**Title: Novel findings of the association between gut microbiota–derived metabolite trimethylamine N‐oxide (TMAO) and inflammation: Results from a systematic review and dose - response meta-analysis**

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**Key words:** Stroke, dose-response meta-analysis, trimethylamine *N*-oxide (TMAO), gut microbiota metabolite, risk factor.

**Supplementary Material**

**Sup. Table 1.** PRISMA Checklist (Moher et al. 2009)

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Section/topic** | **#** | | | **Checklist item** | **Reported on page #** |
| **TITLE** | | | | |  |
| Title | 1 | | | Identify the report as a systematic review, meta-analysis, or both. | Page 1, lines 2-3 |
| **ABSTRACT** | | | | |  |
| Structured summary | 2 | | | Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number. | Page 2, lines 28-45 |
| **INTRODUCTION** | | | | |  |
| Rationale | 3 | | | Describe the rationale for the review in the context of what is already known. | Page 3, 4 |
| Objectives | 4 | | | Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS). | Page 4, lines 84-86 |
| **METHODS** | | | | |  |
| Protocol and registration | 5 | Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number. | | | Page 4, lines 88- 90 |
| Eligibility criteria | 6 | Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale. | | | Page 5, lines 104- 112 |
| Information sources | 7 | Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched. | | | Page 4, lines 92-103 |
| Search | 8 | Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated. | | | Page 4, lines 92-103 |
| Study selection | 9 | State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis). | | | Page 5, lines 104-112 |
| Data collection process | 10 | Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators. | | | Page 6, Lines 113-127 |
| Data items | 11 | List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made. | | | Page 5, lines 104- 112 |
| Risk of bias in individual studies | 12 | Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis. | | | Page 6, lines 148-151 |
| Summary measures | 13 | State the principal summary measures (e.g., risk ratio, difference in means). | | | Page 6, lines 132-142 |
| Synthesis of results | 14 | Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I2) for each meta-analysis. | | | Pages 5-7; lines128-162 |
| Risk of bias across studies | 15 | | Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies). | | Page 9; Lines 221- 225 |
| Additional analyses | 16 | | Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified. | | Pages 5-7; lines128-162 |
| **RESULTS** | | | | |  |
| Study selection | 17 | | Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram. | | Page 7; Lines 163- 178 |
| Study characteristics | 18 | | For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations. | | Page 7; Lines 163- 178 |
| Risk of bias within studies | 19 | | Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12). | | Page 9; Lines 221- 225 |
| Results of individual studies | 20 | | For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot. | | Table 1 |
| Synthesis of results | 21 | | Present results of each meta-analysis done, including confidence intervals and measures of consistency. | | Table 2, figures 3-15 |
| Risk of bias across studies | 22 | | Present results of any assessment of risk of bias across studies (see Item 15). | | Sup. Figure 1 |
| Additional analysis | 23 | | Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]). | | Table 2 |
| **DISCUSSION** | | | | |  |
| Summary of evidence | 24 | | Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers). | | Page 9, 10 |
| Limitations | 25 | | Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias). | | Page 11; lines 256- 267 |
| Conclusions | 26 | | Provide a general interpretation of the results in the context of other evidence, and implications for future research. | | Page 11; Lines 268- 278 |
| **FUNDING** | | | | | Page 12; Lines 280 |
| Funding | 27 | | Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review. | | Page 12; Lines 280 |

|  |  |  |
| --- | --- | --- |
| **Sup. Table 2.** Search strategies and the number of studies according to different electronic database | | |
| **Database** | **Search strategy** | **Number of records** |
| PubMed | Search (((Trimethylamine N-oxide[Title/Abstract]) OR TMAO[Title/Abstract])) AND ((((((((((("Fibrinogen"[Mesh]) OR "Interleukin-17"[Mesh]) OR "Interleukin-2"[Mesh]) OR "Interleukin-1beta"[Mesh]) OR interleukin 1[Title/Abstract]) OR ("Cytokines"[Mesh] OR "Chemokines"[Mesh] OR "Cytokinesis"[Mesh])) OR "Tumor Necrosis Factor-alpha"[Mesh]) OR ("Receptors, Interleukin-6"[Mesh] OR "Interleukin-6"[Mesh])) OR hs crp[Title/Abstract]) OR "C-Reactive Protein"[Mesh]) OR ("Anti-Inflammatory Agents, Non-Steroidal"[Mesh] OR "Anti-Inflammatory Agents"[Mesh] OR "Inflammation Mediators"[Mesh] OR "Inflammation"[Mesh])) Sort by: Best Match | 55 |
| ProQuest | [MESH.EXACT("C-Reactive Protein") OR ab (C-Reactive Protein) OR ti(C-Reactive Protein)](https://search.proquest.com/recentsearches.recentsearchtabview.recentsearchesgridview.scrolledrecentsearchlist.checkdbssearchlink:rerunsearch/4CAADCB8B8044D95PQ/None?t:ac=RecentSearches) OR [MESH.EXACT ("Interleukin-1beta") OR ab(Interleukin-1beta) OR ti(Interleukin-1beta) OR (MESH.EXACT("Cytokines") OR MESH.EXACT("Cytokinesis")) OR ti(Cytokines) OR ab(Cytokines) OR MESH.EXACT("Tumor Necrosis Factor-alpha") OR ti(Tumor Necrosis Factor-alpha) OR ab(Tumor Necrosis Factor-alpha)](https://search.proquest.com/recentsearches.recentsearchtabview.recentsearchesgridview.scrolledrecentsearchlist.checkdbssearchlink:rerunsearch/69804F48A9EB457EPQ/None?t:ac=RecentSearches) OR [MESH.EXACT("Interleukin-1beta") OR ab(Interleukin-1beta) OR ti(Interleukin-1beta) OR (MESH.EXACT("Cytokines") OR MESH.EXACT("Cytokinesis")) OR ti(Cytokines) OR ab(Cytokines) OR MESH.EXACT("Tumor Necrosis Factor-alpha") OR ti(Tumor Necrosis Factor-alpha) OR ab(Tumor Necrosis Factor-alpha)](https://search.proquest.com/recentsearches.recentsearchtabview.recentsearchesgridview.scrolledrecentsearchlist.checkdbssearchlink:rerunsearch/9514DB5C29F94C21PQ/None?t:ac=RecentSearches) OR [(MESH.EXACT("Inflammation Mediators") OR MESH.EXACT("Inflammation")) OR ab(Inflammation) OR ti(Inflammation) OR MESH.EXACT("Fibrinogen") OR ti(Fibrinogen) OR ab(Fibrinogen) OR MESH.EXACT("Interleukin-2") OR ti(Interleukin-2) OR ab(Interleukin-2)](https://search.proquest.com/recentsearches.recentsearchtabview.recentsearchesgridview.scrolledrecentsearchlist.checkdbssearchlink:rerunsearch/7EC146BAB4E4E1APQ/None?t:ac=RecentSearches) AND [ab (Trimethylamine N-oxide) OR ti(Trimethylamine N-oxide) OR ti(TMAO) AND ab (TMAO)](https://search.proquest.com/recentsearches.recentsearchtabview.recentsearchesgridview.scrolledrecentsearchlist.checkdbssearchlink:rerunsearch/D3F90563D174C20PQ/None?t:ac=RecentSearches) | 155 |
| Scopus | ( ( TITLE-ABS-KEY ( trimethylamine  AND n-oxide )  OR  TITLE-ABS-KEY ( tmao ) ) )  AND  ( ( TITLE-ABS-KEY ( inflammation )  OR  TITLE-ABS-KEY (fibrinogen)  OR  TITLE-ABS-KEY ( interleukin\* )  OR  TITLE-ABS-KEY ( c-reactive  AND protein )  OR  TITLE-ABS-KEY ( crp )  OR  TITLE-ABS-KEY ( hs-crp )  OR  TITLE-ABS-KEY ( cytokine\* )  OR  TITLE-ABS-KEY ( tumor  AND necrosis  AND factor-alpha )  OR  TITLE-ABS-KEY ( tnf-α )  OR  TITLE-ABS-KEY ( interleukin  6 )  OR  TITLE-ABS-KEY ( interleukin  2 )  OR  TITLE-ABS-KEY ( interleukin  AND 1β ) ) ) | 165 |
| Embase | ('trimethylamine n-oxide':ti,ab OR 'tmao':ti,ab) AND ('inflamm\*':ti,ab OR 'c reactive protein':ti,ab OR 'tumor necrosis factor':ti,ab OR 'interleukin 6':ti,ab OR 'interleukin 1beta':ti,ab OR 'interleukin 2':ti,ab OR 'cytokine\*':ti,ab OR 'fibrinogen':ti,ab) | 211 |

**Sup. Table** **3. Newcastle–Ottawa quality assessment scale (NOS) for cohort studies**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Selection** | | | | **Comparability** | **Outcome** | | | **Final score** |
| **First author,**  **(year)** | **Study type** | **Representativeness of the exposed cohort** | **Selection of the non-exposed cohort** | **Ascertainment of exposure** | **Demonstration that outcome of interest was not present at start of study** | **Comparability of cohorts on the basis of the design or analysis** | **Assessment of outcome** | **Was follow-up long enough for outcomes to occur** | **Adequacy of follow up of cohorts** |
| **Chou et al. (2019)** | **Cohort** | **\*** | **\*** | **\*** | **\*** | **\*** | **\*** | **\*** | **\*** | **8** |
| **Svingen et al. (2018) a** | **Cohort** | **\*** | **\*** | **\*** | **\*** | **\*** | **\*** | **\*** | **\*** | **8** |
| **Svingen et al. (2018) b** | **Cohort** | **\*** | **\*** | **\*** | **\*** | **\*** | **\*** | **\*** | **\*** | **8** |
| **Haghikia et al. (2018) c** | **Cohort** | **-** | **\*** | **\*** | **\*** | **\*** | **\*** | **-** | **-** | **5** |
| **Haghikia et al. (2018) d** | **Cohort** | **\*** | **\*** | **\*** | **\*** | **\*** | **\*** | **\*** | **\*** | **8** |
| **Tang et al. (2014)** | **Cohort** | **\*** | **\*** | **\*** | **\*** | **\*\*** | **\*** | **\*** | **\*** | **9** |
| **Missailidis et al. (2016)** | **Cohort** | **\*** | **\*** | **\*** | **\*** | **\*** | **\*** | **\*** | **\*** | **8** |
| **Tang et al. (2017)** | **Cohort** | **\*** | **\*** | **\*** | **\*** | **\*\*** | **\*** | **\*** | **\*** | **9** |
| **Troseid et al. (2015)** | **Cohort** | **-** | **\*** | **\*** | **\*** | **\*** | **\*** | **\*** | **-** | **6** |
| **Stubbs et al. (2016)** | **Cohort** | **-** | **\*** | **\*** | **\*** | **\*** | **\*** | **\*** | **-** | **6** |
| **Meyer et al. (2016)** | **Cohort** | **\*** | **\*** | **\*** | **\*** | **\*\*** | **\*** | **\*** | **\*** | **9** |

**a, Western Norway Coronary Angiography Cohort (WECAC) b, Hordaland Health Study (HUSK) cohort, c, first pilot cohort; d, Prospective Cohort With Incident Stroke (PCWIS)**

|  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  |  | **Selection** | | | | **Comparability** | **Exposure** | | | **Final score** |
| **First author,**  **(year）** | **Type** | **Is the case definition adequate?** | **Representativeness of the cases** | **Selection of Controls** | **Definition of Controls** | **Comparability of cases and controls on the basis of the design or analysis** | **Ascertainment of exposure** | **Same method of ascertainment for cases and controls** | **Non-Response rate** |
| **Yu et al. (2019)** | **Nested- case-control** | **\*** | **\*** | **\*** | **\*** | **\*** | **\*** | **\*** | **-** | **7** |
| **Lent-Schochet et al. (2018)** | **Case-control** | **\*** | **\*** | **\*** | **\*** | **\*** | **\*** | **-** | **-** | **6** |
| **Kaysen et al. (2015)** | **Case-control** | **\*** | **\*** | **\*** | **\*** | **\*** | **\*** | **-** | **-** | **6** |

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **ARHQ Methodology Checklist items for Cross-Sectional study** | **Liu et al. (2018)** | **Aslibekyan et al. (2017)** | **Rohrmann et al. (2016)** | **Randrianarisoa et al. (2016)** |
| 1) Define the source of information (survey, record review) | ⊕ | ⊕ | ⊕ | ⊕ |
| 2) List inclusion and exclusion criteria for exposed and unexposed subjects (cases and controls) or refer to previous publications | ⊕ | ⊕ | ⊕ | ⊕ |
| 3) Indicate time period used for identifying patients | ⊕ | \_ | ⊕ | ⊕ |
| 4) Indicate whether or not subjects were consecutive if not population-based | ⊕ | ⊕ | ⊕ | ⊕ |
| 5) Indicate if evaluators of subjective components of study were masked to other aspects of the status of the participants | U | U | U | U |
| 6) Describe any assessments undertaken for quality assurance purposes (e.g., test/retest of primary outcome measurements) | ⊕ | ⊕ | ⊕ | ⊕ |
| 7) Explain any patient exclusions from analysis | ⊕ | \_ | ⊕ | ⊕ |
| 8) Describe how confounding was assessed and/or controlled. | U | ⊕ | ⊕ | ⊕ |
| 9) If applicable, explain how missing data were handled in the analysis | U | ⊕ | ⊕ | \_ |
| 10) Summarize patient response rates and completeness of data collection | \_ | \_ | ⊕ | \_ |
| 11) Clarify what follow-up, if any, was expected and the percentage of patients for which incomplete data or follow-up was obtained | \_ | \_ | \_ | U |
| Final score | 6 | 6 | 9 | 7 |

**Sup. Table 4.** Agency for Healthcare Research and Quality (AHRQ) checklist to assess quality of the cross-sectional studies

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**Sup. Figure 1.** Funnel diagram of two-class meta-analysis.

**References**

Aslibekyan, S., M. R. Irvin, B. A. Hidalgo, R. T. Perry, E. J. Jeyarajah, E. Garcia, I. Shalaurova, P. N. Hopkins, M. A. Province, H. K. Tiwari, et al. 2017. Genome- and CD4 + T-cell methylome-wide association study of circulating trimethylamine-N-oxide in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN). *Journal of Nutrition and Intermediary Metabolism* 8: 1-7. doi: 10.1016/j.jnim.2017.03.002.

Chou R. H., C. Y. Chen, I. C. Chen, H. L. Huang, Y. W. Lu, C. S. Kuo, C. C. Chang, P. H. Huang, J. W. Chen, and S. J. Lin. 2019. Trimethylamine N-Oxide, Circulating Endothelial Progenitor Cells, and Endothelial Function in Patients with Stable Angina. *Scientific Reports* 9 (1): 4249. doi:10.1038/s41598-019-40638-y.

Haghikia, A., X. S. Li, T. G. Liman, N. Bledau, D. Schmidt, F. Zimmermann, N. Kränkel, C. Widera, K. Sonnenschein, A. Haghikia, et al. 2018. Gut microbiota-dependent trimethylamine N-oxide predicts risk of cardiovascular events in patients with stroke and is related to proinflammatory monocytes. *Arteriosclerosis, Thrombosis, and Vascular Biology* 38 (9): 2225-35. doi: 10.1161/ATVBAHA.118.311023.

Kaysen, G. A., K. L. Johansen, G. M. Chertow, L. S. Dalrymple, J. Kornak, B. Grimes, T. Dwyer, A. W. Chassy, and O. Fiehn. 2015. Associations of trimethylamine N-oxide with nutritional and inflammatory biomarkers and cardiovascular outcomes in patients new to dialysis. *Journal of Renal Nutrition* 25 (4): 351-6. doi: 10.1053/j.jrn.2015.02.006.

Lent-Schochet, D., R. Silva, M. McLaughlin, B. Huet, and I. Jialal. 2018. Changes to trimethylamine-N-oxide and its precursors in nascent metabolic syndrome. *Hormone Molecular Biology and Clinical Investigation* 35 (2). doi: 10.1515/hmbci-2018-0015.

Liu, X., Z. Xie, M. Sun, X. Wang, J. Li, J. Cui, F. Zhang, L. Yin, D. Huang, J. Hou, et al. 2018. Plasma trimethylamine N-oxide is associated with vulnerable plaque characteristics in CAD patients as assessed by optical coherence tomography. *International Journal of Cardiology* 265: 18-23. doi: 10.1016/j.ijcard.2018.04.126.

Meyer, K. A., T. Z. Benton, B. J. Bennett, D. R. Jacobs Jr, D. M. Lloyd‐Jones, M. D. Gross, J. J. Carr, P. Gordon‐Larsen, and S. H. Zeisel. 2016. Microbiota‐dependent metabolite trimethylamine N‐oxide and coronary artery calcium in the coronary artery risk development in young adults study (CARDIA). *Journal of the American Heart Association* 5 (10): e003970. doi:10.1161/JAHA.116.003970.

Missailidis, C., J. Hällqvist, A. R. Qureshi, P. Barany, O. Heimbürger, B. Lindholm, P. Stenvinkel, and P. Bergman. 2016. Serum trimethylamine-N-Oxide is strongly related to renal function and predicts outcome in chronic kidney disease. *PloS One* 11 (1):e0141738. doi: 10.1371/journal.pone.0141738.

Moher, D., A. Liberati, J. Tetzlaff, and D. G. Altman. 2009. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Annals of Internal Medicine* 151 (4): 264-69. doi:10.7326/0003-4819-151-4-200908180-00135.

Randrianarisoa, E., A. Lehn-Stefan, X. Wang, M. Hoene, A. Peter, S. S. Heinzmann, X. Zhao, I. Königsrainer, A. Königsrainer, B. Balletshofer, et al. 2016. Relationship of Serum Trimethylamine N-Oxide (TMAO) Levels with early Atherosclerosis in Humans. *Scientific Reports* 6: 26745. doi: 10.1038/srep26745.

Rohrmann, S., J. Linseisen, M. Allenspach, A. von Eckardstein, and D. Muller. 2016. Plasma Concentrations of Trimethylamine-N-oxide Are Directly Associated with Dairy Food Consumption and Low-Grade Inflammation in a German Adult Population. *The Journal of Nutrition* 146 (2): 283-9. doi: 10.3945/jn.115.220103.

Stubbs, J. R., J. A. House, A. J. Ocque, S. Zhang, C. Johnson, C. Kimber, K. Schmidt, A. Gupta, J. B. Wetmore, T. D. Nolin, et al. 2016. Serum Trimethylamine-N-Oxide is Elevated in CKD and Correlates with Coronary Atherosclerosis Burden. *Journal of the American Society of Nephrology* 27 (1): 305-13. doi: 10.1681/ASN.2014111063.

Svingen, G. F. T., H. Zuo, P. M. Ueland, R. Seifert, K. H. Loland, E. R. Pedersen, P. M. Schuster, T. Karlsson, G. S. Tell, H. Schartum-Hansen, et al. 2018. Increased plasma trimethylamine-N-oxide is associated with incident atrial fibrillation. *International Journal of Cardiologyl* 267: 100-106. doi: 10.1016/j.ijcard.2018.04.128.

Tang, W. H., Wang, Y. Fan, B. Levison, J. E. Hazen, L. M. Donahue, Y. Wu, and S. L. Hazen. 2014. Prognostic value of elevated levels of intestinal microbe-generated metabolite trimethylamine-N-oxide in patients with heart failure: Refining the gut hypothesis. *Journal of the American College of Cardiology* 64 (18): 1908-14. doi: 10.1016/j.jacc.2014.02.617.

Tang, W. H., Z. Wang, X. S. Li, Y. Fan, D. S. Li, Y. Wu, and S. L. Hazen. 2017. Increased trimethylamine N-oxide portends high mortality risk independent of glycemic control in patients with type 2 diabetes mellitus. *Clinical chemistry* 63 (1): 297–306. doi: 10.1373/clinchem.2016.263640.

Troseid, M., T. Ueland, J. R. Hov, A. Svardal, I. Gregersen, C. P. Dahl, S. Aakhus, E. Gude, B. Bjorndal, B. Halvorsen, et al. 2015. Microbiota-dependent metabolite trimethylamine-N-oxide is associated with disease severity and survival of patients with chronic heart failure. *Journal of Internal Medicine* 277 (6): 717-26. doi: 10.1111/joim.12328.

Yu, D., X. O. Shu, E. S. Rivera, X. Zhang, Q. Cai, M. W. Calcutt, Y. B. Xiang, H. Li, Y. T. Gao, T. J. Wang, et al. 2019. Urinary levels of trimethylamine-N-oxide and incident coronary heart disease: A prospective investigation among urban Chinese adults. *Journal of the American Heart Association* 8 (1): e010606. doi: 10.1161/JAHA.118.01060.