britt, X. Fu, Y.-M. Chung, Y. Wu, P. Schauer, J.D. Smith, H. Allayee, W.H.W. Tang, J.A. DiDonato, A.J. Lusis, S.L. Hazen, Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease, Nature 472 (2011) 57–63, https://doi.org/10.1038/nature09922.Gut.

[5] A. Van Parys, V. Lysne, G.F.T. Svingen, P.M. Ueland, I. Dhar, J. Øyen, J. Dierkes, O.K. Nygård, Dietary choline is related to increased risk of acute myocardial infarction in patients with stable angina pectoris, Biochimie (2019), https://doi.org/10.1016/j.biochi.2019.11.001.

[6] M. Ebbing, Ø. Bleie, P.M. Ueland, J.E. Nordrehaug, D.W. Nilsen, S.E. Vollset, H. Refsum, E.K.R. Pedersen, O. Nygård, Mortality and cardiovascular events in patients treated with homocysteine-lowering, J. Am. Med. Assoc. 300 (2008) 795–804.

[7] T. Lange, K.W. Hansen, R. Sørensen, S. Galatius, Applied mediation analyses: a review and tutorial, Epidemiol. Health 39 (2017) e2017035, , https://doi.org/10.4178/epih.e2017035.

[8] P. Aldana-Hernández, K.-A. Leonard, Y.-Y. Zhao, J.M. Curtis, C.J. Field, R.L. Jacobs,

Dietary choline or trimethylamine N-oxide supplementation does not influence atherosclerosis development in Ldlr−/− and Apoe−/− male mice, J. Nutr. (2019) 1–7, https://doi.org/10.1093/jn/nxz214.
[9] E.D. Lewis, C.J. Field, R.L. Jacobs, Should the forms of dietary choline also be considered when estimating dietary intake and the implications for health? Lipid Technol. 27 (2015) 227–230, https://doi.org/10.1002/lite.201500048.
[10] Z. Li, D.E. Vance, Phosphatidylcholine and choline homeostasis, J. Lipid Res. 49 (2008) 1187–1194, https://doi.org/10.1194/jlr.R700019-JLR200.