Diet-gut microbiota interactions on cardiovascular disease

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Abstract

Cardiovascular diseases (CVD) are a group of disorders of the heart and blood vessels and remain the leading cause of morbidity and mortality worldwide. Over the past decades, accumulating studies indicated that the gut microbiota, an indispensable "invisible organ", plays a vital role in human metabolism and disease states including CVD. Among many endogenous and exogenous factors that can impact gut microbial communities, the dietary nutrients emerge as an essential component of host-microbiota relationships that can be involved in CVD susceptibility. In this review, we summarize the major concepts of dietary modulation of the gut microbiota and the chief principles of the involvement of this microbiota in CVD development. We also discuss the mechanisms of diet-microbiota crosstalk that regulate CVD progression, including endotoxemia, inflammation, gut barrier dysfunction and lipid metabolism dysfunction. In addition, we describe how metabolites produced by the microbiota, including trimethylamine-N-oxide (TMAO), secondary bile acids (BAs), short chain fatty acids (SCFAs) as well as aromatic amino acids (AAAs) derived metabolites play a role in CVD pathogenesis. Finally, we present the potential dietary interventions which interacted with gut microbiota as novel preventive and therapeutic strategies for CVD management.

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1. Introduction

The gut microbiota, the most abundant microbial community living in the human body, has recently been considered an indispensable “invisible organ” that regulates essential functions of host physiology, including nutrients transformation and metabolism, maintenance of intestinal barrier integrity and host immunity homeostasis [1–3]. Numerous studies indicated that dietary nutrient intake, as a key environmental factor impacting gut microbiota composition, can induce “dysbiosis” which is defined as an altered microbial community including richness, diversity and composition. The dysbiosis can accelerate the homeostatic imbalance which contributes to multiple disease states [4–9], including obesity, diabetes, liver diseases, cancer, brain disorders, as well as cardiovascular disease (CVD) which remains the leading cause of morbidity and mortality worldwide [10,11].

In human gut, more than 1,000 bacterial species have been identified with high-throughput metagenomics sequencing techniques (e.g. 16S, Shotgun) and about 160 species are largely shared among individuals [12]. Two major phyla of bacteria, Firmicutes and Bacteroidetes, are predominant with more than 90% of total bacteria, and their ratio is usually considered a relative signature for health state although it is still controversial [13]. Besides, the phyla Actinobacteria, Proteobacteria and Verrucomicrobia are also members of the gut microbiota but to a much lesser abundance [14]. At the genus level, *Eubacterium*, *Ruminococcus*, *Clostridium*, *Lactobacillus*, and also *Bacteroides* are dominant in healthy adult humans [12–14]. Particularly, the concept of “enterotype” firstly emerged in 2011 by comparing large scales of human subjects to demonstrate the stratification of gut microbiota which were divided into three robust clusters including *Bacteroides*, *Prevotella*, and *Ruminococcus* as predominant genera in the different enterotypes. Subsequently, a new addition to enterotypes separates the *Bacteroides* into two sub-enterotypes B1 and B2 in which B2 enterotype is highly associated with systemic inflammation [15,16]. In general, the compositional and functional features of the gut microbiota include α-diversity (richness of community in one sample) and β-diversity (community differences across samples), as well as microbial metabolites production [17].

CVD is a general term for diseases affecting heart and blood vessels encompassing multiple disorders like coronary artery disease (CAD), heart failure, stroke and other conditions [18]. As a chronic progressive health burden, the development of CVD relates to various risk factors including hypertension (HT), dyslipidemia as well as insulin resistance and inflammation, most of which could damage vascular structure and eventually lead to more direct processes like atherosclerosis and thrombosis susceptibility [19]. Besides these physiological factors, dietary nutrients have been highlighted as one of the key modifiable contributors that could interact with the gut microbiota implicating a diet-microbiota-dependent mechanism for the development of CVD [20].

Specifically, the large and diverse intestinal microbial community serves as a “metabolic filter” in the gastrointestinal (GI) tract to what we eat, our predominant environmental exposures [21]. Trillions of microbial cells residing in the intestine contribute to the nutrient digestion and metabolism, in which the bacteria are the most abundant community belonging to thousands of species, but yeasts, fungi, and archaea are also involved [22]. With the variation of pH, the redox potential and adhesion sites, the microbial density gradually increases along the GI tract until reaching the highest level in the distal intestine where the metabolites are produced in response to dietary stimuli, some of which being correlated with the development of CVD [23]. “You are what you eat” – indeed, a growing body of studies have provided strong evidences to support the diet-gut microbiota crosstalk on CVD in both positive and negative ways [24,25]. On one side, unhealthy dietary nutrient intake like high consumption of saturated fat, refined carbohydrates, and dietary choline, has been refined to increase the abundance of pathogenic bacteria or metabolites which trigger systemic inflammation, damage intestinal barrier to promote CVD susceptibility [26]. Contrarily, healthy dietary patterns with high intake of diet rich in fibers such as fruits, vegetables, and whole grain stimulate the growth of beneficial bacteria which might exert potential preventive effects on CVD [26].

Herein, large body of data is reviewed in supporting our understanding of the diet-gut microbiota interactions in CVD. Thus, we precisely discuss the role of gut microbiota in the pathogenic development of CVD, the diet-microbiota metabolites interactions in the development of CVD, as well as the potential dietary interventions through gut microbiota axis as novel preventive and therapeutic strategies for CVD management.

2. Dietary modulation of gut microbiota

Numerous studies have suggested that diet is an essential modulator of the compositional and functional features of the gut microbiota in both humans and animals. The impacts of dietary changes on microbial communities in the gut can be summarized in three major aspects: (1) Rapid/short-term effect: this theme is supported by the research on human subjects who switched between plant-based (high-fiber) and animal-based (high-fat) diet. The microbiota composition of all subjects shifted within 1–2 days with increased abundance of Firmicutes which metabolized dietary fiber with plant-based diet and increased bile-tolerant microorganisms *Alstipes* and *Bilophila* with animal-based diet [27]. However, short-term diet changes had no impact on enterotypes even after 10 days intervention [28]. (2) Long-term effect: despite of rapid modulations of microbial community, long-term dietary interventions are not only associated with the compositional alterations, but also related to physiological changes. For instance, feeding rats with high fat diet (HFD) for 8 or 12 weeks induced increased abundance of *Enterobacteriales* (Proteobacteria phylum) which was coupled with the elevation of systemic inflammation, intestinal permeability and obese phenotype [29]. On the contrary, human cohorts intervened with 3 months low-carbohydrate or low-fat healthy diet resulted in 14 or 12 taxonomic changes correlating with weight loss suggesting that long-term interventions are necessary [30]. Besides, enterotypes are mostly related to long-term dietary effects instead of short-term impacts [28]. (3) Specific microbial changes in response to particular diet: For example, dietary fiber intake promoted the increase of gut microbiota richness or diversity [31] as well as phylum of Firmicutes [27]. Bacterial species *Ruminococcus bromii* bloomed under the intervention of resistant-starch diet [32]. Interestingly, not only the microbial composition but specific microbial metabolism is correlated with specific diet and disease patterns. For instance, subjects with red meat enriched diet had more trimethylamine-N-oxide (TMAO) (intestinal microbial metabolite of choline present in red meat) in plasma than vegetarians. The elevation of TMAO levels has been identified in human subjects with the enriched proportions of enterotype *Prevotella* and is associated with increased risk of CVD [33].

3. Microbial alterations in CVD

Extensive investigations have identified that the gut microbiota plays an essential role in CVD. Indeed, early studies suggested that the depletion of gut microbiota by antibiotic treatment raised blood pressure in rats [34]. These discoveries have been further confirmed in germ-free (GF) rats implicating the crucial role of gut microbiota in the regulation of blood pressure [35]. Also, the
The alteration of gut microbiota associated with CVD conditions in human cohorts.

| CVD Condition | Cohorts | Sequencing Method | Diversity | Increased Abundance | Decreased Abundance | Ref. |
|---------------|---------|-------------------|-----------|---------------------|---------------------|------|
| Hypertension  | 99 HT   | Shotgun           | $\gamma$-diversity, $\beta$-diversity | Klebsiella | Faecalibacterium Oscillibacter | [40] |
|               | 56 Pre-HT |                 |           | Prevotella           | Roseburia           |      |
|               | 41 Controls |                |           | Desulfovibrio       | Bifidobacterium     |      |
|               | 67 HT   | 16S               | $\gamma$-diversity, $\beta$-diversity | Acetobacteroides Alistipes | Butyrylovibrio | [41] |
|               | 62 Controls |               |           | Bacteroides Barnesiella | Lactobacillus |      |
|               |         |                   |           | Clostridium          | Olsenella           |      |
|               |         |                   |           | Desulfovibrio        | Prevotella          |      |
|               |         |                   |           | Megasphaera          | Romboutsia          |      |
|               |         |                   |           | Microvirgula Parabacteroides | Ruminococcus |      |
|               |         |                   |           | Catabacter           | Akkermania          | [42] |
|               |         |                   |           | Veillonella           | Ruminococcus        |      |
|               |         |                   |           | Clostridium          | Anaerovorax         |      |
|               |         |                   |           | Oscillibacter        | Sporobacter         |      |
|               |         |                   |           | Robinsoniella        | Asaccharobacter     |      |
|               | 13 Patients | Shotgun | NA         | Collinsella          | Bacteroides         | [43] |
|               | 12 Controls |                 |           | Clostridales         | Roseburia           |      |
|               |         |                   |           | Clostridium          | Eubacterium         |      |
|               | 218 Patients | Shotgun | NA         | Escherichia coli     | Faecalibacterium    | [44] |
|               | 187 Controls |               |           | Klebsiella spp.      | Bacteroides spp.    |      |
|               |         |                   |           | Enterobacter aerogenes | Prevotella cogri |      |
|               | 223 Patients | 16S        | $\gamma$-diversity, $\beta$-diversity | Streptococcus anginosus | Alistipes shahii | [45] |
|               | 181 Controls |               |           | Atopobium parvulum   | Bacteroides xylanisolvens |      |
|               |         |                   |           | Actinomyces gravevanizii | Odoribacter splanchinicus |      |
|               |         |                   |           | Streptococcus mits/oralis/pneumonia | Eubacterium eligens |      |
| Atherosclerosis | 13 Patients | Shotgun | NA         | Enterodermobacteriaceae | Roseburia insulivorans | [46] |
|               | 12 Controls |                 |           | Enterococcus         | Rosenburia          |      |
|               |         |                   |           | Streptococcus        | Lachnospiraceae     |      |
| CAD           | 39 Patients | 16S        | $\beta$-diversity | F/B ratio | Bacteroides          | [47] |
|               | 30 High-risks |             |           | Lactobacillales      | Prevotella          |      |
|               | 50 Controls |         | $\gamma$-diversity, $\beta$-diversity | Escherichia-Shigella | Faecalibacterium    |      |
|               | 70 Patients | 16S        |           | Enterococcus         | Subdiligranulum     | [48] |
|               | 98 Controls |         |           |                    | Roseburia           |      |
|               |         | $\gamma$-diversity, $\beta$-diversity | $\gamma$-diversity, $\beta$-diversity | Roseburia           | Eubacterium rectale |      |
|               |         | $\gamma$-diversity, $\beta$-diversity |           | Streptococcus        | Faecalibacterium    |      |
| Heart Failure | 161 Patients | 16S        | NA         | Enterobacteriaceae   | Roseburia           | [49] |
|               | 40 Controls |         |           | Entrobacteriaceae    | Lachnospiraceae     |      |
| Stroke        | 60 Patients | Culture | NA         | Candida              | NA                  | [50] |
|               | 20 Controls |         |           | Campylobacter        |                     |      |
|               |           |           |           | Shigella             |                     |      |
|               |           |           |           | Verruia              |                     |      |
|               |           |           |           | Succiniclasticum     | Lachnospiraceae     | [51] |
|               |           |           |           | Prevotella           | Eubacterium hallii |      |
|               |           |           |           | Actinobacteria       | Lachnospiraceae     | [52] |
|               |           |           |           | Bifudobacterium      | Eubacterium hallii |      |
|               |           |           |           | Escherichia/Shigella | Megamonas           | [53] |
|               |           |           |           | Enterobacter         | Bacteroides         |      |
| Stroke        | 30 Patients | 16S        | NA $\gamma$-diversity, $\beta$-diversity | Actinobacteria       | Faecalibacterium    | [54] |
|               | 30 Controls |         | $\beta$-diversity | Bacteroides         |                    |      |
| Stroke        | 140 Patients | 16S        | $\gamma$-diversity | F/B ratio | Roseburia           | [55] |
|               | 92 Controls |         |           | Lactobacillaceae     | Bacteroides         |      |
|               |           |           |           | Akkermansia          | Lachnospiraceae     |      |
|               |           |           |           | Enterobacteriaceae   | Faecalibacterium    |      |
|               |           |           |           | Porphyromonadaceae   | Blautia, Anaerotipes |      |

The absence of microbiota in ApoE$^{-/-}$ mice model in standard diet accelerated the formation of atherosclerotic plaques in the aorta and the development of heart diseases when compared with conventional ones [36]. Interestingly, the opposite effect of the microbiota was observed with mice infused with Angiotensin II in which the absence of gut microbiota attenuated arterial HT and vascular dysfunction [37]. In addition, the imbalance or maladaptation of the microbial community, defined as “dysbiosis”, has been discovered to be associated with the incidence of CVD risk and to affect the progression of CVD. For instance, a significant dysbiosis has been identified in hypertensive animals and was characterized by decreased microbial richness and diversity, and increased Firmicutes/Bacteroidetes (F/B) ratio [38]. These alterations in microbiota were also discovered in Ldlr$^{-/-}$ mice (a hypercholesterolemic
atherosclerosis and stroke (Table 1). For instance, alterations of the gut microbial dysbiosis in patients with HT, atherosclerosis, CAD, heart failure and stroke (Table 1). For instance, alterations of the gut microbiota in patients with HT include a lower α-diversity and a significant shift of β-diversity with a higher abundance of potential pathogenic bacteria including Parabacteroides, Desulfovibrio, Prevotella and Oscillo bacter which are gram-negative bacteria that may produce endotoxins (e.g. lipopolysaccharides, LPS) associated with inflammatory status. Some gram-positive bacteria such as Clostridium have also been found in higher abundance in patients with HT [40–42]. Concurrently, lower abundance of beneficial bacteria including SCFAs producing bacteria like Lachnospiraceae, Lactobacillus, Faecalibacterium, Ruminococcus was detected in patients with HT [40–42]. Similar findings (Table 1) were also discovered in most of the human studies related to atherosclerosis, CAD, heart failure and stroke [43–54]. Additionally, alterations of some different bacterial groups were also found in CVD cohorts such as a decreased proportion of Bacteroides and Roseburia [43–48,52,54]. Besides, a gut microbial dysbiosis was also correlated with cardiac risk parameters in plasma. For instance, the alterations of microbiota were associated to the lipid metabolism dysfunctions like lower levels of High-density lipoprotein (HDL) in plasma in CVD patients [48]. Moreover, increased abundance of Escherichia/Shigella was positively correlated to the elevated plasmatic levels of TMAO, a gut microbial metabolite contributing to CVD pathogenesis [51]. However, some CAD and stroke patients have also been identified with higher presence of potential beneficial bacteria such as Lactobacillales or Akkermansia which was associated with the depletion of other SCFAs producers [48,53,54]. This discrepancy in human studies might be explained by the following aspects: (1) The methodology of gut microbiota analysis: multiple factors during the gut microbiota analysis could affect the taxonomic resolution including different protocols for fecal samples collection, storage, DNA extraction as well as different sequencing platforms or different sequencing methods (e.g. 16S or shotgun) [55–57]. (2) Population level related parameters in clinical studies: numerous covariates of the subjects recruited in the clinical studies could interfere with the fecal microbiota variation such as blood parameters, dietary habits, lifestyle, anthropometrics as well as stool consistency [58], (3) Medication: this is another essential factor which might result in the variation of gut microbiota. It has been reported that individual medication or multimedication as well as the drug dosage exhibited a strong relation to the alteration of the gut microbiota [58,59]. Therefore, clinical studies in human subjects should consider the above aspects to design properly new investigations on gut microbiota related CVD pathogenesis.

4. The signature of diet-gut microbiota crosstalk in CVD pathogenesis

4.1. Intestinal barrier dysfunctions and inflammation

In healthy state, the appropriate intestinal barrier provides a crucial first line of defense against pathogens that is supported by several physiological components including mucus layer, epithelial cells connected by tight junction proteins and immune cells [60]. However, CVD patients in heart failure or HT were often observed with dysfunctional intestinal barrier accompanied with increase of the systemic microbial component LPS and inflammation [61–63]. What are the risk factors to trigger the gut leakiness and inflammation in the process of CVD? One of the hypotheses is that long-term consumption of western diet or HFD could induce dysbiosis and impair the gut barrier which enhanced LPS translocation and systemic inflammation resulting in increase of CVD risk [64–66].

In view of this, higher intake of diet rich in saturated fat or trans fatty acids is highly associated with the increase of CVD risk while a lower intake of dietary saturated fats reduced CVD by about 30% [67]. In large cohort studies, long-term (6-months) consumption of HFD induced a microbial dysbiosis with increased proportion of Gram-negative bacteria such as Alistipes and Bacteroides accompanied with higher levels of genes involved in LPS biosynthesis [64]. Meanwhile, dietary fats have been identified to impair intestinal barrier through activating the secretion of pro-inflammatory cytokines (e.g. TNF-α, IFN-γ and IL-1β) [68,69]. The upregulation of the pro-inflammatory cytokines further activated MLCK (Myosin light-chain kinase) signaling pathway which reorganizes the tight junction proteins including occludin, ZO-1 (Zonula occludens-1) and results in the leaky gut [70–72].

When the intestinal barrier is disrupted, LPS or pathogens could translocate into the circulation causing endotoxemia that stimulates the release of systemic pro-inflammatory cytokines [73]. Once translocated in the bloodstream, endotoxin can trigger the damage of endothelial cells through interaction with TLR-4 (Toll-like receptor 4) on cellular surface and enhance the generation of ROS (Reactive oxygen species) to reduce endothelial NO (Nitric oxide) bioavailability resulting in the formation of plaque and atherosclerosis lesion [73,74]. This hypothesis has been confirmed in animal models, in which ApoE−/− mice under western diet displayed an aggravation of atherosclerotic lesions with a significant increase of Proteobacteria (Gram-negative pro-inflammatory bacteria) and systemic LPS levels [75]. Moreover, western diet promotes the upregulation of inflammatory cytokines (e.g. TNF-α and IL-1β) and increases intestinal permeability coupled with modification of tight junction proteins (e.g. occludin) in ApoE−/− mice as well [75,76]. However, there is still a lack of data in human cohorts to decipher at what point the impaired intestinal barrier and associated increased endotoxemia due to western diet can induce CVD pathogenesis.

4.2. Lipid metabolism disorders

In addition to diet-microbiota interactions on inflammation and gut barrier functions, intestinal microbes also influence CVD via the host lipid metabolism. Indeed, a growing body of animal and human studies have suggested that the gut microbiota is implicated in lipid metabolism disorders such as dyslipidemia or hyperlipidemia, which are major risk factors in the development of CVD [77–80]. For example, GF mice have an altered cholesterol metabolism [81] and the depletion of gut microbiota in ApoE−/− mice caused a marked elevation of plasma cholesterol accompanied with larger aortic lesions compared to conventional ApoE−/− mice [77,78]. Moreover, microbiota transplantation from high plasma cholesterol humans to mice instigated the phenotype of upregulated circulating cholesterol coupled with the reduction of hepatic cholesterol synthesis [78]. This could be due to cholesterol-to-coprostanol conversion by the intestinal microbiota which could facilitate the elimination of cholesterol from the body and lower cholesterolemia [82]. This has been confirmed using a mathematical model of cholesterol metabolism, and recently revealed that both bile salt metabolism and cholesterol-to-coprostanol conversion by the gut microbiota can influence blood cholesterol level [83]. In addition, an interesting recent study on human cohorts also confirmed this point and identified that individuals harboring coprostanol-forming microbes such as Eubacterium coprostanoligenes which contain cholesterol metabolizing enzymes isMA have significantly lower fecal cholesterol levels and lower serum total cholesterol [84]. However, controversial results were also discovered in GF ApoE−/− mice with smaller plaque area despite of the
upregulation of plasmatic total cholesterol (TC) which might be due to the absence of endotoxemia linked to the GF status [79].

Interestingly, the absence of gut microbiota seems to attenuate atherogenic effects of long-term dietary lipids consumption. Specifically, HFD-fed GF Ldlr\(^{-/-}\) mice were identified with a significant reduction in thrombus size compared with conventional counterparts [39]. Although there is no difference in plasmatic TC levels in both GF and conventional Ldlr\(^{-/-}\) mice fed with HFD, lipids enriched diet still induced about twice the level of TC in GF mice (TC\(=\)1.6 mg/dl\(\times 10^3\)) versus GF mice fed with chow diet (TC\(=\)0.8 mg/dl\(\times 10^3\)) [39]. In comparison, HFD induced about eight fold increase in plasmatic TC in conventional mice (=1.6 mg/dl\(\times 10^3\)) versus mice raised with chow diet (TC\(=\)0.2 mg/dl\(\times 10^3\)) [39]. Similar discoveries for VLDL were also found in this study [39]. Lipids enriched diet also enhanced the microbiota dysbiosis in Ldlr\(^{-/-}\) mice with an increased abundance of Clostridiaceae, Staphylococcaceae, Bacillales and decreased abundance of Lactobacillales [39]. However, recent study suggested that no significant difference was discovered between GF Ldlr\(^{-/-}\) vs conventional mice on late aortic atherosclerosis [85]. Taken together, the different studies show different impacts of the gut microbiota on blood lipid metabolism. Whether this impact has a protective or aggragating effect on CVD development is still not clear. This discrepancy might depend on the animal model, the age of animals, the type of diet, the feeding period as well as the housing conditions. Future studies could take these factors into their consideration for a better investigation.

![Fig. 1](image_url)

**Fig. 1.** Potential mechanisms of dietary metabolites produced by gut microbiota in CVD pathogenesis. Dietary choline or L-carnitine could be metabolized by specific gut microbiota to TMAO which is further oxidized in the liver by FMOs to produce TMAO. TMAO has been identified as an essential biomarker to stimulate foam cell formation, induce inflammation, suppress RCT, as well as accelerate platelet hyperreactivity and thrombosis. Primary BAs are synthesized from dietary fats or cholesterol via enterohepatic circulation. Only about 5% of primary BAs are non-reabsorbed and deconjugated by gut microbiota to produce secondary BAs. BAs can interact with receptors including FXR, PXR or TGR5 (\(^b\)) to stimulate vascular lesion formation, increase inflammation and increased severity of lipid metabolism defects. Dietary proteins rich in AAs such as Phe could be converted into phenylacetic acid via the gut microbiota and then transferred into PAG in the liver. PAG further responded to G-protein coupled receptors including \(\alpha_2A\), \(\alpha_2B\) and \(\beta_2\)-ARs to facilitate platelet responsiveness, thrombosis potential to promote atherosclerotic CVD. The other gut microbial derived metabolites IS from other western style nutrients \(^b\), are primarily metabolized by specific gut microbial enzymes to produce high levels of trimethylamine (TMA) \(^b\). Specifically, TMA lyase containing microbial CntC/D genes is responsible for choline related TMAO transformation [63,66]. TMAO further absorbed into blood stream and oxidized in the liver by flavin monooxygenases (FMOs, mainly FMO3) to generate TMAO [63,92]. Seven different bacterial strains expressing TMA lyase CntC/D were identified in human gut including Anaerococcus hydrogenalis, Clostridium asparagiforme, Clostridium hathewayi, Clostridium sporogenes, Escherichia fergusonii, Proteus penneri, and Providencia rettgeri [87]. Additionally, TMAO can be synthesized from L-carnitine via microbial Rieske-type l-carnitine oxygenase CntA/B [90]. Although CntA/B encoding genes were identified in Proteobacteria, the L-carnitine dependent TMAO formation with commensal gut microbiota has not been demonstrated [93]. Whereas, a very recent study discovered a novel combination of two bacterial strains Emergencia timonensis and.

5. Gut microbiota metabolites in CVD pathogenesis

5.1. TMAO, a dietary induced microbial biomarker for the risk of CVD

_Diet-intestinal microbiota derived metabolite TMAO -_ TMAO (trimethylamine-N-oxide), a gut microbial co-metabolite derived from dietary nutrients, was first discovered and reported to predict the risk for CVD a decade ago [86]. The dietary precursors phosphatidylcholine [86], choline [87], and L-carnitine [33], which are commonly present in cheese, red meat, seafood, egg yolks and other western style nutrients [88], are primarily metabolized by specific gut microbial enzymes to produce high levels of trimethylamine (TMAO) [63,89–91]. Specifically, TMA lyase containing functional microbial CntC/D genes is responsible for choline related TMAO transformation [63,66]. TMAO is further absorbed into blood stream and oxidized in the liver by flavin monooxygenases (FMOs, mainly FMO3) to generate TMAO [63,92]. Seven different bacterial strains expressing TMA lyase CntC/D were identified in human gut including Anaerococcus hydrogenalis, Clostridium asparagiforme, Clostridium hathewayi, Clostridium sporogenes, Escherichia fergusonii, Proteus penneri, and Providencia rettgeri [87]. Additionally, TMAO can be synthesized from L-carnitine via microbial Rieske-type l-carnitine oxygenase CntA/B [90]. Although CntA/B encoding genes were identified in Proteobacteria, the L-carnitine dependent TMAO formation with commensal gut microbiota has not been demonstrated [93]. Whereas, a very recent study discovered a novel combination of two bacterial strains Emergencia timonensis and.
llubacter massiliensis as potential important players in carnitine converted TMAO accumulation [94]. Interestingly, the bacteria *E. timonensis* has lately been identified to promote TMAO production via l-carnitine → γBB (gamma-butyrobetaine, precursors of carnitine) → TMA → TMAO pathway [95]. However, the specific commensal microbiota associated with carnitine-TMA transformation pathways still need to be further discovered.

**Diet-microbiota derived TMAO in the modulation of CVD pathogenesis** – In order to directly discover the diet-microbiota related TMAO pathway in CVD progression, initial studies suggested that mice raised with high choline or carnitine diet showed an elevation in circulating TMAO levels, an increase of macrophage foam cell formation and enhancement of aortic atherosclerotic plaque development (Fig. 1) [33,86]. On the contrary, the capacity to TMAO production, and choline or carnitine diet related atherosclerotic plaque burden were respectively eliminated or suppressed in GF or antibiotic treated ApoE⁻/⁻ mice (C57BL/6 strain) [33,86]. Interestingly, ApoE⁻/⁻ mice were discovered to develop higher choline diet dependent aggregation of aortic lesions when receiving cecal microbiota transplantation from high TMAO/HA mice to produce donor C57BL/6 mice than from low TMAO/TMAO producing donor NZW/Lacj mice [96]. Similarly, transplant of high TMAO producing microbes in GF mice induced platelet hyperresponsiveness and enhanced thrombosis associated with high plasmatic TMAO levels [97]. Therefore, microbiota is necessary for TMAO production which is involved in atherosclerosis progression through several mechanisms: (1) foam cell formation: Microbiota derived TMAO can activate the expression of the stress-induced heat-shock proteins (HSP) HSP70 or HSP90, which may trigger the activation of scavenger receptors (e.g. SR-A1) and CD36 in macrophages to stimulate the uptake of oxidized low-density lipoprotein (ox-LDL) and foam cell formation [33,86,98]. (2) Inflammation: TMAO induced the proatherogenic inflammatory markers expression including IL-6, cyclooxygenase 2 (COX-2), and intracellular adhesion molecule 1 via the activation of mitogen-activated protein kinase (MAPK) and NF-κB signaling pathway in Ldlr⁻/⁻ mice fed a choline-rich diet. [98,99]. In addition, the increase in circulating TMAO was associated with the elevation of pro-inflammatory cytokines TNF-α and IL-1β, and reduction of the anti-inflammatory cytokine IL-10 [98,100]. (3) Lipid Metabolism: TMAO could suppress the reverse cholesterol transport (RCT) which results in the arterial cholesterol deposition to accelerate atherosclerotic lesions [98], (4) Platelet hyperreactivity and thrombosis: Diet induced high levels of microbial TMAO could stimulate platelet to activate sub-maximal stimulus including thrombin, adenosine diphosphate (ADP) and collagen as well as to induce the release of intracellular calcium resulting in platelet hyperresponsiveness [97]. However, some studies have shown opposite results illustrating that dietary TMAO, choline or carnitine did not induce the atherosclerosis in ApoE⁻/⁻ or Ldlr⁻/⁻ mice model [77,101]. This discrepancy might be due to the housing conditions and mice models, but precise reasons still need to be further discovered. Interestingly, Jaworska et al recently illustrated that TMA, but not TMAO reduced cardiomyocytes and vascular smooth muscle cells viability [102,103]. They also found that intravenous infusions of TMA instead of TMAO in rats showed a significant increase of the mean arterial blood pressure indicating the deleterious effect of TMA on CVD [104]. More in vivo and in vitro studies still need to be done to further confirm the role of TMA on CVD pathogenesis and validate the related mechanism.

**Human studies on circulating TMAO in CVD prediction and prognosis** – Numerous human studies have proved the role of gut microbial-derived TMAO in prediction of CVD risk. The original study investigated a human cohort with more than 1800 subjects and found that elevated plasmatic TMAO were related with the occurrence of multiple CVD subtypes including peripheral artery disease (PAD), CAD, and history of myocardial infarction (MI) [86]. In clinical outcome studies, large cohorts of participants indicated that circulating TMAO was positively associated with increased risks of major adverse cardiovascular events, incident mortality and artery infarction [105,106]. The cutoff value of plasmatic TMAO in many studies is over 6 μM to predict the risk of all-cause mortality [21,63] and a recent meta-analysis of over 10,000 subjects proposed 5.1 μM for CVD prognosis [107]. Additionally, high levels of TMAO have been found associated with the increase of pro-inflammatory monocytes and cardiovascular risk in human cohorts [108]. Similarly, a systematic review and dose–response meta-analysis recruited more than 13,000 participants and discovered a non-linear association between the elevation of plasmatic TMAO levels and the increase of inflammatory marker C-reactive protein (CRP) [109]. However, not all the human studies found similar data. For instance, there were no obvious change in gut microbiota and blood TMAO levels in human subjects with asymptomatic atherosclerosis. Whereas, patients with stroke and transient ischemic attack exhibited a significant dysbiosis of the gut microbiota but with a decrease of plasma TMAO levels [52]. Comparatively, there was no significant relation between TMAO concentrations and atherosclerosis progression in a cohort of participants (n = 817) of ages 35–55 over a 10-year follow-up [110]. Interestingly, a recent study uncovered that TMA but not TMAO was related to high blood pressure load and CVD risk factors, and with decreased abundance of genera Akkermansia, Faecalibacterium, Ruminococcus, and Subdoligranulum in human subjects with early-stage chronic kidney disease (CKD) [111]. However, further studies in human cohorts still need to be conducted to investigate whether the TMAO precursor TMA is a forgotten toxin or predictor in the modulation of early-stage CVD pathogenesis.

5.2. Bile acids

Bile acids (BAs) are hydroxylated and saturated steroids, and facilitate the emulsification and intestinal absorption of dietary fat and fat-soluble molecules [112,113]. In human hepatocytes, primary Bas (cholic acid (CA) and chenodeoxycholic acid (CDCA)) are synthesized from cholesterol via catalytic enzymes such as cholesterol 7α-hydroxylase (CYP7A1), steroid-27-hydroxylase (CYP27A1), oxysterol 7α-hydroxylase (CYP7B1) which expressions are regulated by the gut microbiota [114]. Then, primary BAs are conjugated to glycine or taurine, and over 95% primary BAs are reabsorbed and recirculated back to the liver [115]. The non-reabsorbed BAs can be deconjugated by catalyzed enzyme bile salt hydrolases (BSHs), which are expressed by several commensal gut bacteria, including the Gram-positive *Bifidobacterium, Clostridium, Enterococcus, Lactobacillus* and the Gram-negative *Bacteroides* [116,117]. Besides deconjugation, gut microbes such as *Clostridium* and *Eubacterium* are a source of 7-dehydroxylase to generate secondary BAs including lithocholic acid (LCA) from CDCA and deoxycholic acid (DCA) from CA [118]. In addition, the oxidation and epimerization of BAs are catalyzed via hydroxysteroid dehydrogenases (HSDHs), which have been discovered in various bacteria including *Actinobacteria*, *Proteobacteria*, *Clostridium* and others [119,120].

Once the microbial metabolized BAs enter into the circulating blood, BA receptors can mediate the signaling pathways to regulate host metabolism which has been discovered to contribute to CVD development (Fig. 1). One of the most essential BA receptors is Farnesoid X-activated receptor (FXR) that is the main sensor with both primary BAs in the liver and secondary BAs in the intestine [121]. FXR has been identified in the modulation of lipid and glucose metabolism [121]. Interestingly, the activation of FXR in atherosclerosis prone mice showed protective effects in the formation of atherosclerotic lesions [122]. Correspondingly, the deletion
of FXR in ApoE<sup>−/−</sup> caused an increased severity of lipid metabolism defects with enhanced aortic plaque formation [123]. In contrast, other studies in FXR/ApoE or FXR/Ldlr double deficiency mice showed reductions of aortic lesions and plasma LDL cholesterol [124,125]. Of interest, FXR has also been discovered to modulate TMAO pathway via regulating FMO3 activity [126]. Another important BA receptor is Takeda G protein-coupled receptor 5 (TGR5), whose activation by secondary BAs has been identified to attenuate vascular lesion formation via decreased intraplaque inflammation, plaque macrophage content and lipid loading [127]. Pregnane X receptor (PXR) is another nuclear receptor which is associated with BAs metabolism and is activated by secondary BAs (e.g. LCA) [128]. In contrast to other receptors, the activation of PXR elevated the level of lipoproteins VLDL, LDL and CD36 expression to aggregate the atherosclerotic formation in ApoE<sup>−/−</sup> mice [128], while the inhibition of PXR in ApoE<sup>−/−</sup> mice alleviated the aortic lesions area with the decrease of lipid uptake in macrophages and CD36 expression [129].

Although most studies on the mechanism of BAs in CVD pathogenesis are on mice model, circulating levels of BAs have been discovered in association of CVD phenotypes in clinical cohorts. For instance, decreased primary and secondary BAs levels in human subjects have been found with reduced overall survival in chronic heart failure patients [130]. In addition, lower fasting plasmatic total BAs were significantly associated with the severity of CAD, MI and the presence of coronary lesions [131]. Collectively, gut microbiota derived BAs regulate the development of CVD via multiple types of BA receptors, and plasmatic BAs might be another essential predictor for CVD occurrence which still need to be further investigated.

5.3. Short chain fatty acids

Short chain fatty acids (SCFAs) are the major microbial products of dietary fibers (mainly polysaccharides) fermentation, and consist mainly of acetate, butyrate and propionate [132]. Specific members of the gut microbiota participate in particular fermenting pathways for SCFAs synthesis [133,134].

Interestingly, the intestinal microbiota has been identified to modulate the protective association between the diet rich in fibers and CVD risk. Specifically, numerous studies have illustrated the functional role of dietary fibers or SCFAs in alleviating HT or other CVD subtypes (Fig. 1). One of these studies has found that both high fiber diet and acetate supplementation could reduce systolic and diastolic blood pressures, cardiac fibrosis, and left ventricular hypertrophy, which was associated with improved gut dysbiosis and increased abundance of Bacteroides thetaiotaomicron [135]. Similarly, propionate treatment protected the mice from hypertensive cardiovascular damage, while butyrate producing bacteria (e.g. Roseburia intestinalis) decreased the aortic atherosclerotic lesion area [136,137]. The studies discovered that the G-protein-coupled SCFAs olfactory receptor 78 (Olfr78) and G-protein receptor 41 (GPR41) participated in the regulation of host blood pressure and endothelial function [138,139]. In specific, propionate induced an acute hypotensive response in wild-type (WT) mice by modulating the disruption of Olfr78 and GPR41 expression [138]. Whereas, antibiotic treated Olfr78<sup>−/−</sup> mice but not wild-type mice showed an elevation in blood pressure, and GPR41<sup>−/−</sup> mice also had systolic HT compared with WT mice [138,139]. Moreover, a very recent study suggested that both acetate and butyrate improved rat aortic endothelial dysfunction by increasing the bioavailability of NO, through GPR41/43 activation for butyrate only [140]. Further studies still need to be conducted to uncover the mechanistic role of SCFAs in regulation of CVD pathogenesis.

In humans, most of the studies of CVD risk are about SCFAs related blood pressure modulation. Early clinical intervention study found that increase intake of dietary fibers was associated with the reduction of blood pressure in patients with HT [141]. Similar protective effects of viscous soluble fibers on blood pressure were also discovered in a meta-analysis study [142]. By contrast, a recent intervention study reported that high fiber high protein diet might increase the risk of CVD by upregulating circulating SCFAs level [143]. Specifically, high protein high fiber diet induced higher pro-inflammatory level which was associated with upregulation of LDL cholesterol and blood pressure; and higher butyrate level which was correlated with upregulation of glucose and downregulation of HDL cholesterol [143]. However, it is still limited on the direct demonstration of the SCFAs effect on human CVD risk or protection which need to be further clarified.

5.4. Other gut microbial metabolites

Aromatic amino acids (AAAs) are aromatic ring containing amino acids including phenylalanine (Phe), tryptophan (Trp) and tyrosine (Tyr) [144]. The major sources of AAAs are dietary proteins such as beef, pork, chicken or fish [145]. Interestingly, researchers discovered a pathway from the gut microbiota Clostridium sporogenes that generates AAAs metabolites [144]. Recently, several studies uncovered a robust relation between the Phe-derived microbial metabolite phenylacetylglutamine (PAG) and major adverse cardiac events (e.g. myocardial infarction/MI, acute ischemic stroke or coronary artery disease) (Fig. 1) [146–148]. In specific, dietary Phe was converted into phenylacetic acid via the gut microbiota enriched in porA gene and subsequent converted into PAG in the liver. PAG further activated G-protein coupled receptors including α2A, α2B and β2-adrenergic receptors to facilitate platelet responsiveness, thrombosis potential in animal models of arterial injury [146]. Similarly, gut microbial derived metabolite indoxyl sulfate (IS) from Trp and p-cresol sulfate (PCS) from Tyr have also been identified as valuable markers to predict CVD events in patients with CKD [149,150]. This might be due to the deleterious effects of IS and PCS through induction of uremic toxicity and endothelial dysfunction [150–152]. However, some studies discovered that IS, PCS, or PAG were not associated with CVD outcomes [153,154]. The discrepancies might be due to the threshold effect from different studies. Further investigations still need to be conducted to confirm the role of these gut microbial metabolites on CVD progression.

6. Dietary interventions in CVD prevention through gut microbiota

6.1. Dietary patterns

Healthy dietary patterns have been suggested to prevent the CVD progression (Fig. 2) including Mediterranean Diet (Med-diet), Dietary Approaches to Stop Hypertension (DASH) [10] and feeding patterns such as intermittent fasting (IF) [155].

**Types of diets** – Multiple clinical trials have confirmed the protective effect of Med-diet on major vascular events, coronary events, stroke and heart failure [156,157]. This effect is associated with the increase of microbiota diversity and microbial metabolite SCFAs [158] as well as lower levels of gut microbiota derived metabolite TMAO [159] and plasmatic LPS [160]. However, no direct finding has been investigated until very recently that the long-term intervention of Med-diet could protect CVD through gut microbiota modulation [161]. Specifically, long-term intervention of Med-diet could significantly alter the overall gut microbiome profiles with the enrichment of dietary fiber metabolizers such as Faecalbacterium prausnitzii and Bacteroides cellulosilyticus. In particular, Med-diet showed a strong protective effect on CVD...
risk factors including lipid metabolism, inflammation and glucose homeostasis in the absence of Prevotella copri [161]. Although multiple data have illustrated that DASH diet could improve cardiac risk factors with decrease of HT and dyslipidemia [162], there is still lack of data about the direct linkage between DASH diet and microbiota modifications in CVD preventions.

Feeding patterns – Intermittent fasting (IF), one of the important dietary feeding patterns, is a practice of periodic energy restriction which has been discovered to reduce the CVD risk via the alteration of gut microbiota [155,163]. Specifically, spontaneously hypertensive stroke-prone rats showed a striking shift of gut microbiota in β-diversity after 50 days of IF intervention which was associated with the reduction of HT via modulation of the bile acids metabolism [155]. Those findings have been confirmed using fecal transplantation to GF rats [155]. Moreover, clinical interventions in human cohorts with IF during 8 weeks significantly improved vasodilatory parameters and attenuated oxidative stress, inflammation associated with increased SCFAs production by the microbiota and decreased plasmatic LPS [163]. Interestingly, short-term 5 days fasting also reduced the blood pressure and body weight with the modulation of the microbiota including Desulfovibrionaceae, Akkermansia, and Ruminococcaceae [164].

6.2. Dietary components

Dietary polyphenols in fruits and vegetables – Polyphenols are a large family of organic compounds commonly found in plant products, particularly fruits and vegetables. More than 90% of total polyphenols is non-absorbable in the small intestine and further metabolized by the gut microbiota in the large intestine [165]. A growing body of studies supported the effect of dietary polyphenols on the modification of gut microbiota and CVD protection [166–168]. For instance, resveratrol (found in fruits such as grapes, apples and berries) has been identified to attenuate atherosclerosis in ApoE−/− mice by downregulating TMAO levels and upregulating BAs synthesis which was associated with increased abundance of beneficial bacteria Bacteroides, Lactobacillus, Bifidobacterium, and Akkermansia [166]. Oral administration of quercetin (found in vegetables such as onions, broccoli and tomatoes) has been discovered to suppress body weight gains and ameliorate the extent of atherosclerotic lesions with diminished levels of cholesterol, atherogenic lysophosphatidylcholine and reduced abundance of gram-negative bacteria Verrucomicrobia together with the increase of microbiome diversity [167]. In human subjects, interventions with diet rich in polyphenols found that dietary polyphenols could
significantly increase microbial diversity and *Ruminococcaceae* related with the improvement of cardiometabolic risk factors such as plasmatic triglycerides and cholesterol in large VLDL [168]. Collectively, polyphenols in fruits and vegetables might be potential therapeutic interventions for CVD, and a part of their protective effect could be mediated through gut microbiota modifications.

**Dietary fibers and prebiotics** – Dietary fibers are non-digestible carbohydrates including water-soluble or insoluble forms which are commonly presented in fruits, vegetables, whole grains, nuts and legumes etc. [169]. Dietary fibers could not be absorbed in the small intestine and “feed” the healthy gut microbiota leading to increased diversity and production of SCFAs [169]. As mentioned previously, SCFAs activate specific receptors leading to improved HT and aortic endothelial cells dysfunction [138–140]. Indeed, a very recent study discovered that chickpea dietary fiber increased the microbial diversity and the relative abundance of *Bacteroides* and *Lactobacillus* and upregulated levels of propionic acid [170]. Additionally, chickpea dietary fiber could improve hyperglycemia via similar modification of gut microbiota [170]. Whole grain oat also decreased plasma cholesterol levels and improve insulin sensitivity which correlated with increased beneficial *Lactobacillus* in the microbiota [171,172]. Similarly, whole grain products consumption in human cohorts also showed lower levels of total and LDL cholesterol and higher abundance of *Bifidobacterium* [173].

Prebiotics are plant derived or non-digestible food ingredients which stimulate the growth of “friendly” microorganisms in the GI tract [174]. Most prebiotics are dietary fibers, while not all dietary fibers can be categorized as prebiotics [175]. Common prebiotics include oligosaccharides and polysaccharides such as inulin, oligofructose, β-glucans which can ordinarily induce specific modifications of the gut microbiota [174]. Many studies have interestingly investigated the beneficial effects of prebiotics on host metabolism to improve CVD conditions (Fig. 2) through three main aspects: (1) *Reduce blood lipids*: Parnell *et al.* discovered that supplementation of prebiotic fibers (e.g. inulin) could lower the plasmatic cholesterol levels as well as reduce the TAG accumulation in the liver [176–178]; (2) *Diminish endotoxemia and inflammation*: Cani *et al.* found that prebiotic oligofructose could increase the population of *Bifidobacteria* with negative relation to endotoxiaemia and inflammation in plasma and adipose tissues [179]; (3) *Decrease blood pressure*: Kaye *et al.* uncovered very recently that the supplementation of diet rich in prebiotic fibers could diminish both systolic and diastolic blood pressure through GPR43 signaling pathway [180].

**Probiotics** – Probiotics are defined as “live microorganisms that, when administered in adequate amounts, confer a health benefit on the host.” [174]. Large scales of fermented products in human diets such as yogurt, sauerkraut, kefir, Kimchi contain probiotic strains [174]. As prebiotics, probiotic strains have also been identified to protect against CVD progresses (Fig. 2) in more aspects: (1) *Ameliorate vascular endothelial function*: administration of *Lactobacillus plantarum* 299v showed the improvement of endothelium-dependent vasodilation in resistance arteries from patients with CAD [181]. Similarly, *Lactobacillus fermentum* CECT5716 treatment reduced vascular oxidative stress and improved endothelial function in rats [182]; (2) *Decrease blood glucose and oxidant activity*: intervention with probiotic yogurt significantly lowered the blood glucose and increase total antioxidant status [183]; (3) *Reduce cholesterol*: supplementation with *Bifidobacterium longum* BB536 has significant effects on the reduction of total cholesterol, liver lipid deposition and adipocyte size [184]. (4) *Attenuate endotoxemia and inflammation*: oral introduction of *Akermansia muciniphila* has been shown to reduce atherosclerotic lesions by improving systemic endotoxemia-induced inflammation through restoration of the gut barrier function [64]. In addition, supplementation with *Lactobacillus reuteri* V3401 reduced the levels of inflammatory markers such as TNF-α, IL-6, IL-8 which was associated with the reduction of CVD risk [185].

### 6.3. Dietary Chinese medicine

Some natural ingredients from Chinese medicine have also been used as potential CVD therapeutics via the modulation of gut microbiota (Fig. 2). Particularly, berberine (BBR), a bioactive isoquinoline alkaloid which is widely presented and extracted from various Chinese medicinal plants has been proved to exert many beneficial effects. Wu *et al.* recently discovered that high dose of BBR not only improved lipid metabolism via attenuating reduced total cholesterol and VLDL cholesterol levels, but also downregulated pro-inflammatory cytokines TNF-α, IL-1β, IL-6 and upregulated anti-inflammatory IL-10 levels which were correlated with the increased abundance of *Alistipes* and *Roseburia* involved in SCFA production [186]. Additionally, BBR could reduce choline-induced atherosclerosis by suppressing TMAO production through remodeling gut microbiota compositions [187]. Similarly, red yeast rice (RYR), another Chinese folk medicine, could alleviate the plaque formation with the reduction of total cholesterol and LDL levels which are associated with decreased ratio of Firmicutes/ Bacteroidetes as well as reduced abundance of *Alistipes* and *Flavonifractor* [76,188]. RYR intervention also improved gut barrier function, and attenuated inflammation via TLRs signaling pathway [76]. Furthermore, *Ganoderma lucidum* (also known as Lingzhi), a type of medicinal mushroom, has been discovered to reduce obesity, endotoxemia, chronic inflammation as well as restore intestinal barrier function by decreasing endotoxin bearing Proteobacteria levels and increasing beneficial bacteria including *Clostridium* and *Eubacterium* [189].

### 7. Summary and outlook

Over the past decades, accumulating studies demonstrated an essential and complex association between gut microbiota and cardiovascular disease. As one of the important modulators in gut microbiota, dietary components have been identified to modify the microbial compositions which were linked with the systemic endotoxemia, inflammation, gut barrier dysfunction, as well as lipid metabolism dysfunction to increase the CVD risk. While, more research data suggested the principal effect of intestinal microbiota on dietary metabolism in modulating CVD pathogenesis including: (1) metabolizing dietary choline or L-carnitine to induce the release of TMAO which promote the atherosclerotic progression; (2) regulating BAs metabolism which is likely to regulate the atherosclerotic formation via multiple receptors pathway; (3) generating AAAs metabolites PAC, IS, IPA or PCS which can accelerate the atherosclerosis formation; (4) fermenting dietary fibers to generate SCFAs which mostly exert some beneficial effects on CVD progression. These findings provide some excellent opportunities for developing novel potential prevention and therapeutic methods for CVD such as healthy diets and feeding patterns, dietary interventions with healthy components including dietary polyphenols from fruits and vegetables, dietary fibers and probiotics, probiotics as well as the dietary Chinese medicine interventions.

Although numerous intestinal bacteria have been identified in association with CVD risk, much more works remain to discover the specific commensal microbes and the precise mechanisms or pathways behind those complex relationship between the dietary induced gut microbiota modification and CVD pathogenesis. However, there are still some limitations in current research: (1) Regarding gut microbiota analysis, different studies follow different protocols or methodologies for fecal samples collection, storage
and DNA extraction, or choose different sequencing platform or methods. These dissimilarities might impact the consistency and result in the variations of gut microbiota. (2) In the clinical human cohort studies, numerous parameters of the recruited human subjects might affect the consistency of microbiota discoveries, such as dietary habits, lifestyle, and medications. (3) In animal studies, different age, housing condition, or different diet, feeding period could also impact the gut microbiota. Therefore, it is crucial to design the study properly to reach the reliable discoveries for future investigations.

CRediT authorship contribution statement

Xufei Zhang: Conceptualization, Writing – original draft. Philippe Gérard: Conceptualization, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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