Colonic bacterial metabolites and human health

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Abstract (100-120 words)
The influence of the microbial–mammalian metabolic axis is becoming increasingly important for human health. Bacterial fermentation of carbohydrates and proteins produces short-chain fatty acids (SCFA) and a range of other metabolites including those from aromatic amino acid (AAA) fermentation. SCFA influence host health as energy sources and via multiple signalling mechanisms. Bacterial transformation of fibre-related phytochemicals is associated with a reduced incidence of several chronic diseases. The ‘gut–liver axis’ is an emerging area of study. Microbial deconjugation of xenobiotics and release of aromatic moieties into the colon can have a wide range of physiological consequences. In addition, the role of the gut microbiota in choline deficiency in non-alcoholic fatty liver disease and insulin resistance is receiving increased attention.

Highlights:
- Diet-driven changes in microbially-produced SCFA can influence health via signalling
- Gut microbiota mediates the release and transformation of many bioactive phenolics
- Gut microbiota degrades dietary choline to methylamines
- Interactions between the microbiota, inflammasomes and host influence liver disease

Abbreviations: SCFA, short-chain fatty acids; CHO, carbohydrate; FFAR, free fatty acid receptor; WL, weight loss; NSP, non-starch polysaccharide ‘fibre’; AAA, aromatic amino acids; NAFLD, non-alcoholic fatty liver disease; NASH, non-alcoholic steatohepatitis; HMS, hepatic macrovesicular steatosis; PC,
Introduction

The human large intestine is colonised by dense microbial communities that utilise both diet- and host-derived energy sources for growth, predominantly through fermentative metabolism. This highly diverse community has the capacity to perform an extraordinary range of biochemical transformations that go well beyond those encoded by the host genome, and these activities exert an important influence upon many aspects of human health. Metabolites formed by the gut microbiota are largely determined by the composition of the diet and the pattern of food intake, and it is now clear that the species composition of the colonic microbiota is itself altered by the diet [1*,2, 3**]. This review will consider selected examples where recent progress has been made in understanding the links between diet, gut microbial activity and metabolites relevant to health.

Bacterial metabolites derived from the fermentation of plant-derived carbohydrates and their impact on the host

Many carbohydrates (CHOs) present in plant-derived foods are digested slowly, if at all, in the small intestine, making them available for microbial fermentation in the large intestine. Intake of starch that is resistant to digestion in the small intestine (resistant starch) can have benefits for metabolic health [4] and results in changes in the gut microbiota [1*]. Recent work also shows a beneficial influence of whole grain intake upon inflammation, again with concomitant changes in the gut microbiota [5*]. Diet-induced changes in the metabolic activity of the gut microbiota are thought likely to mediate these effects.

Hexose and pentose sugars are fermented by isolated human colonic bacteria via pathways leading to the formation of acetate, succinate, propionate, butyrate, formate, lactate, ethanol, hydrogen and CO₂, depending on the strain and species. Butyrate formation occurs in certain Firmicutes bacteria, either via butyrate kinase (in many Clostridium and Coprococcus species) or via butyryl CoA:acetate CoA transferase [6]. The latter pathway is found in the numerically predominant butyrate-producing species of Roseburia, Eubacterium rectale, E. hallii and Faecalibacterium prausnitzii, and involves the net uptake of external acetate [7]. Acetate is produced by most anaerobes, including acetogens that are able to perform reductive acetogenesis from formate or hydrogen plus CO₂. Producers of succinate and propionate largely belong to the phylum
Bacteroidetes, but also include some Firmicutes. Lactate can be formed by many groups, but is generally converted into acetate, propionate or butyrate by a subset of lactate-utilizing species [8]. Formation of the gases hydrogen and CO₂ varies widely between species in pure culture; in the mixed community these products are partially converted to acetate, methane or hydrogen sulfide [9]. The net outcome of all of these complex cross-feeding interactions for a typical healthy microbiota is that, in faecal samples, acetate is the dominant short-chain fatty acid (SCFA) detected (typically 40 – 70 mM) followed by propionate and butyrate (each 10 - 30 mM) [10]. While alternative products such as ethanol, succinate and lactate are normally found at lower concentrations, they can accumulate in some circumstances and a link has been proposed between endogenous alcohol formation and non-alcoholic steatohepatitis [11].

At these concentrations, SCFA have a major impact on the large intestinal environment and on absorption from the lumen. While butyrate is largely utilised by the gut epithelium, and propionate is largely metabolised in the liver, acetate is the SCFA that reaches the highest concentrations in plasma [10]. There is increasing evidence that acetate plays an important role in controlling inflammation and in combating pathogen invasion [12,13]. Acetate and lactate were also found recently to influence cyclin gene expression and epithelial cell proliferation in a pH-dependent manner in vitro [14]. The importance of butyrate as an energy source for epithelial cells has long been recognised, but its role in regulating inflammation, cellular differentiation and apoptosis, and in helping to prevent colorectal cancer, is still emerging [15]. Interestingly, butyrate was recently found to be the most potent SCFA in activating the AP-1 signalling pathway in epithelial cell lines [16]. Interactions have been recognised between SCFA and the host cell receptors FFAR2 and FFAR3 that might influence satiety, protect against diet-induced obesity and improve insulin sensitivity, with propionate considered to have a potentially important role [17,18]. In view of this it is important to understand how diet and microbiota composition can influence relative, as well as total, SCFA production. Studies in obese subjects on weight loss diets demonstrate that dietary intake of CHO has a major impact on faecal SCFA concentrations [19,20**] presumably reflecting decreased fermentation in the colon (Fig. 1). More surprising, however, is that butyrate per cent responded disproportionately, an effect that correlates with a marked decrease in the Roseburia-E. rectale group of butyrate-producing bacteria [19]. This may be explained by the greater dependence of this group, compared with other members of the microbiota, on intake of resistant dietary CHO, and provides evidence that SCFA relative production rates are responsive to diet composition. An inverse relationship has been noted between faecal pH and butyrate concentration in vivo [21]; this
Formation and metabolism of aromatic compounds

Fibre-related phytochemicals

It is suggested that the inverse relationship between the intake of fibre-rich diets and the incidence of several chronic diseases is mediated in part by the gut microbiota. Microbial release of phytochemical metabolites may be a contributing factor and most widely studied for disease prevention are the aromatic metabolites produced by the phenylpropanoid pathway [25,26]. Increasing the fibre content of the diet from 8.8 to 14 g day\(^{-1}\) in a human volunteer study resulted in significantly increasing certain phenolic acids and their derivatives in the gut, specifically ferulic acid, 4-hydroxy-3-methoxyphenylpropionic acid and 3-hydroxyphenylpropionic acid [20]. Ferulic acid, which is found extensively bound to plant polysaccharides, can be released and metabolised by the gut microbiota [20,27] (Fig. 2). Indeed, the major esters of other phenolic acids such as caffeic acid (chlorogenic and caftaric acid) are also rapidly de-esterified by human faecal microbiota [28]. It appears that the gut microbiota can effectively de-esterify compounds, whether the conjugate is quinic acid, tartaric acid or a sugar moiety to release the aglycone for further metabolism. Gut bacteria can also effectively hydrogenate the \(\alpha,\beta\)-unsaturated bond present on the side chain of phenolic acids [27] and the extent to which this occurs appears to be dependent on additional dietary factors, with high-protein diets decreasing the efficiency of this transformation [20]. Site-specific dehydroxylation and demethylation of the phenolic hydroxyl present in phenolic acids has also been observed [20, 27]. The resultant microbial products of ferulic acid metabolism had differing effects on prostanoid production \textit{in vitro} suggesting that the microbial transformation of dietary compounds will have important consequences for inflammation [27,29].

Aromatic amino acid metabolites

Protein metabolism is a major alternative mechanism for production of aromatic metabolites [30] as observed in recent human dietary interventions involving carefully controlled intakes of CHO and
protein [20]. Until recently, the major metabolites of aromatic amino acid (AAA) fermentation were considered to be phenol, p-cresol and indole, with p-cresol suggested to be a product of phenol catabolism. It has now been demonstrated that a much wider metabolic pathway of metabolism exists for all three AAAs [31]. In particular, phenylacetic acid, 4-hydroxyphenylacetic acid and indole-3-acetic acid were found to be major (de-aminated and chain-shortened) products of phenylalanine, tyrosine and tryptophan, respectively [31]. Bacteria capable of producing these products could effectively metabolise all three AAA substrates. These included Bacteroides thetaiotaomicron, B. eggerthii, B. ovatus, B. fragilis, Parabacteroides distasonis and the Gram-positive bacteria Clostridium bartletii and Eubacterium hallii. Bacterial species that did not substantially produce these de-aminated and chain-shortened products were identified. These included Megamonas hypermegale, Roseburia intestinalis, Ruminococcus obeum, Eubacterium rectale and Faecalibacterium prausnitzii, but strains of these species often produced higher amounts of benzoic acid, 4-hydroxybenzoic acid and indole-3-carboxylic acid and oxidation products including phenylpyruvic acid, phenyllactic acid, 4-hydroxyphenyllactic acid, indole-3-pyruvic acid and indole-3-lactic acid. Given that certain species of gut bacteria can metabolise all three AAAs by specific mechanisms, it is likely that other structural forms of amino acids can undergo these molecular transformations. This will give rise to a range of novel metabolites, which require to be investigated to assess their potential to affect human health.

It is clear that macronutrient balance influences not only the composition of the gut microbiota but also the availability of aromatic metabolites. Certain metabolites such as SCFA and phenyl metabolites can be produced by bacterial metabolism of both CHO and protein in the large intestine, whereas certain branched-chain fatty acids and nitrogen-containing metabolites are considered to be derived from protein metabolism alone. There is a positive association between animal protein consumption (specifically red and processed meat) and colorectal cancer [32]. Evidence is also beginning to emerge that the concentrations of aromatic gut metabolites in the systemic circulation plays a role in vascular health and [33].

**Enterohepatic circulation and β-glucuronidase**

Many diet-derived aromatic compounds, including drugs, are treated as xenobiotics and are conjugated in the liver followed by release into the intestine via the bile. One of the main mechanisms for conjugation is glucuronidation, but it has been known for some time that bacterial β-glucuronidases in the large intestine tend to cleave these conjugates, thus releasing the aromatic moiety and making it available again for re-absorption. The *gus* gene from *Escherichia coli* was originally identified as encoding this activity. A recent survey used degenerate *gus* primers to detect...
related genes among the faecal microbiota from 10 healthy volunteers; this showed a highly uneven
distribution with 60% of sequences accounted for by only 4 operational taxonomic units, while in
total 96% of sequences came from Firmicutes and 3% from *E. coli* [34]. It seems likely that this
activity is associated with enzymes involved in degrading plant polysaccharides. The contribution of
a second putative β-glucuronidase gene identified from metagenomic libraries [35] has still to be
fully established [34].

The ‘gut–liver’ axis, dietary amines, the intestinal microbiota and the
methylamines’ pathway

The ‘gut–liver’ axis

Given the exposure of the liver to intestinal-derived catabolites and the microbiota to biliary/waste
products, the ‘gut–liver axis’ is receiving great attention with respect to host health and its potential
to affect systemic host processes [36]. A recent study has nicely demonstrated the direct
involvement of the gut microbiota in the development of obesity-independent non-alcoholic fatty
liver disease (NAFLD), and the microbiota’s influence on whole body glucose homeostasis and liver
lipid metabolism [37**]. Germ-free mice inoculated with intestinal microbiota from a mouse that
developed hyperglycaemia and had a high plasma concentration of pro-inflammatory cytokines after
being fed a high-fat diet developed hepatic macrovesicular steatosis (HMS) after high-fat feeding,
with increased expression of hepatic genes involved in *de-novo* lipogenesis and lipid uptake (SREBP,
ChREBP, acetyl-CoA carboxylase 1 and CD36) observed. In comparison, germ-free mice inoculated
with faeces from a mouse that was normoglycaemic and had a lower level of systemic inflammation
after being fed a high-fat diet developed low-level steatosis on the same diet [37**]. Differences
were observed in the faecal microbiota of the two groups of mice: *Lachnospiraceae* and *Barnesiella*
(*Porphyromonadaceae*) sequences were significantly overrepresented in the HMS mice, while the
low-level steatosis mice had an increased number of sequences related to *Bacteroides vulgatus*.
Concentrations of isobutyrate and isovalerate, branched-chain amino acids resulting from the
bacterial fermentation of valine and leucine, respectively, were significantly higher in the caecum of
the HMS mice. In addition, these animals had significantly higher fasting glycaemia, fasting
insulinaemia, homeostasis model assessment—insulin resistance index and leptinaemia, and higher
plasma concentrations of aspartate aminotransferase than the animals that developed low-level
steatosis. Taken together, these results demonstrate that the gut microbiota constitutes an
environmental factor driving the progression of NAFLD [37**].
Choline deficiency and NAFLD

Choline is an essential nutrient of the vitamin B complex with numerous roles in the body: acting as a methyl donor in biochemical reactions, as a precursor for the biosynthesis of phospholipids [phosphatidylcholine (PC), lysophosphatidylcholine, choline plasmalogen and sphingomyelin], of acetylcholine and of lipoproteins, and in homocysteine reduction [38,39,40]. The main fate of choline in the body is its incorporation into PC via the Kennedy pathway [41].

Exogenous choline is derived from either dietary choline or, more commonly, PC from plant and animal material [38,39,42]. Foods high in choline include meat and dairy products, fish, soybeans, nuts and whole grains, with PC added to a number of foods as an emulsifier [43]. Endogenous sources of choline, in the form of PC, include biliary lipids, exfoliated epithelial cells and intestinal bacteria [44,45]. De novo synthesis of choline occurs via a reaction catalysed by phosphatidylethanolamine-N-methyltransferase (PEMT) [41].

The intestinal microbiota plays a role in the catabolism of choline in humans and rodents [46,47,48,49,], with trimethylamine (TMA), acetate and ethanol the products of fermentation [50]. Choline degradation by the human intestinal microbiota is temporally stable [47]. TMA produced by intestinal bacteria from choline is absorbed by colonic cells and converted to trimethylamine-N-oxide (TMAO) by flavin mono-oxygenase enzymes [51], demethylated into dimethylamine and (mono)methylamine in the liver, or excreted in the urine. The methylamine pathway is a typical example of microbial–mammalian co-metabolism [52,53] (Figure 3).

Knowledge pertaining to those members of the intestinal microbiota responsible for producing TMA from choline is sparse. In silico predictions have suggested that several members of the human intestinal microbiota (including Clostridium, Anaerococcus, Collinsella, Desulfitobacterium, Klebsiella, Escherichia, Providencia, Yokenella and Proteus spp.) have the ability to degrade choline to TMA via choline TMA-lyase [54**]. In addition to the aforementioned species, many more members of the human intestinal microbiota may be able to degrade choline to TMA using the same mechanism and/or via an alternative pathway(s).
Choline-deficient diets in humans (≤50 mg day\(^{-1}\)) and rodents are known to lead to NAFLD, non-alcoholic steatohepatitis (NASH) and hepatic damage [39,55]. To combat these and other complications (e.g. infertility, renal haemorrhage and hypertension), the Food and Nutrition Board of the Institute of Medicine of America recommends an adequate intake of choline for men is 550 mg per day and for women 425 mg per day [38,43]. Reduced or delayed urinary excretion of TMAO/TMA is specific to hepatic disease, and it has been suggested dysbiosis of the intestinal microbiota in patients with hepatobiliary diseases may delay/decrease conversion of choline to TMA and subsequent urinary excretion of TMAO/TMA [47,48]. Analyses of urinary metabolites produced by mice fed high-fat diets led to the proposals that microbial utilization, and subsequent reduced availability, of dietary choline contributes to the development of NAFLD [56] and insulin resistance [57]. The only study to date comparing the faecal microbiotas of healthy and NAFLD individuals found no difference in their compositions [58]. However, studies in rodents have shown that probiotic [59] and antibiotic administration [60\*,61\**] can offer protection against the onset of NAFLD.

The role for dietary choline in NAFLD can be explained by the bioavailability of free choline to form lipoproteins in the liver (in particular, VLDL), which allows the export of free fatty acids from this organ. If the gut microbiome converts excessive amounts of dietary choline into TMA, this leads to reduced choline bioavailability and, therefore, NAFLD [57].

Recent work has demonstrated that changes in choline levels in a standardized diet modulate the faecal microbiota and can lead to the development of fatty liver in human subjects [61\**]. Fifteen females (BMI 15–34) on a 2-week in-patient study were fed a standardized diet in which choline levels were manipulated. *Gammaproteobacteria* were seemingly inhibited by high levels of dietary choline, and negatively correlated with the per cent change in liver fat/spleen fat ratios. *Erysipelotrichi* sequence numbers were positively correlated with the per cent change in liver fat/spleen fat ratios. This led to the suggestion that baseline levels of these taxa may predict the susceptibility of an individual to fatty liver disease from a choline-deficient diet [61\**]. Combining PEMT promoter SNP rs12325817 phenotype, *Gammaproteobacteria* and *Erysipelotrichi* data proved a powerful method for predicting the physiological effects of choline deficiency, and led the researchers to hypothesize that those with the wild-type version SNP in the *PEMT* gene were better able to produce PC endogenously and were less affected by the composition of their intestinal microbiota in relation to the effects of choline deficiency.
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Interactions between the intestinal microbiota, inflammasomes and NAFLD are known to occur. Deficiencies of the NLRP3 and NLRP6 inflammasomes positively regulated NAFLD progression in mice harbouring a colitogenic intestinal microbiota [60**]. Switching the animals to a choline-deficient diet modulated the faecal microbiota, particularly representation of members of the families Porphyromonadaceae, Erysipelotrichaceae and Prevotellaceae. Modulation of the intestinal microbiota by the choline-deficient diet was thought to promote a TLR4/TLR9 signalling cascade in the liver that led to enhanced hepatic tumour necrosis factor expression that drove progression to NASH in susceptible animals.

Microbial metabolism of phosphatidylcholine and l-carnitine is associated with cardiovascular disease

Choline present in dietary PC is degraded by intestinal bacteria, but is more resistant to degradation than free choline [49,62]. The intestinal microbiota of mice is able to catabolise choline from dietary PC via an unknown mechanism, which led to the proposal of a linear pathway PC → choline → TMA → TMAO [63*]. It is known that human intestinal bacteria (bacteroides, bifidobacteria and clostridia) are able to degrade PC with the release of choline [62].

Following the association of methylamines with murine insulin-resistance phenotypes [57], Wang et al. [63*] proposed a link between degradation of dietary PC, the intestinal microbiota and TMAO in cardiovascular disease. This hypothesis was tested further in a study in which humans were given a PC challenge and their plasma levels of TMAO were measured before and after suppression of the intestinal microbiota with antibiotics [64**]. Time-dependent increases in plasma TMAO levels were observed at the first challenge, with TMAO production suppressed after antibiotic administration. Removal of antibiotics, and ‘release’ of the microbiota, reinstated TMAO plasma levels post-PC challenge, demonstrating the role of the microbiota in increasing circulating levels of TMAO derived from PC. The authors also examined the relationship between fasting TMAO levels in 4007 patients undergoing elective coronary angiography and the occurrence of major cardiovascular events (death, heart attack or stroke) over a three-year follow-up period. An increased fasting plasma level of TMAO was associated with experiencing a major cardiovascular event [64**].

The relationship between TMAO produced from dietary methylamines and cardiovascular disease has recently been extended to include l-carnitine, a compound abundant in red meat [65**]. Antibiotic-induced suppression of the microbiota of humans led to almost-complete absence of
TMAO from plasma and urine after l-carnitine challenge. In addition, it was shown that omnivorous humans have far higher circulating levels of TMAO in their plasma than their vegan and vegetarian counterparts after l-carnitine supplementation, with negligible TMAO formation in vegans post-carnitine challenge. Vegetarians and vegans have higher plasma levels of carnitine compared with their omnivorous counterparts, though it is not known if this is due to reduced microbial metabolism of carnitine to TMA by the intestinal microbiota of the non-omnivores. This suggests that the human intestinal microbiota can be modulated by dietary means with respect to how it processes dietary methylamines.

High levels of plasma carnitine were associated with cardiovascular disease but only in those patients with accompanying high levels of plasma TMAO in a cohort of 2595 patients undergoing cardiac evaluation [64**]. Using an Apoe/− mouse model, Koeth et al. [65**] demonstrated that atherosclerosis plaque formation during carnitine supplementation was microbiota-dependent, being directly related to the presence of bacterially-derived TMAO/TMA in plasma. TMAO is currently thought to induce atherosclerosis by promoting macrophage cholesterol accumulation by increasing cell surface expression of CD36 and scavenger receptor A, pro-atherogenic scavenger receptors [63*,65**], and by repressing reverse cholesterol transport and several bile acid transporters in the liver [65**].

Conclusion

Microbial–mammalian co-metabolism is shaping human health in many ways. In this review, we have covered recent findings on SCFA, AAA and methylamine metabolism and their consequences on human health and disease, which are illustrating particularly well this metabolic symbiosis. With the constant refinement of metagenomics and metabolomics, further insights will become available from cohort studies, bearing promises for personalised nutrition and healthcare in the future.

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** This study shows that consumption of high protein, reduced CHO diets by human volunteers results in fecal metabolite profiles with altered SCFA proportions, decreased concentrations of butyrate and phenolic compounds derived from plant fibre, and increased N-nitroso compounds.


phenylpropanoid-derived metabolites in the human gut can arise from microbial fermentation of


** This study provides a very good example of the potential of the ‘gut–liver axis‘ to affect host health.


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** This study highlights the potential of computational chemistry to identify genes with specific biological functions, with the authors identifying a gene cluster responsible for anaerobic choline degradation in the genome of Desulfovibrio desulfuricans. The choline utilization cluster was found to be present in the genomes of a number of human gut bacteria, though the expression of this cluster in these bacteria has yet to be demonstrated in vitro or in vivo.


* This article further explored the hypothesis of Dumas *et al.* [23] on the association between methylamines and insulin resistance/NAFLD. Wang *et al.* [18] expanded their scope to atherosclerosis and nicely demonstrated that TMAO exposure was associated with markers of inflammation.
The full, proofread version of this review was published by Elsevier and can be found at http://www.sciencedirect.com/science/article/pii/S1369527413000878


** The study described in this article takes the hypothesis from Wang et al. [63*], that microbial-dependent conversion of dietary PC to TMAO is associated with cardiovascular disease, and applies it to a human cohort. It presents the first evidence of an association between a microbiota-dependent product and cardiovascular disease in humans.


** Extends the work of Tang et al. [64**] to include formation of TMAO from dietary carnitine, and demonstrates that TMAO production is dependent on diet. Confirms the association of high levels of plasma TMAO with major cardiovascular events. In addition, the work demonstrates a mechanism for the development of methylamine-dependent atherosclerosis.

[Figure legends]

**Figure 1.** Impact of reduced CHO weight loss (WL) diets in male obese volunteers on fecal SCFA concentrations. Data are from two separate dietary cross-over studies that are reported in [19] (study 1) and [20**] (study 2): M – weight maintenance diet (360-400 g day⁻¹ CHO, 22-28 NSP), HPMC – high protein, moderate CHO WL diet (164-182 g day⁻¹ CHO, 12-13 NSP ), HPLC – high protein, low CHO WL diet (23-24 g day⁻¹ CHO, 6-9 NSP). In addition to the evident decrease in total SCFA, both studies detected a significant decrease in per cent butyrate among SCFA, while in study 2 the per cent of minor SCFA (valerate, isobutyrate, isovalerate) that were derived from amino acid fermentation increased, reflecting the higher protein intake on the WL diets.

**Figure 2.** Concentration of fibre-derived ferulic acid and its major metabolites measured in faecal samples following high protein dietary interventions. Metabolite 1 = 4-hydroxy-3-methoxyphenylpropionic acid, Metabolite 2 = 3,4-dihydroxyphenylpropionic acid, Metabolite 3 = 3-hydroxyphenylpropionic acid. M = maintenance diet (fibre content 22 g day⁻¹), HPMC = high protein moderate CHO diet (fibre content 14 g day⁻¹) and HPLC = high protein low CHO diet (fibre content 8.8 g day⁻¹). Ferulic acid = 4-hydroxy-3-methoxycinnamic acid. Data are given as mean ± standard deviation (n = 8 volunteers). Statistical data were calculated as a one-way ANOVA to compare diet...
with blocking for volunteer and, where significant, are given for comparison between M and HPLC diets. Adapted from [20**].

Figure 3. The methylamines’ pathway and the microbial–mammalian metabolic axis. TMA is derived from microbial degradation of choline, a dietary component that can also be obtained by cleavage of dietary PC, and of L-carnitine. TMA is absorbed by the host to be N-oxidised into TMAO by FMO3 and demethylated into DMA and MMA by cytochrome P450s (CYP) in the liver during first-pass metabolism. Circulating TMAO can reach other cell types, such as arterial epithelial cells and macrophages, leading to atherosclerosis-associated inflammation. PC, synthesized from choline through the Kennedy (CDP-choline) pathway, is essential for exporting fatty acids from the liver to other storage tissues; reduced choline bioavailability leads to lower levels of PC being formed and to NAFLD. PEMT converts phosphatidylethanolamine (PE) into PC, using S-adenosylmethionine as a methyl donor, and a polymorphism in PEMT has been associated with a higher risk of developing NAFLD. When there is sufficient choline in the diet, the Kennedy pathway is responsible for maintaining PC synthesis, with the PEMT pathway contributing ~30% of the hepatic PC. When choline is at low levels in the diet, the PEMT pathway is essential for maintaining the supply of PC in the liver. Adapted from [41,57,63**, 64**,65**].