

# Metabolic endotoxemia and cardiovascular disease: A systematic review about potential roles of prebiotics and probiotics

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## Abstract

Translocation of microbiome-derived lipopolysaccharide (LPS) to the bloodstream (metabolic endotoxaemia) is associated with a significantly increased risk of cardiovascular diseases (CVD); however, the direction of this association is not fully understood. It has been revealed by some studies that alterations in the intestinal microbiota (dysbiosis) lead to increased intestinal permeability and translocation of LPS to the blood circulation. LPS may trigger toll-like receptor 4- (TLR-4) mediated inflammatory responses; this could lead to a chronic low-grade pro-inflammatory condition named metabolic endotoxaemia (ME), which is typically observed in CVD patients. ME is promoted by increased intestinal permeability. Moreover, dysbiosis leads to production of trimethylamine-N-oxide (TMAO), a gut bacterial metabolite suggested as a new risk factor in CVD development. Probiotics, extensively reviewed for decades, are live microorganisms which, when taken in adequate amounts, have beneficial effects on the host metabolism. Prebiotics are a type of dietary fibre that act as nourishment for the good bacteria in the gut and decrease the population of pathogen bacteria that produce greater amounts of endotoxins. Although an association has been postulated between ME and CVD, the results of studies investigating the role of antibiotic therapy in preventing the disease have been inconsistent. In this review, we discuss how prebiotics and probiotics modulate gut microbiota and consequently might help with prevention and/or treatment of CVD associated with ME.

## KEYWORDS

cardiovascular disease, gastrointestinal microbiome, metabolic endotoxemia, probiotics, trimethylamine

## 1 | INTRODUCTION

Cardiovascular diseases (CVD) remain the chief cause of death in both Western and developing societies.<sup>1</sup> Despite the enormous

growth in knowledge and advances in prevention and treatment, approximately one out of three people in the USA still die from CVD.<sup>2</sup> In addition to traditional risk factors such as hypercholesterolemia, homocystinemia, hypertension, hyperglycemia, cigarette smoking,

and aging, metabolic endotoxaemia (ME) has been suggested to contribute to endothelial injury and development of CVD.<sup>3,4</sup> Nowadays attentions have been attracted to the role of ME in many fields of medicine particularly inflammatory diseases like atherosclerosis and other types of CVD.<sup>5</sup> In ME, microbiome-derived lipopolysaccharide (LPS) from the gut microbiota passes through the intestinal mucosa to enter the bloodstream, and may represent an important mediator of low-grade systemic inflammation.<sup>6</sup> Previous studies especially in patients with chronic kidney disease (CKD) have shown that high levels of endotoxin lead to production of pro-inflammatory cytokines and may predispose these patients to CVD.<sup>7</sup> More recently, increased level of trimethylamine-N-oxide (TMAO), a gut bacterial metabolite, has been suggested as a new risk factor in CVD development.

Changes in gut microbiota (dysbiosis) seem to contribute to ME. Under normal conditions, the intestinal epithelium acts as an impervious barrier to prevent LPS translocation; however, some conditions may alter this protective function.<sup>8</sup> Dysbiosis is defined as "any change to the components of resident commensal community relative to the community found in healthy individuals". Each of the following three conditions are generally classified as dysbiosis: (a) loss of valuable microbial organisms, (b) expression of pathobionts of possibly beneficial microorganisms, (c) loss of general microbial variety.<sup>9</sup> In states of dysbiosis, the intestinal barrier increases in permeability as a result of a disruption to the regulation of the epithelial cell-to-cell tight junction protein network.<sup>10</sup> A compromised intestinal barrier can be associated with bacterial translocation from the gut into the systemic circulation increasing the risk of ME.<sup>11,12</sup>

Disruption of the gut barrier and translocation of LPS and other bacterial metabolites have been shown to affect many aspects of human health, through various gut-to-organ axes; some examples include gut-brain axis, gut-heart axis, gut-skin axis, etc. The interaction between the gut and a specific organ has received much attention in current years. Although gut microbiota imbalance has been postulated to be associated with CVD through endotoxaemia, it is yet to be explored whether dysbiosis leads to inflammatory-mediated CVD risk, or CVD dysregulates gut microbiota composition by impairing blood supply of the gut.<sup>13</sup>

In the current review, we will debate findings on probable mechanisms connecting the gut microbiota and onset of endotoxaemia. Additionally, we will discuss the potential relationship between ME and CVD. Finally, we will review the evidence on the potential role of prebiotics/probiotics in modulation of gut microbiota and host metabolism with regard to the development of ME.

## 2 | RESULTS

### 2.1 | Selected articles

A flow diagram of the study selection is summarized in Figure 1. In total, 6895 articles were retrieved, of which 2560 were duplicates,

resulting in 4335 non-duplicated publications. Of these 4335 publications, 4131 articles did not meet the inclusion criteria and were excluded. A further 22 articles were excluded due to insufficient information. After exclusion, 19 articles met the eligibility criteria and were included in this review.

### 2.2 | Study characteristics

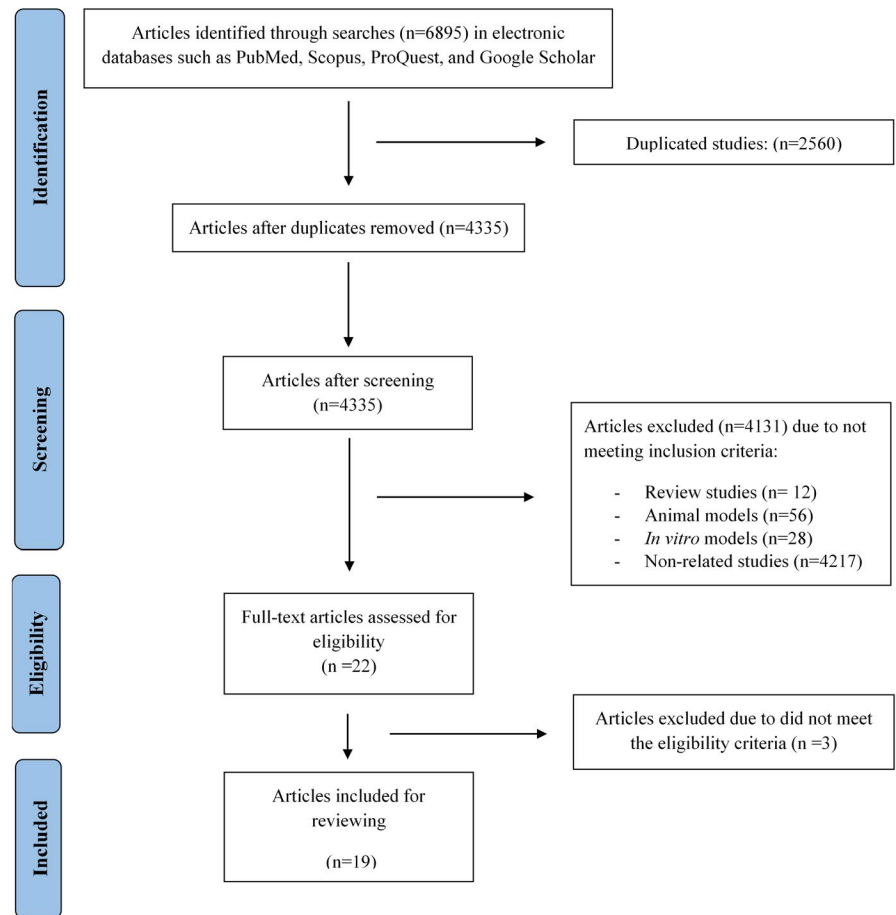
Characteristics and the main outcomes of the 19 articles included in the current review are summarized in Table 1. The studies were conducted between 2007 and 2019. Of all the identified studies, three studies were conducted in animal and 16 studies used a randomized clinical trial design. The trials group ranged in duration from 3 to 28 weeks.

### 2.3 | Gut microbiota

The gut microbiota (formerly called gut flora) is the complex community of microorganisms including bacteria, archaea and eukaryotes that live in the digestive tract of humans.<sup>8</sup> The majority of the GI-tract bacterial composition represent only two bacterial phyla, the Firmicutes and the Bacteroidetes. The gut microbiota offers many profits to the host, through a range of physiological functions such as strengthening gut integrity or affecting the intestinal epithelium, harvesting energy, protecting against pathogens and regulating host immunity.<sup>14,15</sup> However, there is a potential for these mechanisms to be disrupted as a result of altered microbial composition, known as dysbiosis.<sup>16</sup> Many factors can modify the balance of gut microbiota and allow for translocation of luminal contents to the inner layer of the intestinal wall.<sup>15</sup> The normal gut barrier, supported by tight junctions, prevents translocation of whole bacteria or bacterial fragments/products into the sub-mucosal compartment.<sup>14</sup> In the 'leaky gut' situation, infiltration of bacteria or related components into sub-mucosal space results in stimulation of mast cells and lymphocytes. The activation of these immune cells leads to production of pro-inflammatory cytokines, which further induces chronic inflammation and ME.<sup>17</sup>

Balanced gut microbiota plays a critical role in maintaining immune and metabolic homeostasis and protecting against pathogens. However, numerous studies have demonstrated that gut microbiota alteration (dysbiosis) can lead to increased cardiometabolic risk factors such as hypertension, elevated cholesterol, and insulin resistances, which greatly increase the risk of CVD.<sup>8,18</sup> Numerous mechanisms have been proposed to be involved in the role that gut microbiota alterations play in the aetiology of CVD; stimulation of immune system, short chain fatty acid production, chronic low-grade inflammation, lipoprotein and bile acid metabolism, and altered endocannabinoid receptor system tone are among these mechanisms.<sup>19</sup> More recently, more attention has been focused on the effect of metabolic endotoxaemia (ME) in the aetiology of CVD.<sup>20</sup>

**FIGURE 1** Flow diagram of the literature search and study selection process



## 2.4 | Metabolic endotoxaemia

A two to three-fold increase in circulating LPS levels is termed 'metabolic endotoxaemia'.<sup>21</sup> Components from gut microbiota, such as LPS, lipoteichoic acid, peptidoglycan, flagellin and bacterial DNA, can cause immune system activation. An animal model showed that modest rises (~1.5 fold) in endotoxin level or injection of 300 mg/kg/day of LPS could lead to increased fat deposition, insulin resistance, and chronic inflammation.<sup>22</sup> A recent study has demonstrated that systemic LPS administration led to damages in heart mitochondrial DNA and protein by oxidative stress. They revealed that LPS up-regulated endothelial cell adhesion molecules, and LPS associated favourably with the pro-atherogenic fraction.<sup>23</sup> Although endotoxaemia is not necessarily equivalent to increasing LPS, many have defined metabolic endotoxaemia as "a situation of chronically elevated plasma LPS". In patients with septic shock, the concentration of endotoxin level is often elevated a 1000 folds or higher compared to healthy controls.<sup>20</sup> On the contrary, Cani et al defined metabolic endotoxaemia as "a situation of chronically elevated plasma LPS at levels 10–50 times lower than during septic conditions".<sup>21</sup> However, there are more than 20 assays for detection of endotoxin markers, which can lead to cell damage, and theoretically multiple organ failure.<sup>24,25</sup>

Lipopolysaccharide is thought to be a major inducer of inflammatory responses, suggesting a possible association between intestinal LPS and CVD. The gut microbiota is a huge reservoir of

this endotoxin. There are  $10^{12}$  bacterial cells per gram of luminal content. Therefore, more than 1 g of LPS may be detected in the intestinal lumen. LPS is one of the main components of the external cell wall of Gram-negative bacteria. Thus, it is expected that changes in the barrier permeability facilitates translocation of LPS and other endotoxins into the bloodstream, and the following metabolic consequences.<sup>26</sup> LPS binds to LPS-binding protein (LBP). The complex LBP-LPS is presented to cluster of differentiation 14 (CD14) on innate immune cells, which is expressed mainly by macrophages, neutrophils, and dendritic cells; this subsequently mediates signal transduction, including nuclear factor kappa B (NF- $\kappa$  B) activation via TLR4, and contributes to the activation of innate and adaptive chronic inflammatory responses.<sup>27</sup> In addition, results from animal studies suggest that LPS exposure directly induces oxidation of low-density lipoprotein<sup>28,29</sup> (Figure 2).

Increased gut permeability and subsequent elevated circulating LPS has been shown in many cardiovascular conditions.<sup>30</sup> Previous studies have postulated that CVD is accompanied with both alterations in intestinal barrier, and increased microbial translocation. However, it is not yet clear whether dysbiosis is the cause or effect of CVD. Furthermore, some taxa of oral microbiota have also been detected in human atherosclerotic plaques. These data are supported by previous studies that found epidemiological links between periodontal diseases and CVD.<sup>31,32</sup> In other words, periodontal diseases may be associated with CVD.

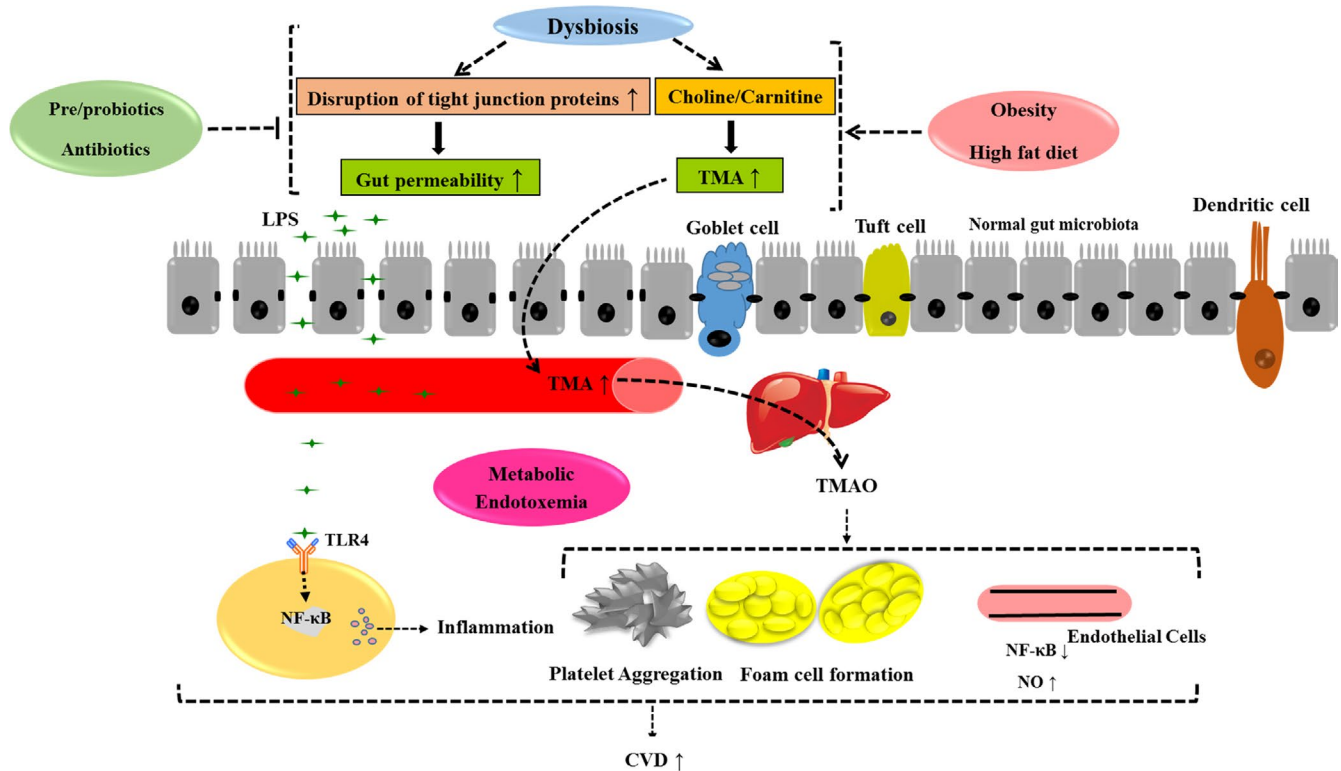
TABLE 1 Effects of gut modulation on endotoxaemia

Type of study	Intervention	Dosage	Duration	Effects	Reference
Animal					
Mice	High-fat diet with prebiotic (oligofructose [OFS])	OFS was added in a proportion of 90:10 (weight of HF diet: weight of OFS)	13 wk	OFS-fed mice had totally restored quantities of bifidobacteria <i>Bifidobacterium</i> spp. positively correlated with improved endotoxaemia	Cani et al., 2007 <sup>67</sup>
Mice with NAFLD	Monosodium glutamate (MSG)-with prebiotic ( <i>Lactobacillus casei</i> , <i>Bifidobacterium animalis</i> )	5 × 10 <sup>9</sup> CFU	2 wk	NAFLD and endotoxaemia prevented by monoprobiotic strains	Kobyliak et al., 2016 <sup>94</sup>
Obese rats with hepatic steatosis	<i>Lactobacillus paracasei</i> CNCM I-4034, <i>Bifidobacterium breve</i> CNCM I-4035 and <i>Lactobacillus rhamnosus</i> CNCM I-4036	10 <sup>10</sup> CFU	30 d	The probiotic strains reduced hepatic steatosis in part by lowering serum LPS, and had an anti-inflammatory effect in obese Zucker rats	Plaza-Diaz, et al., 2014 <sup>95</sup>
Human					
Patients with acute Pancreatitis	Probiotics ( <i>Lactobacillus acidophilus</i> , <i>Bifidobacterium longus</i> , <i>Bifidobacterium bifidum</i> , and <i>Bifidobacterium infantalis</i> with 25 mg of fructooligosaccharide)	Four sachets (2.5 billion bacteria per sachet)	7 d	No effect on gut permeability and endotoxaemia	Sharma et al., 2011 <sup>96</sup>
Patients with alcoholic hepatitis (AH)	Probiotics (cultured <i>Lactobacillus subtilis</i> / <i>Streptococcus faecium</i> )	1500 mg/d	7 d	Restoration of flora Decrease of LPS	Han et al., 2015 <sup>97</sup>
Patients with cirrhosis	Probiotic VSL#3 <sup>®</sup> Pharmaceuticals (contained lyophilized bacteria consisting of four strains of <i>Lactobacillus</i> , three strains of <i>Bifidobacterium</i> and <i>Streptococcus salivarius</i> subsp <i>Thermophilus</i> )	3600 billion bacteria/daily	2 mo	Reductions in endotoxin, No effect on inflammatory index	Tandon et al., 2009 <sup>98</sup>
Patients with cirrhosis	Probiotics ( <i>Escherichia coli</i> Nissle)	Two capsules (2.5-25 × 10 <sup>9</sup> of bacteria per capsule)	42 d	Restores intestinal microflora including <i>Lactobacilli</i> and <i>Bifidobacteria</i> , Decrease endotoxaemia	Lata et al., 2007 <sup>99</sup>
Women with type 2 diabetes mellitus	Prebiotic (Inulin)	10 g/day	8 wk	Decreased inflammation and metabolic endotoxaemia	Dehghan et al., 2014 <sup>91</sup>
Subjects with myocardial infarction (MI)	Probiotics ( <i>Lactobacillus rhamnosus</i> )	1.6 × 10 <sup>9</sup> CFU	3 mo	To determine whether probiotic supplementation will improve metabolic endotoxaemia and gut metabolite in individuals with MI (unpublished results)	Moludi et al., 2019 <sup>100</sup>
46 MetS patients	<i>Lactobacillus casei</i> , <i>Lactobacillus rhamnosus</i> , <i>Bifidobacterium breve</i> , <i>Lactobacillus acidophilus</i>	2 × 10 <sup>8</sup> CFU	12 wk	Decrease in anthropometric measurements and BP Decrease in hs-CRP but not significant	Rabiei, et al 2015 <sup>101</sup>

(Continues)

TABLE 1 (Continued)

Type of study	Intervention	Dosage	Duration	Effects	Reference
50 obese adolescents MetS	Probiotic capsules including <i>Lactobacillus salivarius</i>	10 <sup>10</sup> CFU	12 wk	No change in anthropometric measurements, FBS, BP, Insulin, peptide C, CRP, IL-6, TNF $\alpha$	Gobel, 2012 <sup>102</sup>
30 obese women	Inulin-type fructans (n = 15) or maltodextrin (n = 15)	16 g/day	3 mo	Decrease in LPS levels	Salazar et al., 2015 <sup>103</sup>
Apparently healthy men and women (n = 75)	supplementation with spore-based probiotics <i>Bacillus indicus</i> (HU36), <i>Bacillus subtilis</i> (HU58), <i>Bacillus coagulans</i> , and <i>Bacillus licheniformis</i> , and <i>Bacillus clausii</i>	Two capsules each day probiotic including 4 billion CFU	30 d	A 60% reduction in biomarkers of leaky gut and LPS when compared to the placebo group	McFarlan, et al., 2017 <sup>104</sup>
Obese women	Probiotic mix ( <i>Lactobacillus acidophilus</i> and <i>L. casei</i> ; <i>Lactococcus lactis</i> ; <i>Bifidobacterium bifidum</i> and <i>B. lactis</i> )	2 × 10 <sup>10</sup> CFU	8 wk	Decrease LPS levels	Gomes AC et al 2017 <sup>105</sup>
Adults with T2DM	Twice daily of Ecologic Barrier (multi-strain including <i>Bifidobacterium bifidum</i> W23, <i>Bifidobacterium lactis</i> W52, <i>Lactobacillus acidophilus</i> W37, <i>Lactobacillus brevis</i> W63, <i>Lactobacillus casei</i> W56, <i>Lactobacillus salivarius</i> W24, <i>Lactococcus lacti</i> )	2.5 × 10 <sup>9</sup> cfu/g	6-mo	Decrease endotoxin and inflammatory markers profile	Sabico, S et al 2017 <sup>106</sup>
225 healthy volunteers (BMI 28-34.9)	<i>Bifidobacterium animalis ssp lactis</i> 420 (B420) and the dietary fibre Litesse Ultra polydextrose (LU)	10 <sup>10</sup> CFU	7-mo	Decrease LPS and Zonulin	Stenman LK et al 2016 <sup>107</sup>
Type 2 diabetes patients	probiotic group drank <i>Lactobacillus casei</i> strain Shirota-fermented milk	4 × 10 <sup>10</sup> CFU	16 wk	Probiotic administration reduced bacterial translocation	Sato J et al 2016 <sup>108</sup>
Type 2 diabetes patients	Probiotic contains <i>Bifidobacterium bifidum</i> W23, <i>Bifidobacterium lactis</i> W52, <i>Lactobacillus acidophilus</i> W37, <i>Lactobacillus brevis</i> W63, <i>Lactobacillus casei</i> W56, <i>Lactobacillus salivarius</i> W24	2.5 × 10 <sup>9</sup> cfu/g	12 wk	Decreased metabolic endotoxemia	Sabico S et al 2017 <sup>109</sup>
Obesity and metabolic syndrome patients	After of treatment with VSL#3, a freeze-dried pharmaceutical probiotic containing CFU/capsule of 3 strains of bifidobacteria, 4 strains of <i>Lactobacillus</i> and <i>Streptococcus salivarius</i> subsp thermophilus,	112.5 × 10 <sup>9</sup>	3 mo	A remarkable improvement anthropometric indices, glycaemic control and plasma LPS level	



**FIGURE 2** The gut epithelium is an efficient barrier that prevents absorption of lipopolysaccharide (LPS) derived from Gram-negative gut microbiota. Dysbiosis is associated with higher gut permeability leading to metabolic endotoxaemia. LPS is recognized by TLR4, which is presented to cluster of differentiation 14 (CD14); this subsequently mediates signal transduction, including nuclear factor kappa B (NF-κB), contributing to the activation of innate and adaptive chronic inflammatory responses. Gut microbiota-derived metabolism of dietary PC/choline and L-carnitine produces trimethylamine, which is further metabolized to trimethylamine-N-oxide (TMAO). TMAO, as a pro-atherogenic compound, may increase the risk of developing atherosclerotic heart disease. Probiotics, prebiotics, and antibiotic treatment can reduce LPS absorption and its serum levels. Promotion ----->; Inhibition -----|

As mentioned above, gut microbiome alterations observed in some diseases leads to an increase in serum levels of some gut metabolites such as TMAO.<sup>33</sup> On the other hand, dysbiosis leads to increased production of TMAO, which may also contribute to the pathogenesis of CVD.<sup>34</sup> For the first time, Kallio et al, introduced this metabolite endotoxaemia as the consequence of dysbiosis which was assumed to have a role in CVD development.<sup>35</sup> Animal and epidemiologic studies have shown that higher levels of TMAO are directly linked to the increased incidence of major adverse cardiovascular events (MACE).<sup>36,37</sup> Indeed, some studies have demonstrated that increased TMAO levels may better predict incident cardiovascular events than traditional risk factors such as LDL and C-reactive protein (CRP).<sup>38</sup> In fact, the smallest microbiota changes even without disrupting gut permeability, cause metabolic complications and metabolite endotoxaemia.

*Helicobacter pylori* offers another example of how the gut microbiota of the host can have a major impact on health.<sup>39</sup> Indeed, *H. pylori* is directly or indirectly involved in the development of CVD. Activated release of toxins, pro-inflammatory factors, abnormal lipid metabolism, and altered iron metabolism are the major mechanisms through which *H. pylori* contributes to cardiovascular abnormalities.<sup>40</sup> Although, *H. pylori* infection might play a role in increasing the circulating levels of endotoxaemia in cardiovascular patients,<sup>41</sup>

consequently facilitating the onset of CVD, its main effect in development of heart diseases might be through alteration of immune system, resulting in systemic endotoxaemia.<sup>42</sup>

Small intestine bacterial overgrowth (SIBO), also termed bacterial overgrowth, characterized by the presence of abnormal and excessive numbers of bacteria in the small intestine, has been associated with an increased risk of CVD.<sup>43</sup> Although numerous speculations have been suggested regarding the crosstalk between SIBO and atherosclerosis, the exact underlying mechanism remains unclear. Recently, Ponziani et al provided evidence that SIBO predisposes patients to development of atherosclerosis through reduced matrix Gla-protein (MGP) activation as well as arterial stiffening.<sup>44</sup> Furthermore, Oher et al revealed that SIBO increases endotoxaemia via activation of the Toll-like receptors (TLR) signalling pathway which eventually leads to CVD.<sup>45</sup> In short, despite the association between SIBO and CVD revealed in previous studies, no conclusions can be drawn about causality of the association.

In addition to the bacterial components that cause ME, certain bacterial metabolites such as TMAO can also exert negative effects on the circulatory system and increase chronic inflammation. TMAO is a biological compound produced by gut microbiota from dietary phosphatidylcholine, choline, and carnitine.<sup>46</sup> Alteration of

gut microbiota as identified by increased *Prevotella* and decreased *Bacteroides* species in gut microbiome leads to higher level of TMAO and susceptibility to CVD.<sup>34</sup> In addition, elevated TMAO level is a new prognostic marker in patients with ischaemic and non-ischaemic cardiomyopathy.<sup>47</sup> Moreover, a new study proposed that TMAO may be considered as a biomarker to assess gut barrier permeability.<sup>48</sup>

There is evidence that animals fed with a Western diet have greater plasma TMAO concentrations. The augmented levels of TMAO is known to contribute to over expression of pro-inflammatory cytokines such as tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-1 $\beta$  (IL-1 $\beta$ ) and also attenuation of anti-inflammatory cytokines such as IL-10.<sup>37</sup> Moreover, endothelial dysfunction is another pathologic feature that has been related to TMAO. TMAO also alters cholesterol and sterol metabolism, which could act as an important risk factor for CVD.<sup>34</sup>

## 2.5 | Gut permeability and metabolic endotoxaemia

The gut epithelium is an efficient barrier that prevents absorption of LPS derived from Gram-negative gut microbiota. Diabetes, high-fat diet, obesity, and CVD are associated with higher gut permeability leading to ME.<sup>21</sup> Currently, there are some invasive methods used to detect the gut permeability, which may not be appropriate for clinical purposes. A simple non-invasive method is typically using large molecule oligosaccharide (eg, lactulose or polyethylene glycols (PEGs) of 1500–4000 kDa) and low-molecular-weight sugars such as mannitol and L-rhamnose or concentration ratio of lactulose to mannitol (L/M ratio). The sugar molecules such as mannitol are supposed to permeate both transcellularly and paracellularly, so that the ratio of these sugars in plasma or excreted in the urine reflects intestinal permeation.<sup>49,50</sup> It must be noted that small intestine is technically sterile, and use of L/M ratio as an indicator for small intestinal permeability would be misleading, unless SIBO exists. Sucralose has been used instead of lactulose as a measure of whole gut permeability.<sup>51</sup>

Another indirect method is to assess the tight junction proteins such as occludin, zonulin-1, claudin-1, claudin-4 in serum which are increased in leaky gut.<sup>52</sup> Additionally, LBP has also been used as a gut-blood barrier permeability marker.<sup>53</sup> More newly, TMAO has been proposed as a promising biomarker of gut barrier function.<sup>48</sup> More recently, plasma levels of citrulline, and also assessment of the inflammatory marker calprotectin in faeces have been used as a surrogate marker of small bowel epithelial cell mass.<sup>54</sup> Although many techniques exist for evaluation of intestinal permeability, calculating the excretion ratio of lactulose/rhamnose or lactulose/mannitol are more commonly used.<sup>55</sup>

Assessments of intestinal permeability are regularly used synonymously with the term “gut barrier function,” while these are not the same. For example, intestinal permeability changes do not essentially reveal changes in antimicrobial production, mucus secretion, or IgA secretion.<sup>56</sup> Taken together, results of all these tests

are influenced by changes in many factors including gastric emptying, intestinal peristalsis, gut blood flow, bacterial degradation, and renal clearance. Therefore, there is no single standard way to evaluate the gut permeability, and it is suggested that a combination of these tests be performed for assessment of intestinal permeability.

## 2.6 | Key point

Potential pathways of the association between gut dysbiosis and CVD have been demonstrated in various animal and human studies. The intestinal microbiota has a deep influence on mucosa barrier function and the nutritional/metabolic status of its ‘host’.<sup>19</sup> Dysbiosis allows bacterial products such as lipopolysaccharide, or peptidoglycans to enter the circulation.<sup>17</sup> Furthermore, the dysbiosis can directly impact the cytokine production from epithelial cells and innate immune cells.<sup>21</sup> These mediators also enter the circulation. LPS itself, and also the inflammatory state it causes may induce the production of oxidized low-density lipoprotein.<sup>13,29</sup> In addition to metabolic endotoxaemia, increased TMAO as a gut metabolite may also exert adverse effects on cardiovascular system. TMAO, even in the absence of leaky gut, has been proposed to augment CVD risk. Prebiotics/probiotics could possibly attenuate these adverse effects.<sup>33</sup>

## 3 | DISCUSSION

### 3.1 | Gut dysbiosis and cardiovascular disease

Dysbiosis can be implicated in the pathogenesis of CVD through (a) increased LPS (endotoxaemia) which can promote the formation of atherosclerotic plaque by acting on TLR4,<sup>17</sup> (b) affecting the metabolism of bile acids (BAs), and the production of TMAO which can impair cholesterol catabolism and induce chronic inflammation,<sup>37</sup> and (c) contributing to risk factors such as hypertension and atherosclerosis through chronic inflammation and dyslipidaemia.<sup>5</sup> In the following sections, we will debate findings on probable mechanisms connecting the endotoxaemia and CVDs. Furthermore, we will discuss the evidence on the potential role of prebiotics/probiotics in modulation of gut microbiota and endotoxaemia.

### 3.2 | Endotoxaemia and cardiovascular disease

It is well established that patients with cirrhotic cardiomyopathy have higher LPS levels, and are significantly predisposed to diastolic dysfunction. This finding supports a potential role of ME in the aggravation of cardiomyopathy in cirrhotic patients.<sup>57</sup> In addition, previous studies have shown a relationship between systemic inflammation and increased CVD.<sup>6</sup> However, the potential mechanisms for the observed associations still remain largely unclear. Typically, endotoxaemia is present in early CVD and also at the early phases of some diseases.<sup>57</sup> Additionally, endotoxaemia may activate systemic

inflammatory cascade that can not only have an influence on the cardiovascular systems, but also have a distant effect on intestine and its permeability.<sup>13</sup> Chronic inflammation following endotoxaemia might be a possible mechanism for the association between dysbiosis and CVD.<sup>6</sup> Indeed, increased levels of inflammatory markers and LPS have been found in CVD subjects.<sup>58</sup> It is, however, uncertain whether increased gut permeability can lead to the development of CVD or whether it is a consequence of a cardiovascular condition.<sup>59</sup>

Endotoxaemia (without sepsis) is characterized by presence of LPS, the major glycolipid component of the outer membrane of Gram-negative bacteria in the blood.<sup>8</sup> ME stimulates release of pro-inflammatory cytokines, resulting in systemic inflammation. Components of Gram-positive bacteria's cell wall such as lipoteichoic acid or peptidoglycan are recognized by pattern-recognition receptors (PRRs) such as NOD-like receptors and TLRs. TLRs are PRRs that recognize microbe-associated molecular patterns, and include many types, but TLRs2 and TLRs4 are the most important ones. LPS and peptidoglycan (PGN) trigger TLR4 activation, and TLR2 recognizes lipoteichoic acid (LTA) from Gram-positive bacteria.<sup>60,61</sup> LPS not only induces endothelial damage, but also increases expression of surface adhesion molecules such as CD14 on inflammatory cells, and stimulates the release of pro-inflammatory cytokines.<sup>62</sup> Heightened activation of the immune system in post endotoxaemia may predispose the animals to the development of cardiovascular disease. Epidemiological studies have also shown that ME is associated with CVD.<sup>41</sup> However, the role of ME in CVD remains unknown, if one does not consider the part inflammation plays in this regard; thus, further investigation is warranted.

It has been proposed that ME increases hypertriglyceridaemia, and development as well as progression of fatty liver.<sup>63</sup> Also, LPS seems to increase endothelial lipase, which has been suggested to cause a reduction in HDL.<sup>59</sup> These findings suggest a strong link between ME and increased CVD risk factors. Endotoxins can also induce plaque formation and progression of atherosclerotic lesions, and release of other molecules from endothelial cells involved in pro-inflammatory processes.<sup>64</sup>

Several mechanisms have been proposed to be involved in the role of TMAO (considered as metabolite endotoxaemia) in the aetiology of CVD; activating macrophages to accumulate cholesterol, changing cholesterol metabolism in different organs, and inhibiting reverse cholesterol transport pathway are some of the most important mechanisms.<sup>47</sup> Moreover, elevated TMAO levels promote inflammation and oxidative stress, and impair vascular function.<sup>37</sup>

### 3.3 | Gut microbiota and endotoxaemia

Dysbiosis may contribute to ME, leading to systematic inflammation, and CVD.<sup>5</sup> A healthy intestinal barrier is important to avoid microbial translocation. Evidence from clinical and animal studies show that dysbiosis is associated with an increased risk of CVD.<sup>34,35</sup> Moreover, several lines of evidence suggest that increased gut permeability, as assessed by tight junction proteins in serum, contribute to cardiometabolic risk factors. Surprisingly, hypercholesterolaemia

paradoxically improves survival in cardiac cachexia, and attenuates cardiac cachexia and inflammation, suggesting a hypothesis that a diet with high-fat content, could decrease gut permeability and subsequently metabolite endotoxaemia.<sup>65,66</sup>

As noted before, gut microbiota alterations lead to development of different diseases, such as CVD. Gut microbiota regulates multiple physiological processes of the host; the resident bacteria act as an energy sources in the gut lumen, influence production of leptin and other hormones, regulate immune functions and receptor ligands, and are also substrates for the host enzymes. In order to identify how gut microbiota alterations influence inflammation, high-fat diet was used in experimental settings. High-fat diet increased plasma endotoxin levels and resulted in dysregulation of the gut microbiota by increasing the ration of *Firmicutes* to *Bacteroidetes*. The analyses showed that LPS was responsible for the onset of ME in this animal model.<sup>67</sup>

Germ-free animals have been used to study the probable role of the gut microbiota in development of some disorders.<sup>68</sup> Germ-free animals are animals that have no microorganisms living in or on them. Such animals are raised within germ-free isolators in order to control their exposure to viral and bacterial agents.<sup>69</sup> Germ-free mice fed a normal chow diet had a lower endotoxin production, whereas germ-free mice colonized with LPS-producing germs showed increased fat mass, and developed metabolic diseases.<sup>70</sup> Earlier investigations have revealed that colonization of germ-free mice with microbiota considerably changes the transcription of numerous mediators involved in the regulation of metabolic functions.<sup>71,72</sup> Turnbaugh et al observed that colonization of germ-free mice with the microbiota from the obese mice resulted in a considerably higher percentage of total body fat than that resulting from colonization with a microbiota from lean mice.<sup>71</sup> These results elucidate that gut microbiota is another causal factor in pathophysiology of cardiac risk factors.

To conclude, endotoxaemia and its resultant inflammation is not observed in germ-free mice, but develops only after feeding of high-fat diet or injection of LPS to these animals; this in part demonstrates the effect of gut microbiota dysbiosis in this regard.

There is inconsistency regarding the relationship between high-fat diet and elevated circulating endotoxin. Pendyala et al<sup>73</sup> demonstrated that fasting plasma endotoxin was significantly raised following 30 days of isocaloric, high-fat (40% fat of total energy) feeding in apparently healthy subjects. On the contrary, 2 months of high-fat (45% fat of total energy) diet did not influence fasting plasma LPS in healthy subjects, in another study.<sup>74</sup> Apparently, the association between high-fat diet and ME is more complex in humans, and seems to be influenced by the time course of feeding, the macronutrient (and possibly energy) composition, and the age of the individuals.

This evidence suggests that changes in gut microbiota composition could be responsible for increased endotoxaemia, which in turn would trigger the development of inflammation and cardiovascular risk factors. On the other hand, antibiotic treatment intensely reduces the local intestinal microbiota and LPS. Similar results were observed when a probiotic was administered to mice; *Bifidobacteria*



administration in newborn mice led to lower intestinal endotoxin concentrations and inflammatory cytokine (IL-6, and TNF- $\alpha$ ) production.<sup>75</sup>

### 3.4 | Pre/probiotics and metabolic endotoxemia

Elevated levels of LPS could be the result of increased endotoxin production by a change in gut microbiota; the latter is characterized by decreased proportion of beneficial bacteria (*Lactobacillus* spp., *Bifidobacterium* spp., and *Bacteroides-Prevotella* spp.) to some Firmicutes species.<sup>76</sup> Increased intestinal permeability characterized by an increased expression of epithelial tight junction proteins such as Zonulin and Occludin are involved in this mechanism. This effect can be completely restored by modulation of gut microbiota. Adam et al, demonstrated that specific changes in gut microbiota composition by feeding arabinoxylans oligosaccharides to obese mice led to an increase in *Bifidobacteria* and a decrease in *Lactobacilli*, which consequently improved inflammation and gut barrier integrity. Also, they noticed that the tight junction proteins were up-regulated in the colon after the intervention.<sup>77</sup>

As mentioned above, probiotics can decrease gut permeability and endotoxemia. The mechanisms for probiotics beneficial effects on barrier function are still unknown. Probiotics have been shown to produce bacteriocins, which inhibit pathogenic bacteria and regulate intestinal epithelial cells anti-apoptotic and proliferation responses.<sup>78,79</sup> Moreover, probiotics secrete some proteins that protect intestinal epithelial cells from oxidative stress by inducing cytoprotective heat shock proteins.<sup>80</sup> The beneficial activity of probiotics may be exerted through secreting metabolites of lactic acid bacteria. For example, Ménard et al showed that metabolites of lactic acid bacteria (*Bifidobacterium breve*) may be capable of increasing intestinal barriers function.<sup>81</sup> It is noteworthy that LAB products seem to limit access of LPS to CD14 receptors on monocytes/macrophages. Intestinal macrophages do not express CD14 under basal conditions. This effect was associated with lowered NF- $\kappa$ B signalling in immune cells and decreased inflammation.<sup>82</sup> Taken together, two mechanisms may explain the role of probiotics in the intestinal environment: (a) a direct inhibitory effect on gut permeability; and (b) effect of active bacterial metabolites on epithelial barrier.

Another possible effect of probiotics is restoring the composition of the gut microbiota community. Several studies suggest that dysbiosis may contribute to cardiovascular disease risk, and that probiotic supplementation can have favourable effects by normalizing the gut microbiota.<sup>83</sup> An irregular profile of gut flora with substantially lower ratio of *Bifidobacteria* and *Lactobacilli* to Firmicutes species can affect endotoxin production.<sup>84</sup> Also, previous studies have indicated beneficial therapeutic effects of *Lactobacillus* spp. and other probiotics in patients with CVD.<sup>85</sup> In fact, probiotic interventions with *Bifidobacteria* and *Lactobacillus* spp. restored numbers of beneficial species and led to a significant decrease in endotoxin levels. Another possible mechanism could arise from the putative role of the *Bifidobacterium* spp in maintaining the gut

barrier. *Bifidobacterium* spp do not degrade intestinal mucus glycoproteins like other pathogenic bacteria do, and enhance microvillus environment by averting permeability and bacterial translocation.<sup>86</sup> It has been shown that products of prebiotics including short chain fatty acids (SCFAs) act as an energy substrate for the colonocytes and have a trophic effect on mucosa which in turn increases villus height and crypt depth, and leads to a thicker mucosal layer in the colon.<sup>87,88</sup> Cani et al indicated<sup>17</sup> that prebiotic treatment following high-fat diet led to higher endogenous GLP-2 production and improvement of the mucosal barrier function, consequently improving tight junctions, decreasing plasma LPS concentrations and reducing inflammatory and oxidative stress. Altogether, these data led the authors to hypothesize that there was a positive correlation between GLP and tight junction proteins (ZO-1, occludin), and that probiotics may positively impact ME. Further studies are needed to evaluate the effect of different probiotics strains on gut microbiota profile and endotoxaemia in subjects with CVD.

In vitro models of ME have recently proposed that some probiotics strains such as *Lactobacillus rhamnosus* and *Lactobacillus casei*, protect epithelial barrier function against *Escherichia coli*-induced endotoxaemia.<sup>89</sup> Moreover, treatment with probiotics induced a variety of changes in the expression of different TLRs. In one study conducted by Schmitz et al, administration of probiotics into the intestine of healthy dogs and those with enteropathies led to increased expression of TLR ligands. In addition, production of TNF $\alpha$  and IL-17A proteins decreased in plasma.<sup>90</sup>

The gut microbiota can be restored by non-digestible, fermentable carbohydrates, which are known as prebiotics, including inulin, fructooligosaccharide, oligofructose, and xylose; prebiotics consumption leads to selective stimulation of growth and/or activities of beneficial bacteria in the colon.<sup>91,92</sup> In this regard, gut microbiota modulation by prebiotic increases bacterial fermentation products, mostly SCFAs, which act as an energy substrate for the colonocytes, subsequently having a trophic effect on mucosa.<sup>93</sup> The potential of SCFAs to help form a thicker mucosal layer in the jejunum and colon, may explain their effect on decreasing gut permeability and subsequent ME.<sup>92</sup> On the other hand, prebiotic intake leads to increased proportion of beneficial bacteria in the gut microbiome. A recent study demonstrated that administration of prebiotics (oligofructose) could raise *Bifidobacterium* spp. in gut microbiota, which improved gut permeability.<sup>67</sup> To additionally support our concept, a summary of studies which found changes in levels of endotoxaemia or endotoxin-related markers by prebiotics are presented herein (Table 1). A recent study conducted by Dehghan et al, showed that inulin administration (as prebiotics) for 8 weeks, could modulate inflammation and metabolic endotoxemia in women with type 2 diabetes.<sup>91</sup>

Former studies demonstrated that increased *Bifidobacterium* reduced intestinal endotoxin formation, and improved intestinal barrier function through improving intestinal permeability and a GLP-2-dependent mechanism.<sup>67</sup> Also, available data have shown that a selective gut microbiota change by increasing endogenous GLP-2 production, contributes to improvement of gut barrier permeability.<sup>17</sup> Beside the supposed role of the SCFAs and particular bacterial

strains, the precise mechanism underlying the relationship between prebiotic-induced changes in the gut microbiota and enhanced gut barrier function has not been defined yet.

## 4 | CONCLUSION

Human studies have indicated that endotoxaemia may lead to inflammation and cardiometabolic consequences. This review reported the potential benefits of prebiotics/probiotics therapy for cardiovascular health, probably by reducing endotoxaemia. Although many of these studies have suggested a positive effect of pre/probiotics on ME, we point out that the claim for the favourable effects of these nutraceuticals in cardiovascular diseases is still in its infancy, and requires more comprehensive and well-designed clinical trials. In particular, evidence from human studies on the association between ME and CVDs is insufficient compared to animal studies. As mentioned above, preliminary evidence suggests that antibiotic therapy suppresses endotoxin and TMAO levels; however, the stability of that effect by long-term use of these agents remains unknown. Therefore, seeking for alternative methods for modulating the gut microbial community, either through food additives or prebiotics/probiotics administration is needed. Further studies are warranted to establish whether prebiotics/probiotics therapies can significantly reduce cardiovascular risk through decreasing ME and metabolite endotoxaemia.

## 5 | MATERIAL AND METHODS

To find relevant studies published prior to July 2019, a literature search conducted in the PubMed, Scopus, Embase, Cochrane Library, ProQuest, and Google Scholar electronic databases using the keywords ("probiotic" OR "lactobacillus" OR "bifidobacterium" OR "saccharomyces" OR "Escherichia coli" OR "yeast" OR "prebiotic" OR "inulin" OR "fructooligosaccharide" OR "fructo-oligosaccharide" OR "FOS" OR "galactooligosaccharide" OR "galacto-ligosaccharide" OR "GOS" OR "oligofructose" OR "synbiotic" OR "metabolic endotoxaemia" OR "gut microbiota" OR "dysbiosis" OR "gastrointestinal microbiome" OR "lipopolysaccharide" OR "peptidoglycans") And ("cardiovascular" OR "heart disease" OR "atherosclerosis" OR "hypertension" OR "blood pressure" OR "cholesterol" OR "triglycerides" OR "HDL" OR "LDL" OR "hs-CRP" OR "CRP" OR "inflammation" OR "oxidative stress" OR "LPS" OR "TMAO" OR "TLRs" OR "IL-6" OR "TNF- $\alpha$ " OR "SCFAs"). The search was limited to English language studies published before July 2019.

### 5.1 | Eligibility criteria

The eligibility criteria for entering the study were as follows: (a) all clinical trials which evaluated the effect of prebiotics and probiotics on the metabolic endotoxaemia (ie endotoxin) and cardiovascular

disease. (b) All animal studies which evaluated the effect of prebiotics and probiotics on the metabolic endotoxaemia and cardiovascular disease and (a) in vitro models (b) letters, (c) comments, (d) short communications, and (e) studies with insufficient information (eg, published in non-English-languages or studies that did not provide access to full text) were excluded.

### 5.2 | Data extraction

The titles and abstracts of the eligible papers were screened independently by two researchers and studies were excluded if they did not meet the eligibility criteria. In the next step, full-text articles were examined based on type of study, study subjects, study design, daily dose, and duration of intervention and main outcome.

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### CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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### REFERENCES

1. Townsend N, Wilson L, Bhatnagar P, Wickramasinghe K, Rayner M, Nichols M. Cardiovascular disease in Europe: epidemiological update 2016. *Eur Heart J*. 2016;37(42):3232-3245.
2. Rosamond W, Flegal K, Friday G, et al. Heart disease and stroke statistics—2007 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation*. 2007;115(5):e69-e171.
3. Ahmadi S-F, Streja E, Zahmatkesh G, et al. Reverse epidemiology of traditional cardiovascular risk factors in the geriatric population. *J Am Med Dir Assoc*. 2015;16(11):933-939.
4. McClelland RL, Jorgensen NW, Budoff M, et al. 10-year coronary heart disease risk prediction using coronary artery calcium and traditional risk factors: derivation in the MESA (Multi-Ethnic Study of Atherosclerosis) with validation in the HNR (Heinz Nixdorf Recall) study and the DHS (Dallas Heart Study). *J Am Coll Cardiol*. 2015;66(15):1643-1653.
5. Boutagy NE, McMillan RP, Frisard MI, Hulver MW. Metabolic endotoxaemia with obesity: is it real and is it relevant? *Biochimie*. 2016;124:11-20.
6. Neves AL, Coelho J, Couto L, Leite-Moreira A, Roncon-Albuquerque R. Metabolic endotoxaemia: a molecular link between obesity and cardiovascular risk. *J Mol Endocrinol*. 2013;51(2):R51-R64.
7. Sircana A, De Micheli F, Parente R, et al. Hypertension and chronic kidney disease: recent advances. *Pharmacol Res*. 2019;144:390-408.
8. Carding S, Verbeke K, Vipond DT, Corfe BM, Owen LJ. Dysbiosis of the gut microbiota in disease. *Microb Ecol Health Dis*. 2015;26(1):26191.

9. Petersen C, Round JL. Defining dysbiosis and its influence on host immunity and disease. *Cell Microbiol.* 2014;16(7):1024-1033.
10. Maharshak N, Packey CD, Ellermann M, et al. Altered enteric microbiota ecology in interleukin 10-deficient mice during development and progression of intestinal inflammation. *Gut Microbes.* 2013;4(4):316-324.
11. Mutlu E, Keshavarzian A, Engen P, Forsyth CB, Sikaroodi M, Gillevet P. Intestinal dysbiosis: a possible mechanism of alcohol-induced endotoxemia and alcoholic steatohepatitis in rats. *Alcohol Clin Exp Res.* 2009;33(10):1836-1846.
12. Brun P, Castagliuolo I, Leo VD, et al. Increased intestinal permeability in obese mice: new evidence in the pathogenesis of non-alcoholic steatohepatitis. *Am J Physiol Gastrointest Liver Physiol.* 2007;292(2):G518-G525.
13. Kitai T, Tang WW. Gut microbiota in cardiovascular disease and heart failure. *Clin Sci.* 2018;132(1):85-91.
14. Deitch EA. The role of intestinal barrier failure and bacterial translocation in the development of systemic infection and multiple organ failure. *Arch Surg.* 1990;125(3):403-404.
15. Mariat D, Firmesse O, Levenez F, et al. The Firmicutes/Bacteroidetes ratio of the human microbiota changes with age. *BMC Microbiol.* 2009;9(1):123.
16. Nicholson JK, Holmes E, Kinross J, et al. Host-gut microbiota metabolic interactions. *Science.* 2012;336(6086):1262-1267.
17. Cani PD, Possemiers S, Van de Wiele T, et al. Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut.* 2009;58(8):1091-1103.
18. Tang WW, Hazen SL. The contributory role of gut microbiota in cardiovascular disease. *J Clin Investig.* 2014;124(10):4204-4211.
19. Cani PD, Delzenne NM. The role of the gut microbiota in energy metabolism and metabolic disease. *Curr Pharm Des.* 2009;15(13):1546-1558.
20. Jialal I, Rajamani U. Endotoxemia of metabolic syndrome: a pivotal mediator of meta-inflammation. *Metab Syndr Relat Disord.* 2014;12(9):454-456.
21. Cani PD, Amar J, Iglesias MA, et al. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes.* 2007;56(7):1761-1772.
22. Pirllich M, Norman K, Lochs H, Bauditz J. Role of intestinal function in cachexia. *Curr Opin Clin Nutr Metab Care.* 2006;9(5):603-606.
23. Suliman HB, Welty-Wolf KE, Carraway M, Tatro L, Piantadosi CA. Lipopolysaccharide induces oxidative cardiac mitochondrial damage and biogenesis. *Cardiovasc Res.* 2004;64(2):279-288.
24. Triantafilou M, Triantafilou K. Lipopolysaccharide recognition: CD14, TLRs and the LPS-activation cluster. *Trends Immunol.* 2002;23(6):301-304.
25. Hurley JC. Endotoxemia: methods of detection and clinical correlates. *Clin Microbiol Rev.* 1995;8(2):268-292.
26. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. *Nature.* 2007;449(7164):804.
27. Suganami T, Tanimoto-Koyama K, Nishida J, et al. Role of the Toll-like receptor 4/NF- $\kappa$ B pathway in saturated fatty acid-induced inflammatory changes in the interaction between adipocytes and macrophages. *Arterioscler Thromb Vasc Biol.* 2007;27(1):84-91.
28. Ohlsson BG, Englund MC, Karlsson AL, et al. Oxidized low density lipoprotein inhibits lipopolysaccharide-induced binding of nuclear factor-kappaB to DNA and the subsequent expression of tumor necrosis factor-alpha and interleukin-1beta in macrophages. *J Clin Investig.* 1996;98(1):78-89.
29. Chung SW, Kang BY, Kim SH, et al. Oxidized low density lipoprotein inhibits interleukin-12 production in lipopolysaccharide-activated mouse macrophages via direct interactions between peroxisome proliferator-activated receptor- $\gamma$  and nuclear factor- $\kappa$ B. *J Biol Chem.* 2000;275(42):32681-32687.
30. Jayashree B, Bibin YS, Prabhu D, et al. Increased circulatory levels of lipopolysaccharide (LPS) and zonulin signify novel biomarkers of proinflammation in patients with type 2 diabetes. *Mol Cell Biochem.* 2014;388(1-2):203-210.
31. Beck J, Garcia R, Heiss G, Vokonas PS, Offenbacher S. Periodontal disease and cardiovascular disease. *J Periodontol.* 1996;67(10s):1123-1137.
32. Singer RH. Examining Periodontal Disease and Cardiovascular Disease Risk-Assessing the Feasibility and Acceptability of Screening in the Dental Care Setting. Doctoral dissertation. Available from Public Health Sciences (Medicine). No: 235, 2018.
33. Tang WW, Wang Z, Kennedy DJ, et al. Gut microbiota-dependent trimethylamine N-oxide (TMAO) pathway contributes to both development of renal insufficiency and mortality risk in chronic kidney disease. *Circ Res.* 2015;116(3):448-455.
34. Yin J, Liao S-X, He Y, et al. Dysbiosis of gut microbiota with reduced trimethylamine-N-oxide level in patients with large-artery atherosclerotic stroke or transient ischemic attack. *J Am Heart Assoc.* 2015;4(11):e002699.
35. Kallio KE, Hätönen KA, Lehto M, Salomaa V, Männistö S, Pussinen PJ. Endotoxemia, nutrition, and cardiometabolic disorders. *Acta Diabetol.* 2015;52(2):395-404.
36. Heianza Y, Ma W, Manson JE, Rexrode KM, Qi L. Gut microbiota metabolites and risk of major adverse cardiovascular disease events and death: a systematic review and meta-analysis of prospective studies. *J Am Heart Assoc.* 2017;6(7):e004947.
37. Tang WW, Hazen SL. Microbiome, trimethylamine N-oxide, and cardiometabolic disease. *Transl Res.* 2017;179:108-115.
38. Li XS, Obeid S, Klingenberg R, et al. Gut microbiota-dependent trimethylamine N-oxide in acute coronary syndromes: a prognostic marker for incident cardiovascular events beyond traditional risk factors. *Eur Heart J.* 2017;38(11):814-824.
39. Clemente JC, Ursell LK, Parfrey LW, Knight R. The impact of the gut microbiota on human health: an integrative view. *Cell.* 2012;148(6):1258-1270.
40. Kusters JG, van Vliet AH, Kuipers EJ. Pathogenesis of *Helicobacter pylori* infection. *Clin Microbiol Rev.* 2006;19(3):449-490.
41. Wiedermann CJ, Kiechl S, Dunzendorfer S, et al. Association of endotoxemia with carotid atherosclerosis and cardiovascular disease: prospective results from the Bruneck Study. *J Am Coll Cardiol.* 1999;34(7):1975-1981.
42. Jamkhande PG, Gattani SG, Farhat SA. *Helicobacter pylori* and cardiovascular complications: a mechanism based review on role of *Helicobacter pylori* in cardiovascular diseases. *Integr Med Res.* 2016;5(4):244-249.
43. Adkins C, Small RA. Small intestinal bacterial overgrowth and coronary artery disease: what is in the CARdS? *Dig Dis Sci.* 2018;63(2):271-272.
44. Ponziani FR, Pompili M, Di Stasio E, Zocco MA, Gasbarrini A, Flore R. Subclinical atherosclerosis is linked to small intestinal bacterial overgrowth via vitamin K2-dependent mechanisms. *World J Gastroenterol.* 2017;23(7):1241.
45. Kapil S, Duseja A, Sharma BK, et al. Small intestinal bacterial overgrowth and toll-like receptor signaling in patients with non-alcoholic fatty liver disease. *J Gastroenterol Hepatol.* 2016;31(1):213-221.
46. Albenberg LG, Wu GD. Diet and the intestinal microbiome: associations, functions, and implications for health and disease. *Gastroenterology.* 2014;146(6):1564-1572.
47. Senthong V, Wang Z, Li XS, et al. Intestinal microbiota-generated metabolite trimethylamine-N-oxide and 5-year mortality risk in stable coronary artery disease: the contributory role of intestinal microbiota in a COURAGE-like patient cohort. *J Am Heart Assoc.* 2016;5(6):e002816.
48. Ufnal M, Pham K. The gut-blood barrier permeability—A new marker in cardiovascular and metabolic diseases? *Med Hypotheses.* 2017;98:35-37.

49. Farhadi A, Keshavarzian A, Holmes EW, Fields J, Zhang L, Banan A. Gas chromatographic method for detection of urinary sucralose: application to the assessment of intestinal permeability. *J Chromatogr B*. 2003;784(1):145-154.
50. Van Nieuwenhoven M, de Swart E, van Eijk H, Deutz N, Brouns F, Brummer R-J. Effects of pre- and post-absorptive factors on the lactulose/rhamnose gut permeability test. *Clin Sci*. 2000;98(3):349-353.
51. Odenwald MA, Turner JR. Intestinal permeability defects: is it time to treat? *Clin Gastroenterol Hepatol*. 2013;11(9):1075-1083.
52. Sapone A, de Magistris L, Pietzak M, et al. Zonulin upregulation is associated with increased gut permeability in subjects with type 1 diabetes and their relatives. *Diabetes*. 2006;55(5):1443-1449.
53. Gonzalez-Quintela A, Alonso M, Campos J, Vizcaino L, Loidi L, Gude F. Determinants of serum concentrations of lipopolysaccharide-binding protein (LBP) in the adult population: the role of obesity. *PLoS ONE*. 2013;8(1):e54600.
54. van Vliet MJ, Tissing WJE, Rings EHHM, et al. Citrulline as a marker for chemotherapy induced mucosal barrier injury in pediatric patients. *Pediatr Blood Cancer*. 2009;53(7):1188-1194.
55. Khan MR, Larson J, Dyer R, Singh R, Faubion W, Absah I. The utility of Lactulose/Rhamnose permeability test in celiac disease and high risk children. *Am J Gastroenterol*. 2018;113:S601.
56. Wells JM, Brummer RJ, Derrien M, et al. Homeostasis of the gut barrier and potential biomarkers. *Am J Physiol Gastrointest Liver Physiol*. 2016;312(3):G171-G193.
57. Karagiannakis DS, Vlachogiannakos J, Anastasiadis G, Vafiadis-Zouboulis I, Ladas SD. Frequency and severity of cirrhotic cardiomyopathy and its possible relationship with bacterial endotoxemia. *Dig Dis Sci*. 2013;58(10):3029-3036.
58. McIntyre CW, Harrison LEA, Eldehni MT, et al. Circulating endotoxemia: a novel factor in systemic inflammation and cardiovascular disease in chronic kidney disease. *Clin J Am Soc Nephrol*. 2011;6(1):133-141.
59. Barcia AM, Harris HW. Triglyceride-rich lipoproteins as agents of innate immunity. *Clin Infect Dis*. 2005;41(Supplement\_7):S498-S503.
60. Takeuchi O, Hoshino K, Kawai T, et al. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. *Immunity*. 1999;11(4):443-451.
61. Wiest R, Rath HC. Bacterial translocation in the gut. *Best Pract Res Clin Gastroenterol*. 2003;17(3):397-425.
62. Lu Y-C, Yeh W-C, Ohashi PS. LPS/TLR4 signal transduction pathway. *Cytokine*. 2008;42(2):145-151.
63. Sanz Y, Santacruz A, De Palma G. Insights into the roles of gut microbes in obesity. *Interdisc Perspect Infect Dis*. 2008;2008:1-9.
64. Larsen CG, Anderson AO, Appella E, Oppenheim JJ, Matsushima K. The neutrophil-activating protein (NAP-1) is also chemotactic for T lymphocytes. *Science*. 1989;243(4897):1464-1466.
65. Lourenço AP, Vasques-Nóvoa F, Fontoura D, Brás-Silva C, Roncon-Albuquerque R Jr, Leite-Moreira AF. A western-type diet attenuates pulmonary hypertension with heart failure and cardiac cachexia in rats-3. *J Nutr*. 2011;141(11):1954-1960.
66. Song MJ, Kim KH, Yoon JM, Kim JB. Activation of Toll-like receptor 4 is associated with insulin resistance in adipocytes. *Biochem Biophys Res Comm*. 2006;346(3):739-745.
67. Cani PD, Neyrinck AM, Fava F, et al. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia*. 2007;50(11):2374-2383.
68. Al-Asmakh M, Zadjali F. Use of germ-free animal models in microbiota-related research. *J Microbiol Biotechnol*. 2015;25(10):1583-1588.
69. Hrnčir T, Stepankova R, Kozakova H, Hudcovic T, Tlaskalova-Hogenova H. Gut microbiota and lipopolysaccharide content of the diet influence development of regulatory T cells: studies in germ-free mice. *BMC Immunol*. 2008;9(1):65.
70. Cani PD, Bibiloni R, Knauf C, et al. Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. *Diabetes*. 2008;57(6):1470-1481.
71. Turnbaugh PJ, Hamady M, Yatsunenko T, et al. A core gut microbiome in obese and lean twins. *Nature*. 2009;457(7228):480-484.
72. Hooper LV, Wong MH, Thelin A, Hansson L, Falk PG, Gordon JI. Molecular analysis of commensal host-microbial relationships in the intestine. *Science*. 2001;291(5505):881-884.
73. Pendyala S, Walker JM, Holt PR. A high-fat diet is associated with endotoxemia that originates from the gut. *Gastroenterology*. 2012;142(5):pp. 1100-1. e2.
74. Laugerette F, Alligier M, Bastard J-P, et al. Overfeeding increases postprandial endotoxemia in men: inflammatory outcome may depend on LPS transporters LBP and sCD14. *Mol Nutr Food Res*. 2014;58(7):1513-1518.
75. Griffiths EA, Duffy LC, Schanbacher FL, et al. In vivo effects of bifidobacteria and lactoferrin on gut endotoxin concentration and mucosal immunity in Balb/c mice. *Dig Dis Sci*. 2004;49(4):579-589.
76. Kim K-A, Jeong J-J, Yoo S-Y, Kim D-H. Gut microbiota lipopolysaccharide accelerates inflamm-aging in mice. *BMC Microbiol*. 2016;16(1):9.
77. Adam A, Levrat-Verny M-A, Lopez HW, Leuillet M, Demigné C, Rémésy C. Whole wheat and triticale flours with differing viscosities stimulate cecal fermentations and lower plasma and hepatic lipids in rats. *J Nutr*. 2001;131(6):1770-1776.
78. Tao Y, Drabik KA, Waypa TS, et al. Soluble factors from *Lactobacillus GG* activate MAPKs and induce cytoprotective heat shock proteins in intestinal epithelial cells. *Am J Physiol Cell Physiol*. 2006;290(4):C1018-C1030.
79. Yan F, Cao H, Cover TL, Whitehead R, Washington MK, Polk DB. Soluble proteins produced by probiotic bacteria regulate intestinal epithelial cell survival and growth. *Gastroenterology*. 2007;132(2):562-575.
80. Forsyth CB, Farhadi A, Jakate SM, Tang Y, Shaikh M, Keshavarzian A. *Lactobacillus GG* treatment ameliorates alcohol-induced intestinal oxidative stress, gut leakiness, and liver injury in a rat model of alcoholic steatohepatitis. *Alcohol*. 2009;43(2):163-172.
81. Ménard S, Candalh C, Bambou J, Terpend K, Cerf-Bensussan N, Heyman M. Lactic acid bacteria secrete metabolites retaining anti-inflammatory properties after intestinal transport. *Gut*. 2004;53(6):821-828.
82. Grimm M, Pavli P, Van de Pol E, Doe W. Evidence for a CD14+ population of monocytes in inflammatory bowel disease mucosa—implications for pathogenesis. *Clin Exp Immunol*. 1995;100(2):291-297.
83. Martin F-P, Wang Y, Sprenger N, et al. Probiotic modulation of symbiotic gut microbial-host metabolic interactions in a humanized microbiome mouse model. *Mol Syst Biol*. 2008;4(1):157.
84. Wang Z-T, Yao Y-M, Xiao G-X, Sheng Z-Y. Risk factors of development of gut-derived bacterial translocation in thermally injured rats. *World J Gastroenterol*. 2004;10(11):1619.
85. Bernini LJ, Simão ANC, Alfieri DF, et al. Beneficial effects of *Bifidobacterium lactis* on lipid profile and cytokines in patients with metabolic syndrome: a randomized trial. Effects of probiotics on metabolic syndrome. *Nutrition*. 2016;32(6):716-719.
86. Ruseler-van Embden J, Van Lieshout L, Gosselink M, Marteau P. Inability of *Lactobacillus casei* strain GG, *L acidophilus*, and *Bifidobacterium bifidum* to degrade intestinal mucus glycoproteins. *Scand J Gastroenterol*. 1995;30(7):675-680.
87. Kleessen B, Hartmann L, Blaut M. Fructans in the diet cause alterations of intestinal mucosal architecture, released mucins and mucosa-associated bifidobacteria in gnotobiotic rats. *Br J Nutr*. 2003;89(5):597-606.

88. Bartholome AL, Albin DM, Baker DH, Holst JJ, Tappenden KA. Supplementation of total parenteral nutrition with butyrate acutely increases structural aspects of intestinal adaptation after an 80% jejunioileal resection in neonatal piglets. *J Parenter Enteral Nutr.* 2004;28(4):210-222.
89. Johnson-Henry K, Donato K, Shen-Tu G, Gordanpour M, Sherman P. *Lactobacillus rhamnosus* strain GG prevents enterohemorrhagic *Escherichia coli* O157: H7-induced changes in epithelial barrier function. *Infect Immun.* 2008;76(4):1340-1348.
90. Schmitz S, Henrich M, Neiger R, Werling D, Allenspach K. Stimulation of duodenal biopsies and whole blood from dogs with food-responsive chronic enteropathy and healthy dogs with toll-like receptor ligands and probiotic *Enterococcus faecium*. *Scand J Immunol.* 2014;80(2):85-94.
91. Dehghan P, Gargari BP, Jafar-Abadi MA, Aliasgharzadeh A. Inulin controls inflammation and metabolic endotoxemia in women with type 2 diabetes mellitus: a randomized-controlled clinical trial. *Int J Food Sci Nutr.* 2014;65(1):117-123.
92. Neyrinck AM, Possemiers S, Druart C, et al. Prebiotic effects of wheat arabinoxylan related to the increase in bifidobacteria, Roseburia and Bacteroides/Prevotella in diet-induced obese mice. *PLoS ONE.* 2011;6(6):e20944.
93. Pan X-D, Chen F-Q, Wu T-X, Tang H-G, Zhao Z-Y. Prebiotic oligosaccharides change the concentrations of short-chain fatty acids and the microbial population of mouse bowel. *J Zhejiang Univ Sci B.* 2009;10(4):258.
94. Kobylak N, Falalyeyeva T, Virchenko O, et al. Comparative experimental investigation on the efficacy of mono-and multiprobiotic strains in non-alcoholic fatty liver disease prevention. *BMC Gastroenterol.* 2016;16(1):34.
95. Plaza-Diaz J, Gomez-Llorente C, Abadia-Molina F, et al. Effects of *Lactobacillus paracasei* CNCM I-4034, *Bifidobacterium breve* CNCM I-4035 and *Lactobacillus rhamnosus* CNCM I-4036 on hepatic steatosis in Zucker rats. *PLoS One.* 2014;9(5):e98401.
96. Sharma B, Srivastava S, Singh N, Sachdev V, Kapur S, Saraya A. Role of probiotics on gut permeability and endotoxemia in patients with acute pancreatitis: a double-blind randomized controlled trial. *J Clin Gastroenterol.* 2011;45(5):442-448.
97. Han SH, Suk KT, Kim DJ, et al. Effects of probiotics (cultured *Lactobacillus subtilis*/Streptococcus faecium) in the treatment of alcoholic hepatitis: randomized-controlled multicentre study. *Eur J Gastroenterol Hepatol.* 2015;27(11):1300-1306.
98. Tandon P, Moncrief K, Madsen K, et al. Effects of probiotic therapy on portal pressure in patients with cirrhosis: a pilot study. *Liver Int.* 2009;29(7):1110-1115.
99. Lata J, Novotný I, Příbramská V, et al. The effect of probiotics on gut flora, level of endotoxin and Child-Pugh score in cirrhotic patients: results of a double-blind randomized study. *Eur J Gastroenterol Hepatol.* 2007;19(12):1111-1113.
100. Moludi J, Alizadeh M, Golmohammadi A, Maleki V. The efficacy and safety of probiotics intervention in attenuating cardiac remodeling following myocardial infraction: Literature review and study protocol for a randomized, double-blinded, placebo controlled trial. *Contemp Clin Trials Commun.* 2019;15:100364.
101. Rabiei S, Shakerhosseini R, Saadat N. The effects of symbiotic therapy on anthropometric measures, body composition and blood pressure in patient with metabolic syndrome: a triple blind RCT. *Med J Islam Repub Iran.* 2015;29:213.
102. Gøbel RJ, Larsen N, Jakobsen M, Mølgaard C, Michaelsen KF. Probiotics to adolescents with obesity: effects on inflammation and metabolic syndrome. *J Pediatr Gastroenterol Nutr.* 2012;55(6):673-678.
103. Salazar N, Dewulf EM, Neyrinck AM, et al. Inulin-type fructans modulate intestinal Bifidobacterium species populations and decrease fecal short-chain fatty acids in obese women. *Clin Nutr.* 2015;34(3):501-507.
104. McFarlin BK, Henning AL, Bowman EM, Gary MA, Carbajal KM. Oral spore-based probiotic supplementation was associated with reduced incidence of post-prandial dietary endotoxin, triglycerides, and disease risk biomarkers. *World J Gastrointest Pathophysiol.* 2017;8(3):117.
105. Gomes AC, de Sousa RGM, Botelho PB, Gomes TLN, Prada PO, Mota JF. The additional effects of a probiotic mix on abdominal adiposity and antioxidant status: a double-blind, randomized trial. *Obesity.* 2017;25(1):30-38.
106. Sabico S, Al-Mashharawi A, Al-Daghri NM, et al. Effects of a 6-month multi-strain probiotics supplementation in endotoxemic, inflammatory and cardiometabolic status of T2DM patients: a randomized, double-blind, placebo-controlled trial. *Clin Nutr.* 2019;38(4):1561-1569.
107. Stenman LK, Lehtinen MJ, Meland N, et al. Probiotic with or without fiber controls body fat mass, associated with serum zonulin, in overweight and obese adults—randomized controlled trial. *EBioMedicine.* 2016;13:190-200.
108. Sato J, Kanazawa A, Azuma K, et al. Probiotic reduces bacterial translocation in type 2 diabetes mellitus: a randomised controlled study. *Sci Rep.* 2017;7(1):12115.
109. Sabico S, Al-Mashharawi A, Al-Daghri NM, et al. Effects of a multi-strain probiotic supplement for 12 weeks in circulating endotoxin levels and cardiometabolic profiles of medication naïve T2DM patients: a randomized clinical trial. *J Transl Med.* 2017;15(1):249.

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