Minireview

Short chain fatty acids and methylamines produced by gut microbiota as mediators and markers in the circulatory system

Maksymilian Onyszkiewicz, Kinga Jaworska and Marcin Ufnal

Department of Experimental Physiology and Pathophysiology, Laboratory of Centre for Preclinical Research, Medical University of Warsaw, Warsaw 02-097, Poland

Corresponding author: Marcin Ufnal. Email: mufnal@wum.edu.pl

Impact statement

Despite a progress in the diagnosis and treatment of cardiovascular diseases, there are still significant gaps in understanding complex mechanisms underlying cardiovascular pathology. Increasing evidence suggests that gut microbiota products such as short chain fatty acids or methylamines may affect the circulatory system in health and disease. Hence, the microbiota-derived molecules are potential diagnostic and therapeutic targets in cardiovascular diseases. Therapeutic options may include administration of selected bacterial strains (probiotics) producing desired metabolites or administration of direct gut microbiota products.

Abstract

Ample evidence suggests that gut microbiota-derived products affect the circulatory system functions. For instance, short chain fatty acids, that are the products of dietary fiber bacterial fermentation, have been found to dilate blood vessels and lower blood pressure. Trimethylamine, a gut bacteria metabolite of carnitine and choline, has recently emerged as a potentially toxic molecule for the circulatory system. To enter the bloodstream, microbiota products cross the gut–blood barrier, a multilayer system of the intestinal wall. Notably, experimental and clinical studies show that cardiovascular diseases may compromise function of the gut–blood barrier and increase gut-to-blood penetration of microbiota-derived molecules. Hence, the bacteria products and the gut–blood barrier may be potential diagnostic and therapeutic targets in cardiovascular diseases. In this paper, we review research on the cardiovascular effects of microbiota-produced short chain fatty acids and methylamines.

Keywords: SCFA, TMA, TMAO, bacterial metabolites, blood pressure, cardiovascular diseases

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Gut bacteria in human homeostasis

The mammalian gut and skin is colonized early after birth by bacteria and fungi.[1,2] The number of gut bacteria is at least equal to the number of human body cells and, at the same time, the composition of human microbiota is very unique for each person. The composition of the gut microbiota depends on numerous factors including geography, age, diet, the mode of delivery, and postnatal feeding.[3–7] For example, it has been shown that vaginal birth and breastfeeding are associated with more diverse microbiota than cesarean birth and formula-feeding.[8–10] The most common bacterial phyla in the human gut include Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria.[11,12]

Increasing research provides evidence that human homeostasis depends on reciprocal interaction with gut microbiota. On the one hand, gut microbiota produces vital compounds for human homeostasis such as vitamin K or B group (cobalamin, biotin, pyridoxine, thiamine, folates, riboflavin and nicotinic acid), and contributes to the metabolism of bile acids, steroids and xenobiotics.[13–16] On the other hand, gut bacteria produce toxic substances such as ammonia or trimethylamine and may reduce the availability of essential nutrients for the host.[17–19]

Finally, recent research shows that microbiota produced molecules such as hydrogen sulfide (H_2S), indoles, short chain fatty acids and trimethylamines affect the circulatory system homeostasis, acting on its humoral and nervous control.[20–22] In particular, short chain fatty acids and trimethylamines have attracted a lot of attention, which resulted in the explosion of experimental and clinical papers on the circulatory effects of the two groups of microbiota

products.[23–25] It is also worth noting that some research reported changes in gut bacteria composition also referred to as dysbiosis in cardiovascular diseases (CVDs).[26–28]

Mechanisms of a crosstalk between gut microbiota and the circulatory system

The mechanisms underlying the interaction between gut microbiota and the circulatory system are far from clear. However, increasing experimental data show that at least two potential pathways are likely (Figure 1). Firstly, gut microbiota-derived products may stimulate afferent (sensory) nerves of the enteric nervous system which may modulate the cardiovascular centers in the brain. The latter affects the activity of the autonomic nervous system that controls the function of the heart, vasculature and glands releasing cardiovascular hormones. Secondly, gut microbiota products may enter the bloodstream and thereby affect the function of organs and tissues that contribute to the circulatory system homeostasis. As most of the gut-derived molecules first pass the liver, the systemic effects of microbiota products may also depend on liver metabolites.[29–31]

It has been well established that biological effects of any molecule depend on its concentration. In this regard, the blood concentration of bacterial metabolites depends on several factors including gut bacteria metabolic activity, the function of the intestinal wall (gut-to-blood permeability) and the function of the liver and the kidneys (metabolism, detoxification and excretion). Deteriorated function of the kidneys and the liver in cardiovascular diseases is well established and has been described in numerous papers.[32–37] Recently, the decline of the intestinal function and a "leaky gut" syndrome in cardiovascular diseases has gained a lot of attention.[38–40]

In short, to enter the circulation, gut bacteria products need to cross the intestinal wall that forms the gut–blood barrier (GBB). The GBB is a complex system of several biological layers, including vascular endothelium, epithelial cell lining and mucus layer. The integrity and proper functioning of the GBB depend on numerous, not fully understood factors. The GBB enables the absorption of nutrients and beneficial bacterial metabolites such as SCFAs from intestinal lumen and, at the same time, reduces the passage of potentially toxic substances.[41] A pivotal factor for the proper functioning of the GBB is adequate blood perfusion. There is some experimental and clinical evidence showing that CVDs compromise the GBB function by decreasing intestinal blood flow and disrupting the GBB structure. A leaky gut has been reported in patients with heart failure[42] and in several experimental models of CVDs.[39,43] For example, we have found that hypertension in rats is accompanied by morphological and hemodynamic alterations in the colon, and increased permeability of the colon to TMA, a toxic gut bacteria metabolite.[43]

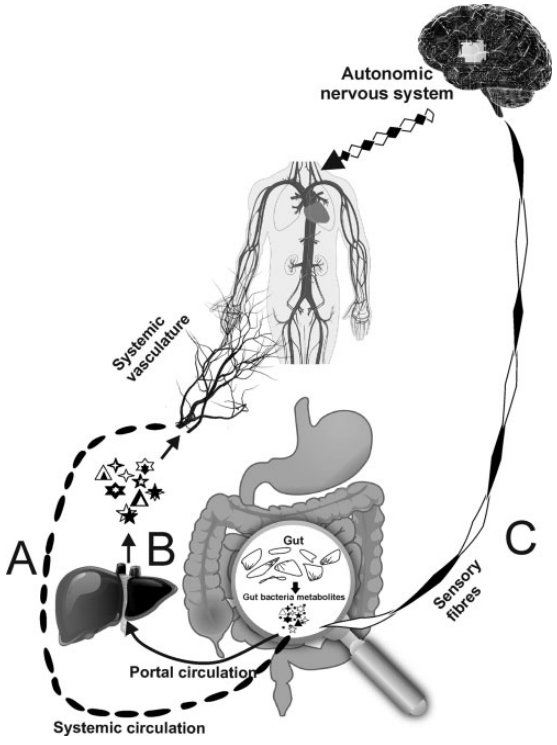

Figure 1. Suggested pathways of cardiovascular actions of gut microbiota products. (A) Gut bacteria products cross the gut–blood barrier, bypass the liver via rectal plexuses and reach cardiovascular tissues. (B) The liver produces derivatives of microbiota metabolites that affect the heart and vasculature. (C) Gut microbiota metabolites stimulate sensory fibers of the enteric nervous system which project to the brain that controls the circulatory system via the autonomic nervous system.

Short-chain fatty acids

Various biological effect of short-chain fatty acids (SCFAs) have been described in medical literature since ancient times. However, only recently it has been recognized that gut microbiota is an important source of SCFAs in mammalian organisms. SCFAs are produced chiefly by the colon microbiota in fermentation process from dietary fiber. This group of fatty acids consists of aliphatic tail with five or fewer carbons.[44,45] There is evidence that SCFAs are also present in intestinal content of germ-free animals, but at lower concentration than in conventional animals.[46,47] In addition, a group of dietary products constitutes a direct source of short chain fatty acids including acetate, propionate and butyrate (Figure 2).

SCFAs play an important role locally in intestines serving as an energy source for enterocytes and inhibiting the growth of pathogens by reducing pH. In addition, SCFAs may affect the local intestinal microcirculation.[48,49] Furthermore, SCFAs originating from gut microbiota metabolism enter the systemic circulation through the GBB. Biological effects of SCFAs including metabolic, hemodynamic and inflammatory effects are thought to be mediated by GPR41 (also known as Free Fatty Acid Receptor 3, FFAR3), GPR43 (Free Fatty Acid Receptor 2, FFAR2), or Olfr78 receptors which are located in the gastrointestinal tract, adipose tissue, immune cells, and peripheral nerves.[30,50–54] Accumulating evidence suggests that SCFAs may exert blood pressure lowering effect by decreasing vascular tone.[55–59] Furthermore, SCFAs may change blood pressure by mechanisms related to

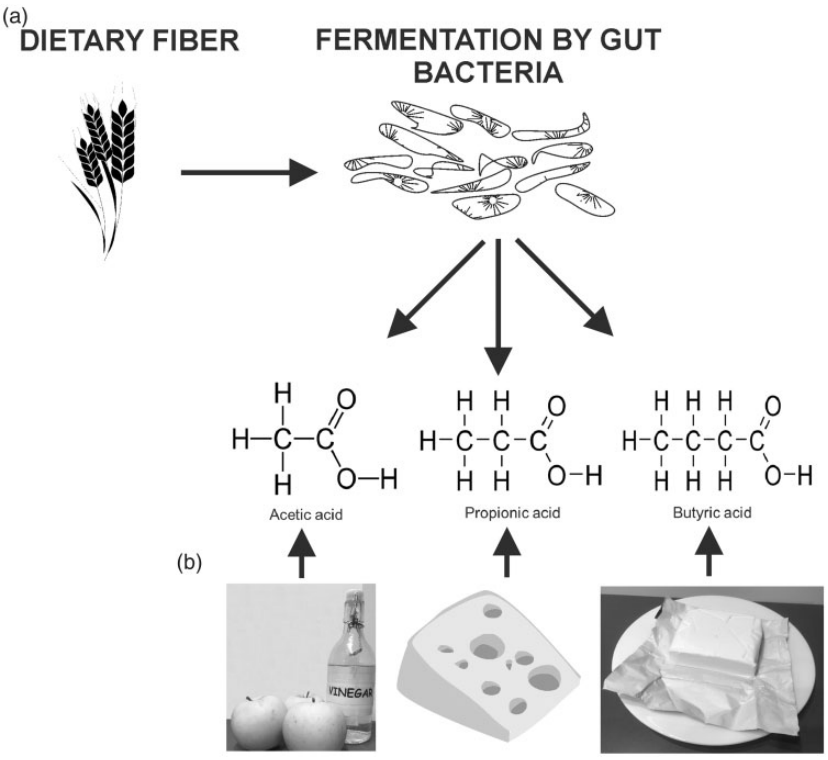

Figure 2. Sources of short chain fatty acids (SCFAs) in human organism. (a) SCFAs are produced during fermentation process from dietary fiber; (b) Dietary products constitute a direct source of SCFAs including acetate, propionate and butyrate.

gut-to-brain nervous signaling[60] renal sensory nerves[31,53] and renin-angiotensin-aldosterone pathway.[53,61–63]

Acetic acid

Acetic acid is a colorless liquid organic compound which consists of methyl group attached to a carboxyl group. It possesses acrid smell and sour taste. Liquid containing 5–20% of acetic acid is called vinegar and was used as a food preservative by Babylonians (c. 5000 BC).[64] Moreover, vinegar was used for dressing wounds by Hippocrates (c. 420 BC) and in American civil war, likely due to its antimicrobial properties.[65] It has also been suggested that acetic acid prevents osteoporosis by increasing calcium intestinal absorption and the concentration of calcium in bones.[66] Moreover, acetic acid was reported to exert antidiabetic effects by improving glucose uptake in skeletal muscles and decreasing fasting and postprandial glucose levels.[67–69] With regard to cardiovascular effects, acetic acid has been found to lower arterial blood pressure in rats.[70,71] The mechanisms behind the hypotensive effect may include the inhibition of ACE activity, decrease in plasma renin activity or increase in tissue level of cAMP.[72–75] In vitro studies report that acetic acid produces vasodilatation in colonic resistance vessels.[76] Additionally, acetate might play a role in flow-mediated vasorelaxation by inducing eNOS phosphorylation in the endothelium.[77] Clinical studies involving patients regularly undergoing hemodialysis with dialysate containing acetate showed a significant decrease in peripheral vascular tone and diminished myocardial contractility.[78–80]

Propionate

Propionic acid is a colorless oily liquid organic compound, miscible with water, with characteristic unpleasant smell fairly resembling body odor. Antimicrobial effects of propionic acid and its salts have been known for a long time. Propionates are used in the food industry as preservatives in many products such as bread or cheese.[81,82] Propionic acid has been found to exert several potentially health-promoting effects which may lower cardiovascular risk. Firstly, propionate may inhibit lipid synthesis in hepatocytes and thereby decrease cholesterol level.[83,84] Secondly, some evidence suggests that propionic acid has anti-inflammatory effect[85] and improves insulin sensitivity.[86–88] Notably, propionic acid is the strongest ligand for the SCFAs receptors Gpr41 and Gpr43.[89,90]

Finally, some studies show that propionic acid exerts direct cardiovascular effects. A dose-dependent vasodilatory effect have been reported in rat mesenteric arteries and in isolated human colonic resistance arteries.[76,91]

Butyric acid

Butyrate is a colorless carboxylic acid produced in the mammalian gut by bacterial fermentation of dietary fiber. However, butyric acid is also present in butter and other dairy products. The taste of butyrate is characterized as pungent, with a sweetish aftertaste which can be linked with the taste of ether. The acid is responsible for the obnoxious smell of human vomit.

Butyrate has been found to affect numerous physiological processes including energy homeostasis,

cardiovascular parameters, immune response, and brain functions. The underlying mechanisms may range from metabolic effects of receptor's signaling to enzymatic inhibition.[92,93] Some evidence suggests that butyric acid exerts anticarcinogenic effect that may be explained by the Warburg effect. The opposing effect of butyric acid on normal and cancer cells sometimes is called as butyrate paradox.[94,95]

Numerous studies show that butyrate may affect the regulation of arterial blood pressure. The hypotensive and bradycardic effect of intravenous administration of butyric acid was described already in 1957 by Wretlind.[96]

Blood pressure lowering effect of butyrate seems to be dependent on vasodilation. Mortensen *et al.* showed that butyric acid and other SCFAs produce a concentration-dependent dilation of human colonic resistance arteries.[76] Likewise, a weak vasodilatory effect of butyric acid at concentrations above 5 mM was reported in the coronary arteries.[57] The vasodilatory effect of butyric acid was also demonstrated in rat caudal artery.[58] Several mechanisms of the butyric acid-dependent vasodilation have been proposed including stimulation of the cyclic AMP second messenger system[97] and increased synthesis of F2 alpha prostaglandins.[59] It has also been suggested that the hypotensive effect of butyric acid may depend on the inhibition of the intrarenal renin-angiotensin system.[62]

Our recent findings suggest that gut-derived butyric acid affect the circulatory system via the following two mechanisms. Firstly, butyric acid stimulates afferent fibers of the vagus nerve which project from the gut to the brain. This decreases tonic sympathetic activity and thereby lowers arterial blood pressure. Secondly, gut-derived butyric acid crosses the GBB, enters the bloodstream and produces vasodilation acting on GPR41/43 receptors. Interestingly, our study showed that physiological concentration of butyric acid in the colon is three orders of magnitude higher than that in systemic blood, which makes the colon a very likely site of butyric acid action under physiological conditions.[60]

Further indirect evidence for the hypotensive effect of butyric acid produced by gut microbiota is provided by studies showing an inverse correlation between arterial blood pressure and the abundance of bacteria producing butyrate in rats.[98] Similarly, in overweight pregnant women the abundance of butyrate-producing bacteria and butyrate production was associated with lower arterial blood pressure.[99]

Apart from the hypotensive action, butyric acid have been suggested to possess a direct antiatherogenic activity by diminishing vascular smooth muscle cells proliferation.[100]

Trimethylamines: TMAO vs. TMA

Trimethylamine (TMA) is a simple tertiary aliphatic amine with the formula N(CH3)3. It is synthetized in colon by several bacterial genera including *Clostridium*, *Collinsella*, *Desulfovibrio*, *Lactobacillus*, and *Proteus* from dietary L-carnitine, choline, and their derivatives. After absorption in large intestine TMA passes through the portal vein to the liver where most of it is oxidized to trimethylamine N-oxide (TMAO) by the flavin-containing monooxygenase (FMO3).[101]

TMAO

In terms of cardiovascular effects, TMAO has gained much more attention than its precursor TMA as recent studies showed positive correlation between plasma TMAO concentration and cardiovascular risk. However, despite hundreds of studies investigating TMAO, it is not clear whether it is a harmful factor, sign of adaptive response or just a confounder.[23]

It has been shown that plasma TMAO may be used in risk assessment in general population[102] and in patients with heart failure,[103–105] acute myocardial infarction,[106,107] coronary artery disease,[108] peripheral artery disease,[109] stroke,[110] and hypertension.[111,112] In particular, the association between TMAO and atherosclerosis burden has been observed.[113–116] It has also been reported that TMAO contributes to platelet hyperreactivity by increasing intracellular Ca(2+) release and enhancing thrombotic potential and it independently predicted incident thrombosis risk.[117] However, there are some issues that call into question the above results. Some research found no evidence linking TMAO to increased cardiovascular risk.[118–121] Other studies suggest that increased TMAO is only a manifestation of decreased kidney function and association with cardiovascular risk become insignificant after adjustment for eGFR.[122–124] It is along with the fact that TMAO is excreted mainly with urine and elevated plasma TMAO level has been widely reported in chronic kidney disease.[114,125,126] It was shown that impaired renal function was the main variable affecting plasma TMAO concentration.[127] It is also worth noting that plasma TMAO concentration may be confounded by the disturbed GBB function[43] or FMO3 activity.[128] FMO3 is mostly active in liver, however, it was detected in lung, kidney and other tissues as well. Besides, its expression is sex- and age-specific,[129,130] which may further interfere with the results. In addition, it has been shown that the association between TMAO and cardiovascular events may vary depending on race[131] or gender.[132] Finally, the connection between TMAO and atherosclerotic progression in patients has been questioned.[133–135]

A lot of experimental studies have been performed to determine a mechanistic insight into how TMAO potentially promotes cardiovascular diseases. Supplementation of TMAO[136,137] or its dietary precursors[116,137–139] augmented atherosclerotic plaque formation in atherosclerosis-prone mice, possibly due to toxic effect on endothelial progenitor cells[140] or acting by CD36/MAPK/JNK pathway.[136] However, some studies do not support these findings[141] and surprisingly, Collins *et al.* suggested even a protective effect of TMAO precursor against atherosclerotic lesion development.[142] Diet enriched by TMAO and its precursors caused a significant increase in heart failure severity in mice[143] and reduction in TMAO concentration resulted in improvement in heart failure symptoms induced by myocardial infarction.[144] Some studies showed that TMAO may induce cardiac fibrosis[145,146] and disturb energy

metabolism in the heart.[147] However, experiments carried out by Querio *et al.*[148] and in our laboratory[149] do not support the damaging effect of TMAO on cardiomyocytes. The connection between TMAO and aging is controversial as well. In mouse model, TMAO treatment for 16 weeks induced vascular aging.[150] On the other hand, TMAO did not increase with age in rat plasma and did not affect smooth muscle cells viability.[151] Indirect evidence of damaging effect of TMAO followed from the fact that its precursors, choline and carnitine, are abundant in red meat which is known to increase cardiovascular risk. However, consumption of fish and other seafood, which are linked to cardio-protective effects, produce substantially greater increase in circulating TMAO than red meat.[152,153] Therefore, the causative role of TMAO in cardiovascular diseases remains under question.

Intriguingly, TMAO has protective functions against osmotic and hydrostatic pressures. Number of biochemical studies show that TMAO stabilizes protein structure and counteracts the effects of urea and other denaturants.[154–156] It may be speculated that TMAO is accumulated as a mechanism of adaptation to hydrostatic pressure or water-electrolyte imbalances which occur in cardiovascular diseases.

TMA, a toxic precursor of TMAO

On the other hand, cardiovascular effects of TMA, a TMAO precursor, have not been sufficiently studied yet. It is rather surprising because natural occurrence of TMA has been recognized for a long time and its toxic properties have been described in medical literature already in 19th century. Nowadays it is mainly known as a compound responsible for fish odor syndrome i.e. trimethylaminuria. Genetic FMO3 deficiency causes TMA accumulation in the body fluids inducing the characteristic smell of urine, breath, and sweat in patients suffering from this disease.[157]

TMA is a pollutant widely present in industry, and several guidelines with exposure limit are available, e.g., the Recommendation from the Scientific Committee on Occupational Exposure Limits.[158] Numerous studies show toxic effect of TMA at relatively low concentrations.[159–162] TMA causes eye[160] and skin[163] irritation, developmental toxicity,[161] epilepsy and behavioral disorders[164–166] and promotes hepatic encephalopathy.[167] Strikingly, to the best of our knowledge, there is no research evaluating the association between TMA and cardiovascular risk. Unfortunately, clinical studies focused on TMAO did not assess TMA concentration but it may be speculated that increased TMAO levels were accompanied by increased level of its precursor. Possibly, it is TMA and not TMAO that contributes to increased mortality. There is some evidence to support this view. Increased TMA level has been observed in cardiovascular patients[149,168] and kidney deterioration seems to play a key role in these changes as demonstrated by a significant inverse correlation between level of TMA and eGFR. It must be stressed that TMA has been considered a uremic toxin for almost 40 years[169,170] contributing to neurotoxicity and uremic breath in patients with end-stage renal disease.[171] Moreover, as demonstrated by Srinivasa *et al.*, TMA and not TMAO is associated with atherosclerotic plaque formation in HIV patients.[133] Recently, we have found that TMA and not TMAO elevates blood pressure, exerts cytotoxic effect on cardiomyocytes and vascular smooth muscle cells as well as disturbs LDH and albumin structure.[149,151,172] It is also worth noting that most of experimental studies showing positive effects of reduction in TMAO level were associated with TMA decrease at the same time because they targeted precursors of TMA.[173,174]

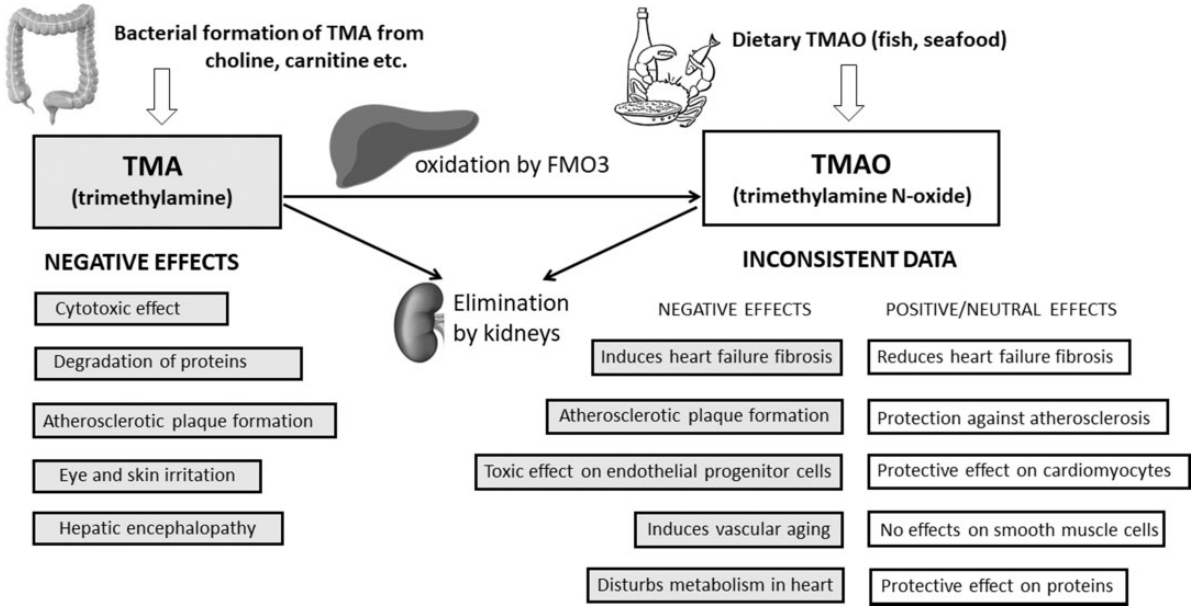

Figure 3. Dietary sources, metabolism and elimination of trimethylamine (TMA) and trimethylamine N-oxide (TMAO) in humans. Suggested main cardiovascular and other effects of both methylamines based on currently available data (details in the text). FMO3: flavin-containing monooxygenase 3.

Formation and major biological effects of TMA and TMAO are depicted in Figure 3.

Conclusions

Increasing experimental and clinical evidence suggests that gut microbiota-derived products affect the circulatory system functions. Both beneficial and toxic effects have been described. For instance, short chain fatty acids have been found to lower blood pressure. On the other hand, trimethylamine has been suggested to increase blood pressure and exert negative effect on the circulatory system. Hence, the bacteria products may have a therapeutic potential in cardiovascular diseases. Further studies are needed to establish the role of gut microbiota products in the circulatory system homeostasis.

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DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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ORCID iD

Marcin Ufnal https://orcid.org/0000-0003-0088-8284

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