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Is increased plasma TMAO a compensatory response to hydrostatic and osmotic stress in cardiovascular diseases?

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A B S T R A C T

Recent clinical studies show a positive correlation between elevated plasma TMAO and increased cardiovascular risk. However, the mechanism of the increase and biological effects of TMAO in the circulatory system are obscure.

Plasma TMAO level depends mostly on the following three factors. First, the liver produces TMAO from TMA, a gut bacteria metabolite of dietary choline and carnitine. Second, plasma TMAO increases after ingestion of dietary TMAO from fish and seafood. Finally, plasma TMAO depends on TMAO and TMA excretion by the kidneys.

Ample evidence highlights protective functions of TMAO, including the stabilization of proteins and cells exposed to hydrostatic and osmotic stresses, for example in fish exposed to hydrostatic stress (deep water) and osmotic stress (salty water).

Osmotic stress and hydrostatic stresses are augmented in cardiovascular diseases such as hypertension. In hypertensive subjects a diastole-systole change in hydrostatic pressure in the heart may exceed 220 mmHg with a frequency of 60–220/min. This produces environment in which hydrostatic pressure changes over 100,000 times per 24 h. Furthermore, cardiovascular diseases are associated with disturbances in water-electrolyte balance which produce changes in plasma osmolarity. Perhaps, the increase in plasma TMAO in cardiovascular diseases is analogous to increased level of plasma natriuretic peptide B, which is both a cardiovascular risk marker and a compensatory response producing beneficial effects for pressure/volume overloaded heart. In this regard, there is some evidence that a moderate increase in plasma TMAO due to TMAO supplementation may be beneficial in animal model of hypertension-related heart failure. Finally, increased plasma TMAO is present in humans consuming seafood-rich diet which is thought to be health-beneficial.

We hypothesize that increased plasma TMAO serves as a compensatory response mechanism which protects cells from hydrostatic and osmotic stresses.

Background

Recent clinical studies show a positive correlation between elevated plasma trimethylamine oxide (TMAO) and increased cardiovascular risk [1–7]. However, the mechanism of the increase and biological effects of TMAO in the circulatory system are obscure.

Plasma TMAO originates from liver oxygenation of trimethylamine (TMA), a gut bacteria product of choline and carnitine [8–10]. Since the latter two nutrients are abundant in red meat and eggs, TMAO has been proposed to be a link between “unhealthy diet” and cardiovascular diseases [11–13].

However, the second source of plasma TMAO is dietary TMAO from seafood and fish. Furthermore choline, a precursor of TMA, is also present in large amount in poultry, fish and many vegetables (Fig. 1). In fact, higher plasma TMAO was found after the ingestion of fish than after ingestion of red meat and eggs [14,15]. This would rather suggest that higher plasma TMAO may have a positive effect as fish-rich diet is beneficial for reducing cardiovascular risk.

Nevertheless, several experimental studies enthused by clinical observations of a positive correlation between high plasma TMAO and increased cardiovascular risk, show a negative effect of TMAO on the circulatory system in rodents [16–18].

On the other hand, ample evidence highlights protective functions of TMAO, including the stabilization of proteins and cells exposed to hydrostatic and osmotic stresses [19–21]. Furthermore, some experimental studies show that a moderate increase in plasma TMAO may be beneficial in animal models of hypertension and heart failure [22,23].

The hypothesis

We believe that increased plasma TMAO is a compensatory and a beneficial response to cardiovascular disturbances. Specifically, our hypothesis assumes that:

1. TMAO serves as a piezolyte and osmolyte in the circulatory system in health and disease.
2. Increased plasma TMAO in cardiovascular diseases reflects the accumulation of TMAO, which is a compensatory and protective mechanism to hydrostatic and osmotic stresses associated with cardiovascular diseases (Fig. 2).

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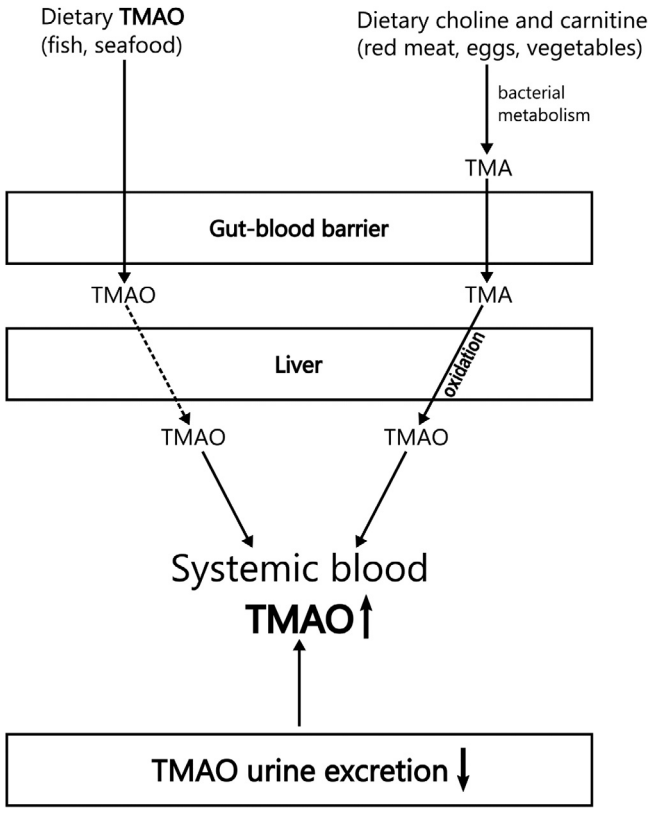

Fig. 1. Factors influencing plasma TMAO levels. Plasma TMAO levels depend on numerous factors including ingestion of a direct dietary TMAO (e.g. fish, seafood), ingestion of substrates for bacterial production of TMA (choline, l-carnitine), intestinal absorption of TMAO and penetration of TMA from the colon to the circulation (the gut-blood barrier), liver metabolism of TMA (TMA to TMAO oxidation), and finally TMAO excretion by the kidneys.

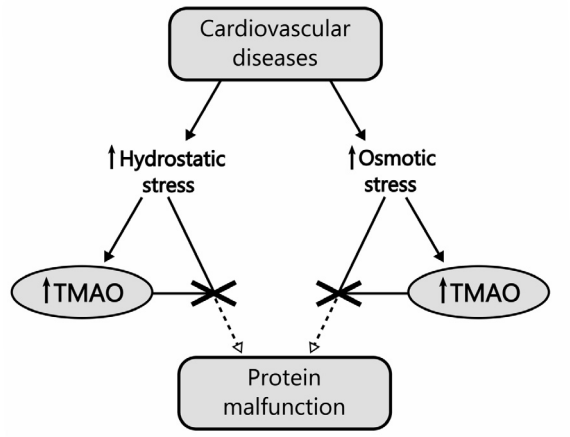

Fig. 2. TMAO in cardiovascular diseases. High hydrostatic and osmotic pressure (hydrostatic and osmotic stresses) produce malfunction of proteins. It has been found that TMAO protects proteins against hydrostatic and osmotic stresses [20,21,55–57]. Since cardiovascular diseases are associated with hydrostatic and osmotic stresses, increased TMAO level may serve as a protective mechanism.

3. The increase in plasma TMAO in cardiovascular diseases strongly resembles increased level of plasma natriuretic peptide B (BNP), which is both a cardiovascular risk marker and a compensatory response producing beneficial effects for pressure/volume overloaded heart.

Evaluation of the hypothesis

Increased plasma TMAO and increased cardiovascular risk. Association is not a causation

Accumulating evidence suggests that plasma TMAO level is associated with cardiovascular risk, however, data from clinical studies are somehow contradictory. Most importantly, interventional clinical studies showing the effect of plasma TMAO manipulation on cardiovascular events are lacking.

Increased plasma TMAO is associated with cardiovascular risk

A positive correlation has been found between increased plasma TMAO and major adverse cardiovascular outcomes (MACE) in patients suspected of coronary artery disease (CAD) [1,3]. Furthermore, increased plasma TMAO concentrations have been linked with increased mortality in CAD [6] and peripheral artery disease [2]. It has also been shown that plasma TMAO concentration could be used in a secondary risk stratification in patients presenting with acute coronary syndrome (ACS), as the concentration has been linked to MACE incidence, all-cause mortality and recurrence of ACS [5,24]. In Chinese population, an association between TMAO and CAD occurrence has been shown [5,25].

In heart failure patients plasma TMAO levels could serve as a negative marker, predicting unfavorable course of heart failure and overall mortality, especially in patients with reduced ejection fraction [7,26,27]. The levels differ between patients with decompensated and compensated heart failure and may be caused by gut dysbiosis [28].

Moreover, Randrianarosa and collaborators stated that TMAO can serve as an increased carotid intima-media complex thickness marker, regarded to be a sign of early atherosclerosis [29]. Optical coherence tomography-guided studies show association between unfavorable morphology of atherosclerotic plaque in coronary arteries [30]. In addition, some data suggest that plasma TMAO concentrations may predict brain ischemic injury in patients with atrial fibrillation or the ones undergoing carotid artery stenting [31,32].

Besides, TMAO plasma concentrations are increased in patients with chronic kidney disease. As the evidence is being unveiled, plasma TMAO levels seem to emerge as an independent marker of all-cause mortality, but also of CKD complications, like CAD progress and cardiovascular ischemic events [33–36].

Furthermore, high TMAO concentration has been found to relate to unfavorable metabolic pattern and metabolic syndrome [37,38]. The link between TMAO and diabetes, one of most common metabolic diseases, has also been shown. Namely, risk of diabetes development and incidence of cardiovascular complications may be related to high plasma TMAO levels [37,39–41].

Increased plasma TMAO is not associated with cardiovascular risk

While in several clinical studies TMAO concentration is considered as a marker of negative prognosis, there are a few ones which seem to stand in opposition to this assumption. Results described by Gruppen and collaborators show TMAO association with mortality was seen only when patients' renal function (i.e. GFR and urine albumin) was not included in statistical model [42]. Having in mind the elimination of TMAO with urine, some studies in patients with CKD draw attention. The increase in plasma TMAO level following L-carnitine intake had beneficial effects on vascular injury in end-stage CKD patients [43]. In another cohort of CKD patients, no association between TMAO and all-cause mortality and other clinical events was observed [44].

What is more, in contrary to findings shown by Zhu and collaborators [45], there may be no association between plasma TMAO concentration and platelet hyperreactivity [46]. Furthermore, it has been found that in patients suffering from the stroke or transient

ischemic attack TMAO levels were lower in comparison to subjects with asymptomatic atherosclerosis, and the data show relation between gut bacteria dysbiosis and the severity of the disease [47]. In young adults TMAO concentration seems not to be related with intima-media thickness and coronary disease [48]. Similarly, analysis performed in Canadian population showed no link between TMAO and carotid artery intima-media thickness [4]. On another hand, in patients with exacerbated chronic obstructive pulmonary disease TMAO levels seem to be associated with mortality, but this link is no longer seen, when taking comorbidities into account [49].

Evidence supporting the hypotheses. TMAO as a protective factor

TMAO as an osmolyte and piezolyte. Protective functions of TMAO in animals

Piezolytes and osmolytes are small cosolutes that stabilize proteins against high hydrostatic and osmotic pressure, respectively [21,50–52]. The group includes trimethylamine oxide, glycine, taurine, sarcosine, betaine, glycerol, myo-inositol, sorbitol, GPC- glycerophosphorylcholine. A number of biophysical studies show that osmolytes and piezolytes protect structural and functional proteins from denaturants such as NaCl, urea, high temperature and high osmotic and hydrostatic pressures. The molecules enable cell proteins to work over a wider range of environmental conditions (changes in osmolarity and hydrostatic pressure) [19,21,50–54]. Some of those molecules e.g. TMAO may play a role of both a piezolyte and osmolyte.

The importance of piezolytes and osmolytes has been recognized in marine animals exposed to high hydrostatic stress (deep water) and osmotic stress (salty water) [50,51]. Marine animals accumulate TMAO and show over 100–1000-fold higher TMAO concentrations than mammals.

Research suggests that TMAO may help evolve resistance to high hydrostatic pressure. In shallow marine animals, TMAO is absent or present in less than 100 mmol kg-1 wet weight (except for ureosmotic fish such as sharks). In turn, deep-sea fish have up to 300 mmol kg-1 TMAO, increasing with depth. In osmocononformers, high levels of TMAO essentially replace ordinary osmolytes of shallow relatives [55].

In vitro TMAO was shown to neutralize the pressure-dependent effects in a number of lactate dehydrogenase homologs, actinic polymerization, enzyme-substrate binding for two enzymes and the growth of live yeast cells [20,21].

Recent studies also confirm that TMAO acts in a protective manner, preventing protein degeneration and structural changes in osmotic or thermal stress conditions [19,56,57]. Some of these effects can be observed when TMAO concentrations are close to the ones present in marine organisms [56].

Due to its protective properties, TMAO is used as a stabilizing agent during crystallization and protein purification [58]. TMAO can also be used as an additive to improve the quality of macromolecular crystals and has recently been shown to be an effective cryoprotective agent [58].

Finally, it has been shown that the administration of TMAO as a chemical protective device reduces experimental diabetic peripheral neuropathy [59], asthma [60], and cataract formation [61]. TMAO can also reduce the rates of oxidative damage in neuroblastoma [62], improve protein folding and secretion of miocilin mutant protein in spongificular reticulum scintigraphy [63,64]. TMAO, with caring activity, may facilitate the folding and secretion of mutant myocillin Asp384Asn, which can be used to treat glaucoma [63,65].

TMAO stabilizing action may not always be beneficial

While TMAO may serve as a protein stabiliser, a question emerges whether the stabilising action of TMAO is always beneficial. The stabilisation in the absence of a counteracting agent, leading to "overstabilisation" may cause a deleterious effect instead of an advantageous one. This phenomenon is called by some researchers "yin and yang of stabilisation" [51].

As shown by some research [66,67] high TMAO concentration in the absence of disturbing agents may produce highly rigid molecules, creating aggregates lacking their primary function. For example, the stabilization of enzymes by TMAO may affect energy gain from ATP lysis [68]. Furthermore, the protective activity of TMAO against thermal damage that increases the resistance to denaturation caused by heat may at the same time decrease the catalytic rate of LDH [69].

The circulatory system as a group of organs exposed to hydrostatic and osmotic stresses

Some of mammalian cells constantly or intermittently operate in the environment of significantly increased osmotic and hydrostatic stresses.

For example, cells of the kidney medulla or red blood cells passing the kidney medulla in vasa recta encounter the environment with 3–4-fold higher osmolarity than osmolarity of plasma.

Other cells such as cardiomyocytes operate under conditions of significant and frequent changes in hydrostatic pressure. For example, in a healthy heart hydrostatic pressure rapidly changes from zero to 120–130 mmHg, with a frequency of 60–220/min in humans, and higher in small animals.

Importantly, osmotic and hydrostatic stresses are significantly augmented in cardiovascular diseases such as hypertension. In hypertensive subjects a diastole-systole change in hydrostatic pressure in the heart may exceed 220 mmHg. Furthermore, cardiovascular diseases are associated with disturbances in water-electrolyte balance which produce changes in osmolarity and hydrostatic pressure in body water compartments reflected in the formation of central and peripheral edema.

So far, several compensatory, neuro-hormonal mechanisms to such distresses have been described. For example, it has been found that volume and pressure overloaded heart releases natriuretic peptides that decrease the overload by increasing natriuresis and diuresis. One of these peptides i.e. BNP is now commonly used in clinical practice as a marker of heart failure. This peptide is a good example of a cardiovascular marker, which increased plasma level correlates with increased mortality, however, the peptide itself plays a beneficial role and an increase in its plasma level is a compensatory response.

Studies suggesting beneficial effects of TMAO in the circulatory system in experimental studies

Recently, we have showed that high-salt diet, a cardiovascular risk factor, increases blood concentration of TMAO by decreasing its urine excretion in rats [22]. Furthermore, we have found that 60-week-old hypertensive rats had approximately 20% higher plasma TMAO level than healthy controls [23]. These may suggest that increased plasma concentration of TMAO may be a response to noxious factors, i.e. high-salt intake (osmotic stress) high blood pressure (hydrostatic stress).

Strikingly, 4–5-fold increase in plasma TMAO due to a chronic (12-month) dietary TMAO supplementation reduced diastolic dysfunction in pressure-overloaded heart in the hypertensive rats. Namely, in comparison to controls, TMAO-treated rats showed significantly lower plasma NT-proBNP and vasopressin, significantly lower left ventricular end-diastolic pressure and cardiac fibrosis [23]. These findings strongly suggest that a moderate increase in plasma TMAO does not have a negative effect on the circulatory system. In contrast, our studies seem to demonstrate the positive effect of TMAO.

Studies suggesting beneficial effects of TMAO in the circulatory system in humans

Fishes and seafood are important components of Mediterranean diet

thought to be a beneficial for human health [70,71]. Interestingly, increase in TMAO plasma concentration was described to be more pronounced after fish intake in comparison to eggs or red meat [14]. Also, the results published by Cheung and collaborators support the link between seafood diet and TMAO [72]. Lean-seafood diet has been shown to be beneficial in terms of metabolic state of human body, decreasing levels of circulating metabolites linked with insulin resistance and parallel to raised plasma TMAO concentration [44,73]. In other dietetic intervention trials, positive changes in TMAO plasma concentrations were not correlated with the rise of high sensitive C-reactive protein or low-density lipoprotein levels [74,75].

A study performed by Reiner and collaborators showed no association between bleeding and TMAO plasma levels in patients with venous thromboembolism [76]. What is interesting, the researchers also found a U-shaped association between mortality and TMAO concentration.

According to WHO reports, age-dependent mortality in the Japanese population, comparing to the North American one is lower [77,78]. Moreover, there is data showing opposite trend, when comparing urine TMAO concentrations between Japanese and Americans [79], which may be caused by the differences in TMAO intake due to fish-rich diet in Japanese population.

Evidence against the hypotheses

Studies suggesting negative effects of TMAO in the circulatory system in experimental studies

Yu and collaborators reported that TMAO increased the instability of atrial electrophysiology in normal canines, however, TMAO was injected locally i.e. atrial ganglionated plexi of the heart and in situ concentration of TMAO was not established [80]. Savi et al. in in vitro experiments showed that TMAO worsened cardiomyocyte mechanics [18]. Makrecka-Kuka and collaborators in in vivo experiments showed that TMAO impairs pyruvate and fatty acid oxidation in cardiac mitochondria in mice treated with TMAO that increased plasma TMAO level by 22-23-fold [17]. Importantly, the effect of such a high TMAO concentration in drinking water on water-electrolyte balance and energy balance was not reported. Finally, Organ and collaborators showed that heart failure severity is significantly enhanced in TAC mice fed TMAO-rich diet which increased plasma TMAO by 16-17-fold [16].

We believe that the discrepancy between our findings showing the positive effect of TMAO and studies showing the negative effect of TMAO may result from several factors, such as tested doses of TMAO and experimental settings (i.e. in vitro experiments). It is worth stressing that high doses of any compound may produce the opposite effects than smaller doses. This is in particular true for cardiovascular mediators such as norepinephrine, epinephrine, angiotensin II or natriuretic peptides. Therefore, it seems that the effect of TMAO on the circulatory system should be tested under experimental conditions which resemble concentrations of TMAO in cardiovascular diseases i.e. 2–4 times higher than in healthy subjects [7,25]. In this regard, paraphrasing Paracelsus (1493–1541), all agents are poisons, and only the dose makes them a cure.

Implications of the hypotheses

Despite a significant progress in diagnosis and treatment, the mortality and cost of care due to cardiovascular diseases is very high worldwide. Recent decades are characterized by a rapid growth of interventional cardiology but a little progress in prevention and pharmacological treatment. This partially results from significant gaps in the knowledge on physiological and pathological processes occurring in the circulatory system.

The role of TMAO in the circulatory system has not been established, whereas there is a number of studies showing a significant protective effect of TMAO in marine animals.

Further studies on the role of TMAO are needed to establish its physiological or perhaps pathological role in the circulatory system. Long-term experimental studies evaluating the effect of TMAO at concentrations found in cardiovascular diseases are lacking.

If our hypothesis is true i.e. if TMAO is accumulated as a compensatory response of the organism to disturbances in the circulatory system, new preventive (e.g. dietary) and pharmacological strategies based on TMAO supplementation may be developed.

Conclusions

There is a growing interest in the role of TMAO in the circulatory system. We hypothesize that increased plasma TMAO may be a marker of cardiovascular risk and that increased level of plasma TMAO is a compensatory response, which may exert beneficial effects i.e. protect cells from hydrostatic and osmotic stresses in cardiovascular diseases.

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Declaration of Competing Interest

None.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mehy.2019.109271.

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