ALINEA

NUTRITION

SEPTEMBER 2020

www.alineanutrition.com

TABLE OF

CONTENTS

Crimarco A, Springfield S, Petlura C, Streaty T, Cunanan K, Lee J, et al. A randomized crossover trial on the effect of plant-based compared with animal-based meat on trimethylamine-N-oxide and cardiovascular disease risk factors in generally healthy adults: Study With Appetizing Plantfood—Meat Eating Alternative Trial (SWAP-MEAT). Am J Clin Nutr. 2020;(7):1–12.

What We Know, Think We Know, or Are Starting to Know

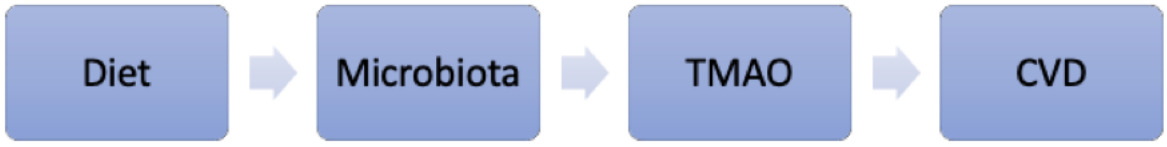
Stepping back from the role of diets, specific foods or nutrients in human health, of this we can be certain: the production of meat contributes more than any other aspect of the human food supply to anthropogenic global warming [(1)]. There is, therefore, incentive to reduce meat consumption across the population to address climate change, a consideration which may be considered distinct from any health debates.

In relation to the health debate regarding meat - and animal produce in general - one marker that has become popular in the Diet War™ between plant and animal is trimethylamine - N - oxide: TMAO.

Production of TMAO in humans requires gut bacteria; the microbiota metabolises certain dietary nutrients - carnitine, choline, and betaine - into trimethylamine [TMA]. TMA is then metabolised by liver enzymes into TMAO, which appears in plasma [(2)]. Pre-formed TMAO is also found in high amounts in fish, and is directly absorbed [(3)]. Evidence is now also emerging that underlying metabolic dysfunction in the liver and kidney increases plasma TMAO levels [(4)].

In 2011, the first prospective study was published which found elevated plasma TMAO was associated with a significant increase in CVD risk [(5)]. Subsequent cohort studies have also reported that elevated plasma levels of TMAO are associated with increased CVD risk [(6)]. This has placed the focus on foods like red meat and eggs, respectively sources of carnitine and choline, although interesting no one has mentioned the betaine, which is generally found in plant foods. This hypothesised causal chain can be illustrated as follows:

Proposed Causal Chain Between Diet, TMAO, and CVD



However, there are rather a few holes in this story which may be sufficient to sink said causal chain, in particular whether TMAO is an independent causal risk factor or systems biomarker that is more innocent bystander*. One of these potential holes is whether the actual role of specific foods and nutrients is as hypothesised, given that choline is an essential nutrient, carnitine is conditionally essential, and foods like fish contain high levels of pre-formed TMAO.

The present study tested the effects of eating plant-meat substitutes for 8 weeks and animal meats for another 8 weeks on plasma TMAO levels, and other cardio-metabolic risk factors.

*Geek Box: Causal Risk Factors vs. Systems Biomarkers

Studies investigating diet and health outcomes may use any number of measures to test effects of diet. Whether the outcome is composition of gut bacteria or blood cholesterol levels, it is important to distinguish between a physiological parameter that has been established as causative in a given disease process, or acts more as a "systems biomarker", a term I first heard from Professor Chris Packard in a discussion about blood lipids and cardiovascular risk. So, what is the difference? We can delineate between the two as follows:

- *Independent risk factor: biomarkers in a causal pathway between the exposure and outcome;*
- *Systems biomarkers: biomarker which provide indications of underlying cardio-metabolic processes, but are not causal independently.*

This distinction is not academic. For example, LDL-cholesterol is an independent risk factor which is the causal pathway through which elevated cholesterol drives atherosclerosis. A systems biomarker, however, may not necessarily be causal of itself, but provides important additional granularity to the risk equation. For example, high HDL-cholesterol is generally associated with lower risk for CVD, however, deliberately raising HDL-C does not reduce CVD risk, indicating that HDL-C is not directly causal of lower risk. But it remains an important systems biomarker; for example if two individuals had the same moderately elevated LDL-C levels, but one high and one low HDL-C, the individual with low HDL-C would likely be at higher CVD risk. Thus, HDL-C is this context is providing additional information to the risk assessment. In the context of the present study, the question is whether TMAO is in the causal pathway driving cardio-metabolic disease processes, or is TMAO a biomarker for something else, perhaps underlying disease itself or the activity of the gut microbiota? And is diet in this causal chain? In addition to other lines of research to determine whether a risk factor has a causal role, a powerful tool in research design to look at potential independent causality is Mendelian randomisation studies [see the next Geek Box].

The Study

The Study With Appetizing Plantfood—Meat Eating Alternatives Trial [MEAT-SWAP] was a randomised crossover intervention trial comparing 8-weeks consuming a plant-based meat alternative ['Plant-meat'] and 8-weeks of consuming animal meats ['Animal-meat'].

Generally healthy omnivorous adults who habitually consumed >1 serving meat per day were randomised to diet order:

- Plant-meat x 8-weeks > Animal-meat x 8-weeks
- Animal-meat x 8-weeks > Plant-meat x 8-weeks

There was no washout period between diets: one phase was immediately followed by the other.

Participants were instructed to consumed >2 servings per day of either the Plant-meat or Animal-meat, depending on diet phase. Participants were requested to track types of burger buns, garnishes, and condiments used, and to keep these constant through both diet phases.

Plant-meats and Animal-meats were provided to participants, however participants purchased all other foods and prepared meals. The plant-meats were provided by Beyond Meat and animal meats provided by an organic grass-fed food service.

The primary outcome was changes in TMAO between the Plant-meat and Animal-meat diets. Secondary outcomes included IGF-1, blood pressure, blood lipids, glucose, and insulin.

Results: 24 women and 12 men completed the intervention. Baseline TMAO levels were 3.5uM in the Plant>Animal and 3.4uM in the Animal>Plant groups, respectively. Both groups consumed ~2.5 servings per day of the respective Plant-meats or Animal-meats.

- ***TMAO:*** Overall, the mean difference between diet groups was significantly different: 2.7uM in the Plant-meat diet vs. 4.7uM in the Animal-meat diet [2.0uM difference].

  However, there was a significant effect of diet order on the results. In the Plant>Animal group, there was no significant difference between the end of the Plant-meat diet [2.5uM] compared to the end of the following Animal-meat diet [3.0uM].

  In the Animal>Plant group, there was a significant difference between the end of the Animal-meat diet [6.4uM] compared to the end of the Plant-meat diet [2.9uM].
- ***LDL-C:*** The mean overall baseline LDL-C level was 3.1mmol/L [122mg/dL]. At the end of the Plant-meat phase, LDL-C levels were 2.8mmol/L [109mg/dL] compared to 3.1mmol/L [120mg/dL] in the Animal-meat groups. There was no significant effect of diet order on LDL-C.
- ***Other secondary outcomes:*** There were no significant differences in any other outcome measure between the Plant-meat vs. Animal-meat diets.

The Critical Breakdown

Pros: The study recruited otherwise healthy participants, with no evidence of cardio-metabolic disease; this is a positive given metabolic dysfunction may influence TMAO levels. The crossover design meant that each participant served as their own control [i.e., the comparison between diet phases was within the same person, minimising potential inter-individual differences]. Providing key study foods for both diet phases may have enhanced adherence.

Cons: Using TMAO as the primary outcome measure [more under ***Key Characteristic***, below] may have biased the results toward the Plant-meat diet. Diets were not controlled and it is not known what effects other constituents of diet - and there are numerous which influence TMA and TMAO production - could have played. Finally, while I don' t usually flag up funding sources in studies, Beyond Meat provided an "unrestricted research gift" to the lead investigator, and the selection of TMAO as the primary outcome could read like the study was an attempt to set the products up for the win.

Key Characteristic

Selecting TMAO as the primary outcome measure. This is strange given that there is sufficient evidence to suggest that the diet>TMAO link may be more red herring than causal risk factor [7].

In particular, a well-conducted Mendelian randomisation* study examined the causal relationship between genes that increase levels of TMAO [or dietary precursors] and cardio-metabolic disease [8]. The analysis demonstrated that genetically higher TMAO levels were not associated with increased cardio-metabolic disease risk; however, the presence of Type-2 Diabetes or Chronic Kidney Disease were both associated with higher TMAO levels, indicating that underlying metabolic dysfunction may be a cause of elevated TMAO.

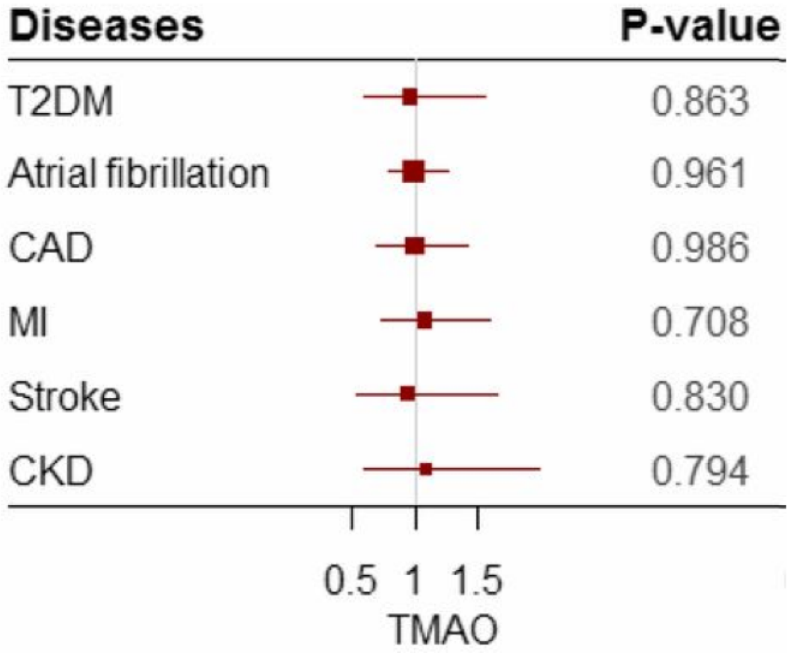


Figure *from Jia et al.* [8] *indicating relationship of TMAO to cardio-metabolic diseases based on genetic predisposition to higher TMAO in Mendelian randomisation analysis.*

In addition, most dietary interventions to date indicate that the effects of diet on plasma TMAO are short term, and results from short-term interventions are inconsistent in relation to specific foods and nutrients [9]. This means that short-term elevations have the potential to be 'false positives' .

Moreover, fish contains more pre-formed TMAO than could be generated from precursors in red meat or eggs together, i.e., carnitine and choline, yet fish is consistency associated with reduced risk for CVD [3,10].

Based on previous knowledge, comparing meat to plant-meats would be expected to bias the result toward the Plant-meat exposures. The fact that this result was inconsistent in relation to order of diet may in fact add another knock on the diet>TMAO>CVD hypothesis.

*Geek Box: Mendelian Randomisation

Mendelian randomisation [MR] is a principle of using genetics to mimic a long-term randomised controlled trial, particularly where a long-term intervention study may be unethical or practically infeasible. Because an individuals' genes are 'assigned' when they are conceived, this in effect it is the purest form of randomisation, i.e., the genetic lottery from Mom and Pops. Well conducted MR can provide an unconfounded estimate of the relationship between an exposure and an outcome. It is unconfounded because the genetic variant results in a certain physiological response that is independent of other considerations. Thus, to be properly conducted, a MR study has to satisfy three criteria. 1) The genetic variant must be associated with the specific mediating factor, e.g., LDL-C or TMAO; 2) The genetic variant must not be associated with any potential confounders that could influence the outcome, and; 3) The genetic variant must only influence the disease outcome through the specific mediating factor, not through other mechanisms. In keeping with this study example, the genetic variants examined were those associated with increased gut microbiota-dependent metabolites, i.e., TMAO, that also did not potentially influence disease risk by other pathways. Similarly with LDL-C, the genetic variants examined are those that specifically influence cholesterol clearance from the blood. When long-term randomised studies are not possible, Mendelian randomisation is a powerful tool to examine potential cause-effect relationships.

Interesting Finding

The effect of order of diet on the outcomes is interesting, and as stated above, it may also serve to be another hole in the TMAO hypothesis.

First, let' s consider the fact that after consuming the Plant-meat first there was no change in TMAO, but after consuming the Animal-meats first TMAO levels elevated and then declined to similar levels as during the Plant-meat phase. However, significant inter-individual variability was evident, and in a number of individual plot lines it is evident that large increases in TMAO after 2-4 weeks began to regress to the mean after 8-weeks. This is consistent with other research indicating significant individual variability in TMAO responses to diet, and that short-term dietary changes may not lead to lasting elevated TMAO levels [3,11].

Secondly, this could reflect short-term responsiveness of the gut microbiota to diet, which we know can shift in as little as 3-days but reverts to stable composition once an intervention ends [12]. In fact, the gut microbiota may itself be the culprit. For example, human studies have demonstrated that administration of broad-spectrum antibiotics essentially eradicates the production of TMAO, which production returned once bacteria recolonised the gut after 1-month cessation of antibiotics [6].

In a study comparing the effects of fish, beef, eggs, and fruit on TMAO responses, Cho et al. also demonstrated that high-TMAO producers had a 2:1 Firmicutes:Bacteroidetes ratio, while low-TMAO producers had 1:1 Firmicutes:Bacteroidetes ratio, and high-TMAO producers displayed less microbial diversity [3]. This indicates that an individual' s microbiota composition modulates response to diet, and it may be that increased risk is more associated with TMA-producing bacteria and microbial composition than plasma TMAO levels per se. Thus, plasma TMAO is the 'innocent bystander' .

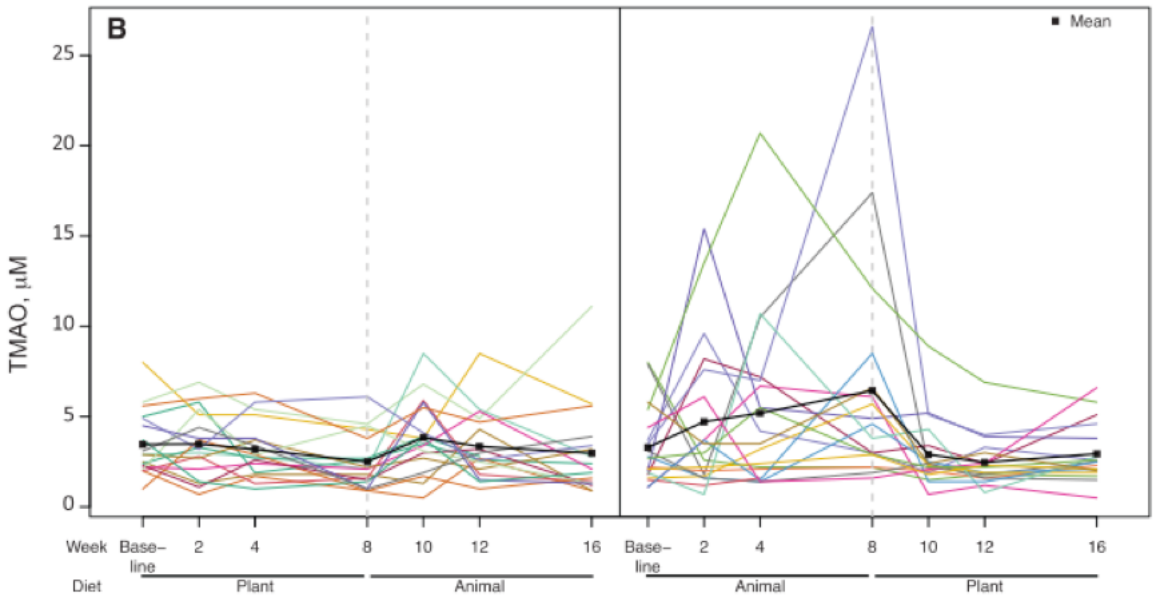
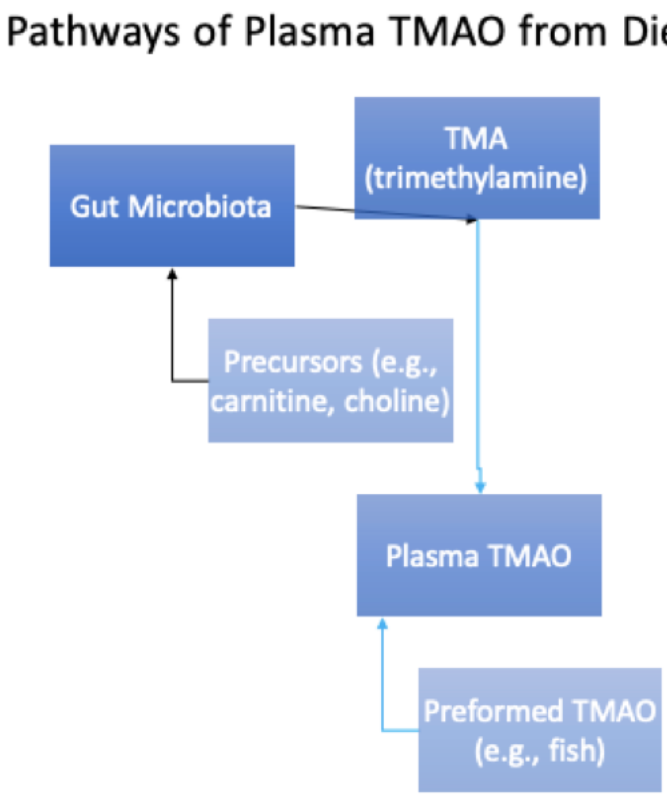


Figure *from paper illustrating the individual responses [**coloured lines**] and mean response [**solid black line**] during the Plant>Animal intervention group [**left**] vs. the Animal>Plant intervention group [**right**].*

Finally, the fact that TMAO from fish is absorbed intact into the plasma and increases circulating levels ~50 times higher than beef or eggs may also support that TMAO is a proxy, i.e., the conversion of TMA to TMAO in the liver creates the 'innocent bystander' in plasma TMAO levels, but the real culprit in increased cardio-metabolic disease risk is TMA-producing bacteria in the gut.



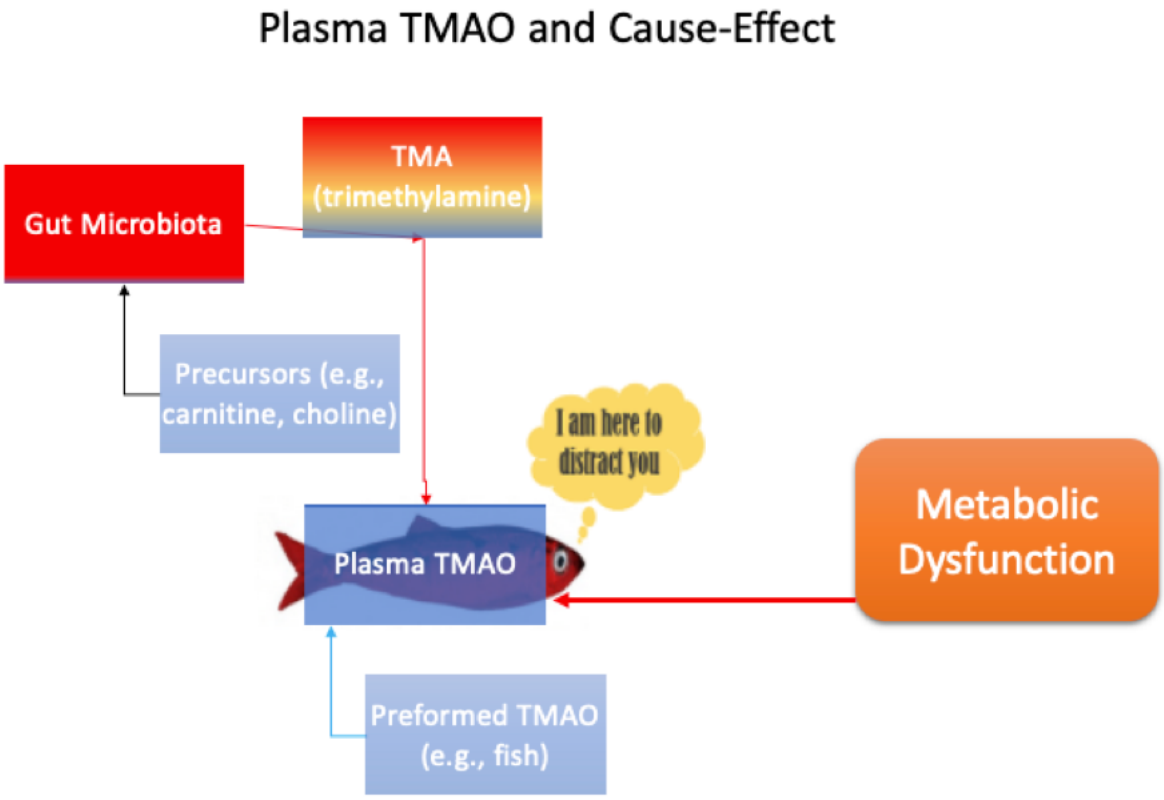
Relevance

Is TMAO an independent risk factor on a causal chain from diet to cardio-metabolic disease risk? It doesn't appear so. The authors even describe TMAO as an "emerging" risk factor, which is being generous. There is sufficient evidence to consider TMAO red herring [3,4,7,8,9,11].

From the perspective of TMAO as a reliable risk factor, consider both between-person and within-person variability. Short term studies have indicated variation in TMAO response between individuals of between 30% to 270% [3]. In addition, studies specifically investigating within-person variability over periods of 1yr have demonstrated low reproducibility of TMAO and high within-person variability [11], which suggests TMAO in long-term prospective studies may not be a reliable biomarker to relate diet to health outcomes.

Let's also consider the some of the foods that have been shown to increase TMAO levels: fish, resistant starch, and prebiotic fibres - all associated with positive health outcomes [9]. And, let's consider that impaired metabolic function observed in underlying disease states may increase plasma TMAO levels [4].

Finally, let' s consider the production of TMA from precursors requires activity of microbiota [3], and that TMAO reflects liver detoxification of TMA [2], i.e., microbial composition may be the factor modulating risk of disease. All of this points to circulating free TMAO being a proxy for either metabolic dysfunction or activity of certain bacteria in the gut, but not a direct cause of disease of itself [7].



Mueller et al. found no significant differences in plasma TMAO between patients with or without angiographically documented coronary heart disease, and no relationship between further adverse CVD events at 8-years follow-up [4]. And, in a subgroup of the EPIC-Heidelberg cohort, with low habitual fish consumption, low-moderate red meat [~40-70g/d] and egg [~13g/d] was not associated with TMAO levels [11].

Taken together, it is difficult to justify TMAO as an independent direct cause of disease, and consequently difficult to justify specific dietary modifications to address TMAO itself.

Application to Practice

In those who consume meat, there are distinct environmental considerations for swapping some weekly servings for plant-meat alternatives, which appear to be well tolerated in terms of taste and texture. However, increases in TMAO have been proposed as a reason why foods like meat and eggs directly increase risk of CVD. Of this, the evidence is underwhelming to the point of entirely unconvincing. The present study adds yet another piece of unconvincing evidence. The TMAO ship set sail in 2011 looking rather formidable; a decade later it has numerous holes and is taking water fast.

References

1. Pimentel, D., and Pimentel, M. (2003). Sustainability of meat-based and plant-based diets and the environment. The American Journal of Clinical Nutrition 78, 660S-663S.

2. Bennett, B., Vallim, T., Wang, Z., Shih, D., Meng, Y., Gregory, J., Allayee, H., Lee, R., Graham, M., and Crooke, R. et al. (2013). Trimethylamine-N-Oxide, a Metabolite Associated with Atherosclerosis, Exhibits Complex Genetic and Dietary Regulation. Cell Metabolism 17, 49-60.

3. Cho, C., Taesuwan, S., Malysheva, O., Bender, E., Tulchinsky, N., Yan, J., Sutter, J., and Caudill, M. (2017). Back cover: Trimethylamine-N-oxide (TMAO) response to animal source foods varies among healthy young men and is influenced by their gut microbiota composition: A randomized controlled trial. Molecular Nutrition & Food Research 61, 1770016.

4. Mueller, D., Allenspach, M., Othman, A., Saely, C., Muendlein, A., Vonbank, A., Drexel, H., and von Eckardstein, A. (2015). Plasma levels of trimethylamine-N-oxide are confounded by impaired kidney function and poor metabolic control. Atherosclerosis 243, 638-644.

5. Wang, Z., Klipfell, E., Bennett, B., Koeth, R., Levison, B., DuGar, B., Feldstein, A., Britt, E., Fu, X., and Chung, Y. et al. (2011). Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. Nature 472, 57-63.

6. Tang, W., Wang, Z., Levison, B., Koeth, R., Britt, E., Fu, X., Wu, Y., and Hazen, S. (2013). Intestinal Microbial Metabolism of Phosphatidylcholine and Cardiovascular Risk. New England Journal of Medicine 368, 1575-1584.

7. Landfald, B., Valeur, J., Berstad, A., and Raa, J. (2017). Microbial trimethylamine-N-oxide as a disease marker: something fishy?. Microbial Ecology in Health and Disease 28, 1327309.

8. Jia, J., Dou, P., Gao, M., Kong, X., Li, C., Liu, Z., and Huang, T. (2019). Assessment of Causal Direction Between Gut Microbiota–Dependent Metabolites and Cardiometabolic Health: A Bidirectional Mendelian Randomization Analysis. Diabetes 68, 1747-1755.

9. Papandreou, C., Moré, M., and Bellamine, A. (2020). Trimethylamine N-Oxide in Relation to Cardiometabolic Health—Cause or Effect?. Nutrients 12, 1330.

10. Mozaffarian, D., and Rimm, E. (2006). Fish Intake, Contaminants, and Human Health. JAMA 296, 1885.

11. Kühn, T., Rohrmann, S., Sookthai, D., Johnson, T., Katzke, V., Kaaks, R., von Eckardstein, A., and Müller, D. (2017). Intra-individual variation of plasma trimethylamine-N-oxide (TMAO), betaine and choline over 1 year. Clinical Chemistry and Laboratory Medicine (CCLM) 55.

12. David, L., Maurice, C., Carmody, R., Gootenberg, D., Button, J., Wolfe, B., Ling, A., Devlin, A., Varma, Y., and Fischbach, M. et al. (2014). Diet rapidly and reproducibly alters the human gut microbiome. Nature 505, 559-563.