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Review

Gut microbiota metabolism of L-carnitine and cardiovascular risk

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ABSTRACT

In recent years, a number of studies have alluded to the importance of the intestinal microflora in controlling whole-body metabolic homeostasis and organ physiology. In particular, it has been suggested that the hepatic production of trimethylamine-*N*-oxide (TMAO) from gut microbiota-derived trimethylamine (TMA) may enhance cardiovascular risk via promoting atherosclerotic lesion development. The source of TMA production via the gut microbiota appears to originate from 2 principle sources, either phosphatidylcholine/choline and/or L-carnitine. Therefore, it has been postulated that consumption of these dietary sources, which are often found in large quantities in red meats, may be critical factors promoting cardiovascular risk. In contrast, a number of studies demonstrate beneficial properties for L-carnitine consumption against metabolic diseases including skeletal muscle insulin resistance and ischemic heart disease. Furthermore, fish are a significant source of TMAO, but dietary fish consumption and fish oil supplementation may exhibit positive effects on cardiovascular health. In this mini-review we will discuss the discrepancies regarding L-carnitine supplementation and its possible negative effects on cardiovascular risk through potential increases in TMAO production, as well as its positive effects on metabolic health via increasing glucose metabolism in the muscle and heart.

Contents

1. Introduction

Cardiovascular disease is a leading cause of death world wide, of which a significant portion can be attributed to ischemic heart disease, often as a result of underlying coronary artery disease due to atherosclerosis. Risk factors for atherosclerosis include dyslipidemia (i.e. elevated serum cholesterol, triglycerides, and low-density lipoproteins), hypertension, obesity, smoking, and diabetes [1,2]. Current therapies for atherosclerosis that target these risk factors, such as the first-line therapy statins (which inhibit 3-hydroxy-3-methyl-glutaryl-CoA reductase to reduce cholesterol production) are quite effective in preventing and treating this

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disease [3]. However, there remains a large number of patients that are refractory to statin therapy and other conventional therapies who will see their atherosclerotic disease worsen, and will ultimately die of other cardiovascular diseases such as myocardial infarction (MI) or stroke [4]. Therefore, there is a growing need to better understand the mechanisms contributing to the formation of the atherosclerotic plaque, and how to reverse its progression. In recent years, knowledge pertaining to the role of the intestinal microbiota as an external factor contributing to obesity and its associated co-morbidities has considerably grown [5].

Of interest, recent developments suggest that metabolism of dietary nutrients by the intestinal microbiota may contribute to atherosclerosis and subsequent cardiovascular disease in obese rodents and humans [6–8]. The aims of this mini-review are: (1) to highlight the role of the intestinal microbiota in atherosclerosis development, (2) to discuss the dependency of this effect on microbiota-derived trimethylamine-*N*-oxide (TMAO), and (3) to challenge the notion that enhanced L-carnitine metabolism in obesity may increase cardiovascular risk, due to L-carnitine acting as the major driver of TMAO production via the gut microbiota.

2. Gut microbiota-derived trimethylamine-*N*-oxide and cardiovascular risk

Trimethylamine (TMA) produced via the gut microbiota is oxidized with hydrogen peroxide via the enzymatic activity of hepatic flavin monooxygenase (FMO), resulting in the production of the organic compound, TMAO (Fig. 1). As such, consumption of compounds that can produce TMAO, such as phosphatidylcholine (PC) and L-carnitine, has the potential to result in elevated circulating TMAO levels [6,8]. TMAO is also commonly found in large amounts in saltwater fish and various members of the *Elasmobranchii* fish subclass (i.e. sharks and rays), and studies suggest that it is a proatherogenic compound that is positively associated with increased cardiovascular risk in both rodents and humans [6–8].

Support for a proatherogenic action of TMAO was first demonstrated by Wang et al., who demonstrated that consumption of a choline-enriched diet (1% in food wt/wt) for 16 weeks increased macrophage foam cell formation and atherosclerotic lesion area in C57BL/6J-*Apoe*$^{-/-}$ mice [8]. Illustrating the dependence of the gut microbiota for this effect, these effects were eliminated in both male and female C57BL/6J-*Apoe*$^{-/-}$ mice supplemented with antibiotics in the drinking water during choline diet-enrichment. Similarly, Koeth et al. demonstrated that consumption of an L-carnitine-enriched diet (1.3% in drinking water) for 15 weeks exacerbated atherosclerotic lesion area in C57BL/6J-*Apoe*$^{-/-}$ female mice [6]. Once again, these effects were prevented in female mice supplemented with antibiotics in the drinking water during L-carnitine diet-enrichment. Alluding to the clinical relevance of these findings, humans subjected to a dietary PC-choline challenge (consumption of 2 hard-boiled eggs with yolk containing approximately 500 mg total choline combined, plus a gelatin capsule containing 250 mg of deuterium-labeled PC) exhibited a significant increase in both urine and plasma levels of TMAO and radiolabeled-TMAO [7]. Consumption of broad-spectrum oral antibiotics for 1 week in these same participants resulted in an almost complete suppression of detectable TMAO and radiolabeled-TMAO during an identical PC-choline dietary challenge, which reverted back to normal during a third PC-choline dietary challenge following antibiotic withdrawal for at least a month [7]. In addition, a 3-year follow up of over 4000 patients undergoing coronary angiography examining the relationship between fasting plasma levels of TMAO and incident major cardiovascular events including MI and death, reported a significant positive correlation between the two variables [7].

The mechanisms by which TMAO promote atherosclerosis and increase cardiovascular risk are not completely understood, but it has been demonstrated that TMAO arising from both choline and L-carnitine diet supplementation in mice may inhibit reverse cholesterol transport (RCT) [6]. This reduction in RCT was attenuated in mice treated with antibiotics, highlighting the importance

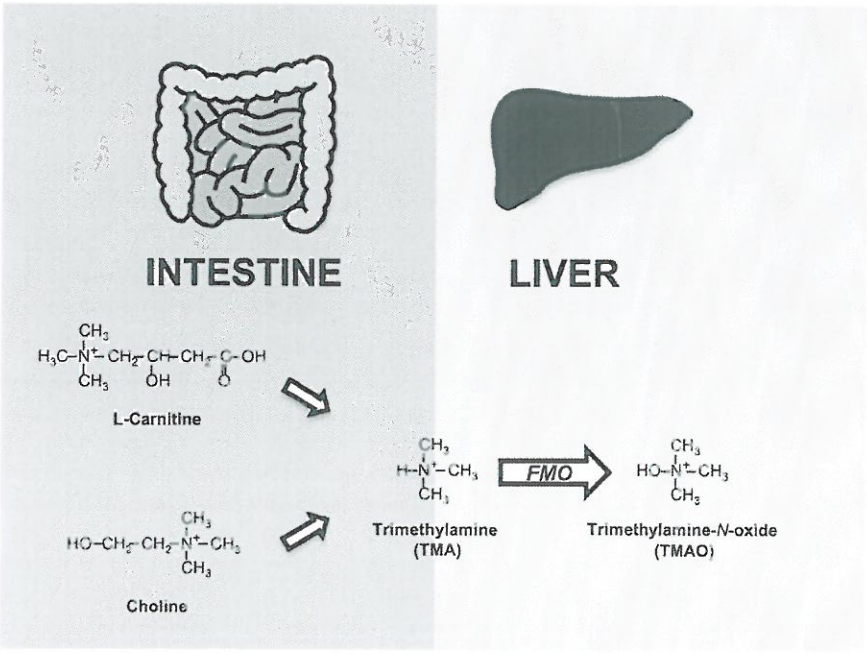

Fig. 1. Gut microbiota-dependent production of TMAO. The bacteria inhabiting the intestine are able to produce TMA from the TMA-containing compounds choline and L-carnitine, which are obtained in significant quantities from dietary red meats, eggs, and dairy products. This gut microbiota-derived TMA is subsequently converted into TMAO in the liver by the enzymatic activity of FMO. FMO – flavin monooxygenase, TMA – trimethylamine, TMAO – trimethylamine-*N*-oxide.

of gut microbiota-derived TMAO for this effect. Furthermore, mice supplemented with a diet enriched in TMAO also exhibited a marked reduction (35%) in RCT [6]. TMAO may additionally impact cholesterol metabolism via decreasing bile acid synthesis, as TMAO supplementation in mice reduced hepatic mRNA expression of the bile acid synthesizing enzymes, Cyp7a1 and Cyp27a1 [6].

3. Different sources of trimethylamine-*N*-oxide

As discussed, dietary TMA-containing compounds such as PC and L-carnitine are significant sources of gut microbiota-derived TMAO [6,8]. Furthermore, as TMAO is enriched in saltwater fish, consumption of fish such as cod represents a significant source of TMAO. Indeed, an 8-ounce cod contains more than 1 g of TMAO, with squid containing even more [9]. The different exogenous sources that may result in TMAO production via the gut microbiota, and the dietary means by which they can be consumed by humans are as follows:

3.1. Phosphatidylcholine/choline (PC)

PC, also commonly referred to by its generic term, lecithin, represents a class of phospholipids containing a choline headgroup. It is a naturally occurring substance found in many foods, some of which include red meat (especially liver), eggs, soybeans, and peanuts, and it is an essential component for the stability of biological membranes. In addition, PC is the primary phospholipid component of circulating very low-density and low-density lipoproteins (VLDL/LDL) [10]. As a TMA-containing compound, upon dietary consumption, PC is cleaved into choline, and the gut microbiota release TMA, which is converted into TMAO by FMO in the liver. Previous studies have observed a positive correlation between total plasma choline levels and the risk of developing cardiovascular disease [11], whereas choline deficiency has also been linked to atherosclerosis [12], with choline-deficient rats predisposed to cardiovascular disease and exacerbated cardiovascular lesions [13]. In contrast, *Apoe*$^{-/-}$ mice deficient for phosphatidylethanolamine *N*-methyltransferase, an enzyme responsible for approximately one-third of PC synthesis in the liver, are protected against the formation of atherosclerotic lesions and exhibit a marked improvement in systolic function [14]. This cardioprotection is associated with a modest reduction in hepatic PC content, in addition to significant reductions in triacylglycerol and cholesterol levels in the VLDL and LDL fractions, respectively.

Of interest, PC is a significant component of bile, and biliary PC has been shown to constitute up to 40% (~4–6 g) of the organic material of bile [15]. Based on this, one would anticipate that circulating TMAO levels in humans would consistently be detectable at high levels and increase one's risk for atherosclerosis, which is clearly not the case [7]. Thus, it is likely that the majority of PC present in bile is reabsorbed in the ileum and does not reach the cecum/colon to act as a precursor for TMAO. Nevertheless, whether there is a fraction of biliary PC that reaches the cecum/colon, and whether there are differences in dietary PC versus biliary PC as a source for gut microbiota-derived TMAO remains to be determined and something that needs to be considered in future studies.

3.2. L-Carnitine

L-Carnitine is a quaternary ammonium compound biosynthesized from the amino acids methionine and lysine. It plays an essential role in energy metabolism, particular in the catabolism of fatty acids, as fatty acyl CoA esters must be esterified into their respective fatty acylcarnitine moieties in order to be transported into the mitochondria for subsequent fatty acid oxidation and production of ATP [16,17]. L-Carnitine also plays an important role in carbohydrate metabolism, as acetylcarnitine transport out of the mitochondrial matrix into the cytosol via the enzymatic activity of carnitine acetyltransferase relieves acetyl CoA mediated inhibition of pyruvate dehydrogenase, which promotes glucose oxidation and is a key step in the metabolic flexibility associated with the fasting-to-refeeding transition [18,19]. L-Carnitine is generally found in red meats and dairy products such as milk and cheese, with other sources including peanut butter and asparagus. Similar to PC/choline, L-carnitine is a TMA-containing compound that upon dietary consumption will result in the release of TMA via the gut microbiota, which is then converted into TMAO by hepatic FMO. Despite recent studies suggesting that gut microbiota-dependent metabolism of L-carnitine can promote atherosclerosis due to increased production of TMAO [6], other studies suggest that L-carnitine may confer a number of different metabolic health benefits (see below) [18–22]. Another aspect to consider regarding L-carnitine as a source for gut microbiota-derived TMAO production involves the kinetics of cellular L-carnitine transport. Cellular L-carnitine levels are significantly higher than plasma L-carnitine levels, and thus L-carnitine is actively transported into cells in a slow sodium-dependent manner [23,24]. Whether the same processes are involved in L-carnitine transport into microbial cells remains to be determined, though Koeth et al. demonstrated detection of TMAO in plasma via liquid chromatography-mass spectrometry within minutes following an L-carnitine challenge in humans [6]. However, these L-carnitine supplementation studies were performed in combination with eating an 8-ounce sirloin steak. As steak is also a rich source of choline, which is a major source of gut microbiota-derived TMAO [8], this may account for the rapid detection of TMAO in plasma.

3.3. Fish

As mentioned previously, TMAO is found in significant quantities in marine fish including cod and other teleosts, as well as members of the animal subclass, *Elasmobranchii* (i.e. sharks and rays). In general, members of the teleost infraclass contain anywhere from 20 to 70 μmol/g wet wt muscle, whereas members of the *Elasmobranchii* subclass can accumulate levels up to 140 μmol/g wet wt muscle [25]. Regarding its physiological role in marine fish, TMAO may act as an important osmoregulatory compound affecting buoyancy in elasmobranches [26], whereas in teleosts it may play a role in protecting protein function against the effects of high pressure [9].

Much of the original work investigating the metabolism of TMAO was carried out in cod [27,28]. However, it has been suggested that despite the significant amounts of TMAO observed in saltwater fish, the majority of this TMAO may be derived from their natural food supply, as most fish exhibit very low to non-detectable FMO activity [29]. Indeed, the zooplankton, *Calanus finmarchicus*, which is a primary food source for fish and whales in the North Atlantic, has significant soluble monooxygenase activity with a high specificity towards TMA, and may represent a plentiful source of dietary TMAO [27]. On the other hand, in addition to cod, a number of marine fish that comprise a significant portion of the world population's seafood diet (including salmon, trout, and tilapia) all exhibit significant FMO activity and measurable TMAO levels [30–32]. Despite the controversy regarding whether TMAO is produced endogenously in marine fish or obtained via dietary means, this does not change the fact that marine fish represent a significant source of dietary TMAO for humans. In a study involving 44 men, urinary levels of TMAO were positively correlated with weekly fish intake [33].

4. L-Carnitine and cardioprotection

Despite the observations reported by Koeth et al. suggesting that L-carnitine may promote atherosclerosis via acting as a source of gut microbiota-derived TMA for hepatic TMAO production [6], these results must be interpreted with caution. In the rodent studies suggesting that L-carnitine supplementation promotes atherosclerosis in a gut microbiota-dependent fashion, C57BL/6J-*Apoe*$^{-/-}$ mice were supplemented for 15 weeks with L-carnitine in the drinking water at a concentration of 1.3%. At this concentration, a 25 g mouse consuming ~4 mL of water a day would be ingesting 2.08 g/kg/day of L-carnitine, a dose 1000 times higher than that taken by a 70 kg human eating an 8-ounce sirloin steak, which contains 180 mg of L-carnitine. Hence, whether these findings are translationally relevant in humans is unknown. Furthermore, the extrapolation from C57BL/6J-*Apoe*$^{-/-}$ mice to human studies is problematic because of significant differences in: (1) cholesterol metabolism and lipid profiles, (2) cardiovascular physiology, (3) plaque pathology, and (4) the relative lack of lesion progression leading to thrombotic occlusion and clinical events in rodents [34]. Combined with the massive dose of L-carnitine utilized, the interpretation of the findings of Koeth et al. is challenging. As mentioned previously, further complicating this issue is that the L-carnitine supplementation studies in humans were performed in combination with consuming an 8-ounce sirloin steak. Steak is also a rich source of choline, which is a major source of gut microbiota-derived TMAO [8]. Therefore, the link between L-carnitine supplementation and plasma TMAO levels is inconclusive. In addition, a recent systematic review and meta-analysis of 13 controlled trials ($N = 3629$) showed that, compared to placebo, L-carnitine actually causes a 27% reduction in all-cause mortality, a 65% reduction in ventricular arrhythmias, and 40% reduction in angina symptoms in patients experiencing an acute MI [35]. The majority of the studies analyzed used an oral dosage of L-carnitine ranging from 2 to 6 g per day, a much lower dose than that used by Koeth et al. in C57BL/6J-*Apoe*$^{-/-}$ mice (~25–70 times less) [6].

The overall conclusions of the systematic review by DiNicolantonio et al. are consistent with a number of studies suggesting that carnitine may have beneficial effects on metabolic health and cardiovascular function (see Fig. 2 for potential cardioprotective properties of L-carnitine). As such, direct treatment of the isolated working rat heart with L-carnitine increases glucose oxidation rates and protects against *ex vivo* ischemia/reperfusion injury [20]. In the heart, an elevation of glucose oxidation results in a corresponding decrease in fatty acid oxidation, which has been postulated to protect the ischemic heart by enhancing the efficiency of conversion of energy into mechanical work [36,37], and this translates to improvements in cardiac function and a reduction in infarct size *in vivo* [38]. Furthermore, mice fed a 45% high fat diet supplemented with L-carnitine in their drinking water at a dose of 300 mg/kg/day exhibited marked improvements in glucose tolerance and insulin sensitivity compared to non-supplemented mice [19]. These findings have been recapitulated in humans, as a 6-month supplementation with 2 g/day oral L-carnitine lowered plasma glucose levels, plasma insulin levels, and HOMA scores in elderly obese patients [18].

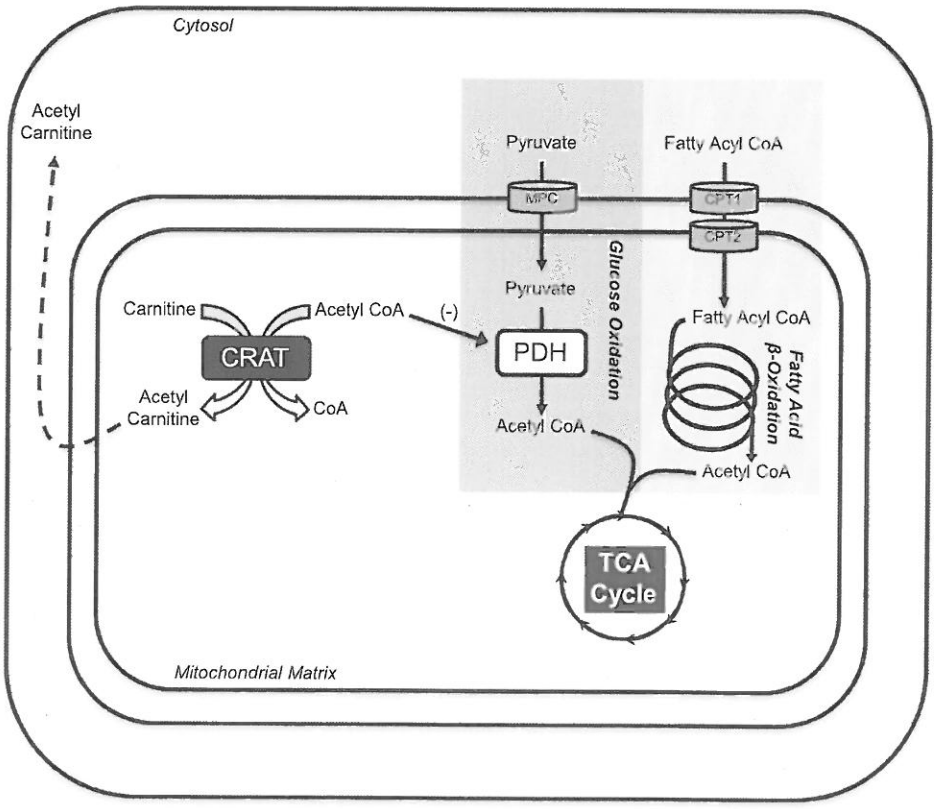

Fig. 2. Potential beneficial metabolic properties of L-carnitine. Carnitine has been postulated to have beneficial effects on metabolism via stimulation of glucose oxidation. As fatty acid β-oxidation-derived acetyl CoA inhibits PDH, the rate-limiting enzyme of glucose oxidation, supplementation with carnitine increases levels of carnitine substrate for CRAT, which lowers the acetyl CoA/CoA ratio in the mitochondrial matrix, thereby relieving inhibition of PDH and increasing glucose oxidation. CPT – carnitine palmitoyl transferase, CRAT – carnitine acetyltransferase, MPC – mitochondrial pyruvate carrier, PDH – pyruvate dehydrogenase, TCA – tricarboxylic acid.

These aforementioned protective effects of L-carnitine on metabolism and cardiac function are in contrast to the dose-dependent positive associations between plasma carnitine concentrations and risk of prevalent coronary artery disease and overall cardiovascular disease in humans observed by Koeth et al. [6]. However, this is just a correlation and not conclusive of whether L-carnitine has a negative effect on cardiovascular health. In the setting of inborn mitochondrial diseases, it has been suggested that acylcarnitine production and subsequent tissue export may act as a detoxifying system that enables efflux of excess acyl groups from the mitochondria [39]. Thus, it is also important to determine the accumulation of different carnitine esters (i.e. long-chain acylcarnitines) in tissues with high mitochondrial activity, such as the heart, versus just assessing plasma levels, to better understand the metabolic status/health of the tissue [40]. Nevertheless, based on the evidence to date, there does not appear to be an overwhelming amount of evidence to support the notion that L-carnitine supplementation has direct actions on the body that increase cardiovascular risk.

5. Fish consumption and cardioprotection

Since fish are a major source of the TMAO that has been implicated in increasing cardiovascular risk, it is important to discuss dietary fish consumption in the context of cardiovascular health (which is an area of active investigation [41]). Indeed, there have now been a number of randomized clinical trials examining whether fish consumption, and in particular, whether omega-3 fatty acid (i.e. fish oils) supplementation, can improve cardiovascular outcomes [42]. While a number of studies have demonstrated beneficial effects of omega-3 fatty acid supplementation on cardiac function and cardiovascular risk factors [43,44], the evidence from recent meta-analyses do not support a cardioprotective role against cardiovascular diseases including MI, heart failure development, sudden cardiac death, and stroke [45–47]. Whether this overall lack of positive findings could potentially be due to the aforementioned possible atherogenic-promoting effects of TMAO at this stage is too preliminary to determine. First, a number of the randomized clinical trials utilize omega-3 fatty acid supplements rather than enforcing strict dietary fish regimens, and thus the reported findings would be TMAO-independent. Second, in those trials whose aim is to look strictly at dietary fish consumption and cardiovascular risk, it is not clear whether the fish consumed are those that are high in both omega-3 fatty acids and TMAO. Furthermore, to our knowledge, no dietary fish consumption and cardiovascular risk trial to date has measured TMAO levels in humans. As we enhance our understanding of the role of TMAO in humans and its potential effects on RCT and progression of atherosclerosis, TMAO may become an important biomarker to measure in these trials. It is worth noting, however, that one trial examining the relationship between consumption of a Southern European Atlantic diet (SEAD) and the occurrence of non-fatal acute MI reported a lower risk for acute MI occurrence [48]. As both cod and red meats/dairy (significant source of L-carnitine) make up key components of the SEAD, it suggests that both L-carnitine and TMAO may not increase cardiovascular risk in humans. Nevertheless, this study was a population-based case–control study, and therefore definitive conclusions cannot be drawn from it, illustrating once again the challenges faced in determining whether increasing dietary fish consumption will confer cardiovascular benefit in humans.

6. Summary

Recent studies by Hazen and colleagues [6–8] allude to the potential contribution of gut-microbiota-derived metabolism of dietary PC/choline and L-carnitine towards production of TMAO, which may increase the risk of developing atherosclerotic heart disease. Such observations contrast with a multitude of studies demonstrating beneficial effects of L-carnitine on metabolic health, including protection against obesity-induced insulin resistance and myocardial ischemia/reperfusion injury in both the non-diabetic and diabetic heart. A possible explanation for these discrepant findings by Koeth et al. [6], may be due to extremely large doses of L-carnitine supplementation, and the use of steak during L-carnitine challenges, which is also a source of choline that may contribute to the appearance of TMAO. Further complicating the issue is the fact that both PC/choline and L-carnitine are components of bile. Therefore, the intestinal transport/metabolism of PC/choline and L-carnitine, and whether they actually reach the cecum/colon and contribute to the potential generation of gut microbiota-derived TMAO, independent of their dietary contributions, also needs to be considered. Moreover, recent findings from the European prospective investigation into cancer and nutrition (EPIC), a prospective cohort study of over 400,000 men and women, demonstrated a positive association between processed red meat consumption and mortality due to cardiovascular diseases after multivariate adjustment [49]. Of interest, this positive association was not observed for unprocessed red meat consumption [49], which has the highest L-carnitine content of the red meats [50]. Such findings suggest that perhaps L-carnitine supplementation and subsequent TMAO generation are not the root cause for the associations between red meat consumption and cardiovascular risk. In the USA, processed meats may comprise on average 400% more sodium than unprocessed red meats [51], which may be another critical component of red meats promoting cardiovascular disease. Nonetheless, gut microbiota-derived metabolism of dietary nutrients and how they may impact metabolic health is an exciting new area of research still in its infancy, and due to this, it would appear premature to think that both L-carnitine metabolism and TMAO production are the major mechanisms via which the gut microbiota can enhance cardiovascular risk.

Disclosures

The authors have no conflicts to disclose.

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