Beyond gut feelings: how the gut microbiota regulates blood pressure

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Abstract | Hypertension is the leading risk factor for heart disease and stroke, and is estimated to cause 9.4 million deaths globally every year. The pathogenesis of hypertension is complex, but lifestyle factors such as diet are important contributors to the disease. High dietary intake of fruit and vegetables is associated with reduced blood pressure and lower cardiovascular mortality. A critical relationship between dietary intake and the composition of the gut microbiota has been described in the literature, and a growing body of evidence supports the role of the gut microbiota in the regulation of blood pressure. In this Review, we describe the mechanisms by which the gut microbiota and its metabolites, including short-chain fatty acids, trimethylamine N-oxide, and lipopolysaccharides, act on downstream cellular targets to prevent or contribute to the pathogenesis of hypertension. These effects have a direct influence on tissues such as the kidney, the endothelium, and the heart. Finally, we consider the role of the gut microbiota in resistant hypertension, the possible intergenerational effect of the gut microbiota on blood pressure regulation, and the promising therapeutic potential of gut microbiota modification to improve health and prevent disease.

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High blood pressure (BP) is a complex and multifactorial condition influenced by both genetic and environmental factors. Genome-wide association studies in >300,000 individuals have identified several single genetic variants linked to BP control and hypertension, but these variants only explained a very small proportion (<5%) of the interindividual variation in systolic BP[1–3]. Lifestyle and environmental factors are well-established contributors to increased BP[4]. These factors include, among others, physical inactivity, obesity, high alcohol consumption, tobacco use, high stress levels, and poor diet (including high intake of fat, sugar, and salt)[4,5].

Over the past decade, increasing evidence has linked the activity and composition of the gut microbiota with human health and disease. Specifically, the composition of the gut microbiota is likely to affect many organ systems, including the cardiovascular, immune, neural, and metabolic systems. The composition of gut microbiota is altered in many disease states, including cardiovascular disease (CVD)[6], colitis[7], malignancy[8], type 2 diabetes mellitus[9], obesity[10], psychiatric disorders[11,12], asthma[13], and numerous immune disorders[14,15]. The pathogenesis of most of these diseases are closely influenced by lifestyle factors, such as diet and exercise[14]. These observations provide a new perspective on human disease, and suggest that the gut microbiota and its metabolites might be as important as genetic factors for determining (or preventing) a whole host of human diseases. The importance of the gut microbiota is further underscored by epidemiological studies that have drawn associations between differences in disease rates with variations in the composition of the gut microbiota[16]. Many diseases, including CVD, seem to fit a model whereby adverse environmental factors, such as insufficient intake of dietary fibre, excessive consumption of food preservatives, and inappropriate use or overuse of antibiotics can alter the composition of the gut microbiota. This proposed disease model also suggests that major improvements in health might be achievable through new approaches to alter the composition of the gut microbiota, for example, by the use of prebiotics (beneficial foodstuffs) or probiotics (beneficial bacteria for gut health). In this Review, we summarize the evidence supporting a role for the gut microbiota in the development and maintenance of elevated BP, and the potential benefit of targeting the gut microbiota and its metabolites to regulate BP and prevent or treat hypertension. We also discuss reports of a growing family of metabolite-sensing G-protein-coupled receptors (GPCRs) that have a fundamental role in sensing gut bacterial metabolites and transducing its effects into many systems. Moreover, certain gut microbiota metabolites are also epigenetic modifiers of host DNA, adding another layer of complexity in determining the mechanisms that underlie the effect of gut microbiota on BP regulation.

Key points

- High dietary intake of fruit, vegetables, and fibre is associated with lower blood pressure levels
- Short-chain fatty acids, such as acetate and propionate, released by the fermentation of fibre by the gut microbiota are linked to lower blood-pressure levels in experimental models of hypertension
- A growing body of literature supports a role for the gut microbiota in the development and maintenance of high blood-pressure levels
- Limited evidence suggests that the manipulation of the gut microbiota (such as through faecal transplants, or the use of antibiotics or probiotics) might be a novel therapeutic approach for the treatment of hypertension
- The composition of human gut microbiota in the setting of high blood-pressure levels should be assessed to determine the complex nature of essential hypertension, given that gut microbiota can interact with the the host's environment and genome

The gut microbiota

The body of a healthy individual is thought to contain approximately 3.8×10^{13} bacterial cells; although this number is similar to the number of total human cells in the body, the total mass of bacterial cells makes up only 0.3% of an individual's total body weight[17]. The human microbiome contains 9 million genes, which is approximately 450 times the number of genes in the human genome[18,19]. In humans, the vast majority of the bacteria inhabit the colon, typically in a symbiotic fashion[17]. The community of commensal bacteria that lives within the gut adds a new complexity to an organism, composing of at least 1,000 prevalent species, with each individual having >160 different species[20]. Assessment of these bacterial species was only made possible in the past 12 years owing to the advances in techniques that permit the unbiased sequencing of the gut microbiome[21], allowing researchers to identify and study microorganisms that cannot be cultured. The role of these microorganisms in the development or prevention of disease is an ongoing area of research.

Diet alters gut microbiota populations. The composition of the gut microbiota depends on many factors, the most important of which is diet. Short-term and long-term dietary choices have been shown to influence the human gut microbiota[22–25]. Both animal-based and plant-based diets can rapidly alter the microbial communities that inhabit the colon[25]. One important role of the gut microbiota is to harvest nutrition and energy for the host through digestion of dietary fibre, as vertebrates do not have the enzymatic machinery to digest dietary fibre on their own. A type of fibre called resistant starches are considered prebiotics, as these fibres resist digestion in the upper gastrointestinal tract and pass to the colon, where they are metabolized by commensal bacteria as an energy source (FIG. 1). Therefore, these gut bacteria work symbiotically with the host to provide energy, metabolites and vitamins. Energy from digestion of dietary fibre is extremely important in some animal species, and even within human populations major differences exist in reliance on fibre as an energy source. In one often-cited study, African children from rural areas in Burkina Faso who consumed a high-fibre diet were compared with European children who consumed a typical Western diet[24]. The faeces of the African children contained higher amounts of bacteria from the phylum Bacteroidetes and lower amounts of the phylum Firmicutes compared with the faeces of European children[24]. This finding was consistent with early studies demonstrating that both patients with obesity and mouse models of obesity had higher rates of Firmicutes and lower rates of Bacteroidetes compared with their lean counterparts[10,26]. Later studies, however, were not able to replicate these findings[27]. Although the Firmicutes-to-Bacteroidetes ratio, the two most prevalent bacterial phyla that inhabit the gut, continues to be used as a measurement of health or disease state in the literature, each phylum contains hundreds of species that inhabit the gut and not all species from the Firmicutes are likely to have a detrimental effect on health.

Consumption of a diet rich in fibre increases gut microbiota populations that generate short-chain fatty acids (SCFAs) through the fermentation of fibre. Acetate, propionate, and butyrate (found in roughly 60:25:15 ratio in the colon) account for 80% of the SCFAs produced by the gut microbiota[28]. These SCFAs are produced in enormous amounts by bacteria in the colon (and in the caecum and rumen, depending on the species). For example, the concentration of butyrate is ~20 mM in colonic contents, but ~3 uM in peripheral blood. Butyrate is mostly used by colonocytes, whereas the remaining SCFAs and smaller proportions of butyrate are transported to the liver through the portal vein (FIG. 1). Propionate is metabolized by hepatocytes, whereas acetate and smaller proportions of propionate and butyrate are released into the systemic circulation[29]. Importantly, intestinal and serum levels of SCFAs, especially acetate and propionate, rise with an increase in consumption of fibre in animals and humans[13,24,25], whereas gnotobiotic (germ-free) mice have few or no SCFAs[7]. The extremely high concentrations of SCFAs in the gut affects the physiology of the local gut mucosa, including gut epithelial integrity[30]. In addition, numerous leukocytes continuously circulate throughout the gut and, at least transiently, must be exposed to high concentrations of SCFAs many times per day.

The relative role of numerous environmental factors in shaping the gut microbiota is still under investigation. Some of the strongest environmental influences on gut microbiota composition relate to Western lifestyle factors[31], including high-fat and low-fibre diets, emulsifiers in processed food[32], artificial sweeteners[33], caesarean versus vaginal birth[34], antibiotic use (including those in food)[35], and exercise[36]. For example, noncalorific artificial sweeteners, such as saccharin, sucralose, or aspartame, induce changes in the gut microbiota that contribute to glucose intolerance[33]. Data from a study to assess the effects of faecal transplants from lean and obese discordant twin pairs of mice into germ-free mice supported a role for the gut microbiota in the development of obesity, but this effect was dependent on diet[37]. Although the genetic background of the host organism can influence the composition of the gut microbiota[38,39], one study reported that genetic factors were estimated

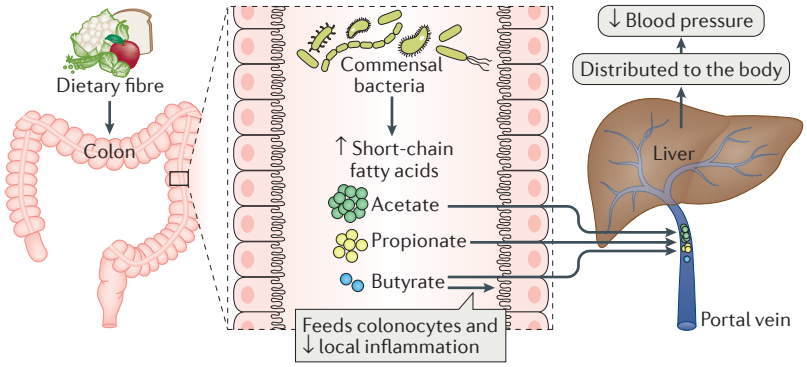

Figure 1 | **Influence of diet on gut microbiota and blood pressure.** Dietary fibre is mostly undigested until it reaches the colon, where it feeds commensal bacteria, decreasing the proliferation of pathogenic bacteria. The fermentation of fibre generates short-chain fatty acids (SCFAs). Acetate, propionate, and butyrate account for 80% of the SCFAs produced by the microbiota, found in roughly 60:25:15 ratios, respectively, within the colon. Butyrate is used by colonocytes to maintain the intestinal barrier and decrease local inflammation, but small amounts are transported with acetate and propionate to the liver through the portal vein. Propionate is metabolised by the hepatocytes, whereas acetate and smaller proportions of propionate and butyrate are released into the systemic circulation, where they can reach organs involved in the regulation of blood pressure.

to account for only 12% of the gut microbiota, whereas diet accounted for 57%[40]. Bacteria used to ferment food, such as those in cheese and cured meats, can be detected in faecal samples[25]. Therefore, changes in diet can modify the profile of the gut microbiota by changing the prevalence of certain bacterial species, in addition to introducing new species.

Regulation of immune pathways. A growing body of evidence supports the role of the immune system and exaggerated inflammatory responses in the development of hypertension[41]. In particular, preclinical models indicate that subsets of T lymphocytes such as T helper (T_H) 1, T_H2, T_H17, and regulatory T (T_{reg}) cells are involved in the regulation of BP and end-organ damage[41,42]. The gut microbiota seems to be a crucial modulator of the immune and inflammatory response[7,22]. Germ-free mice, for example, have fewer T_H7 (REF. 43) and T_{reg} cells than conventionally raised mice[44], and tend to have higher levels of T_H2 cells than T_H1 cells[45], all relevant to the development of hypertension. Interestingly, almost half of the genes expressed in the colon that are induced by the microbiota are mapped to the immune system[46]. One important connection between the gut microbiota and the immune system relates to the actions of SCFAs[7,14,22]. SCFA-binding GPCRs, such as G-protein coupled receptor 109A (GPR109A; also known as hydroxycarboxylic acid receptor 2) and GPR43 (also known as free fatty acid receptor 2), are highly expressed in immune cells, and signalling through these receptors affects the biology of such cells. SCFAs also inhibit the expression or function of histone deacetylases, particularly in immune cells. The beneficial role of SCFAs in inflammatory diseases can be explained, at least in part, by the direct effect of SCFAs on immune cells, particularly T_{reg} cells and inflammatory cells such as neutrophils[13,14]. Gut microorganisms can also stimulate the immune system through activation of Toll-like receptors (TLRs)[22].

Gut microbiota and hypertension

Numerous epidemiological studies have shown that a high intake of fruit and vegetables is associated with a lower incidence of cardiovascular mortality[47] and reduced BP[48,49]. Short-term intervention studies to assess diets such as the Dietary Approaches to Stop Hypertension (DASH) diet (which emphasizes the intake of vegetables, fruit, and low-fat dairy products[50]) and the traditional Mediterranean diet[51,52] have reported similar findings. Even combined with a diet high in fat (37% fat), a diet rich in fruit and vegetables was inversely associated with BP levels[53]. These findings have led to investigations on several micronutrients, including potassium, nitric oxide and, of most relevance to this Review, fibre. As mentioned previously, some types of fibre are considered prebiotics, as they feed commensal bacteria in the colon and stimulate their growth. Despite reports indicating that every 7 g of fibre consumed could lower the risk of CVD by 9%[54], the recommended targets for fibre intake have mostly not been met[55,56]. In a prospective study that involved 388,122 participants followed up for approximately 9 years, fibre intake (especially from grains) was associated with lower risk of both cardiovascular and all-cause death[57]. A meta-analysis reported that greater fibre consumption was associated with lower risk of CVD and coronary heart disease[54], and two other meta-analyses found that interventions to increase total intake of dietary fibre significantly reduced systolic and diastolic BP in patients with hypertension[58–60]. Different types of dietary fibre might exert different beneficial outcomes for CVD, given that these fibres modulate diverse pathophysiological mechanisms[61,62]. No studies thus far have specifically assessed the role of resistant starches, the preferred type of substrate of commensal bacteria that inhabit the human colon, in preventing CVD.

Experimental models of hypertension

Some of the pioneering studies on the influence of the microbiota in the cardiovascular system were conducted in the 1960s and 1970s[63,64]. These investigations found that compared with control rats, germ-free rats had 30% lower cardiac output[64] and a markedly decreased microvasculature response to catecholamines such as noradrenaline and vasopressin[63]. A study published in 1990 found that caecum from germ-free rats contained five times as many bioactive and vasoactive substances as caecum from control rats, which might be responsible for the changes in cardiovascular parameters such as reduced cardiac output, blood volume, and heart weight[65].

Dahl-salt sensitive rats and SHRs. Several studies using genetic models of hypertension, including the Dahl-salt sensitive rat[66] and the spontaneously hypertensive rat (SHR)[67,68], have reported alterations in gut microbiota compared with sham animals. The ratio of Firmicutes-to-Bacteroidetes has been frequently used as a marker of gut dysbiosis, and was reported to be nearly 5-fold higher in SHRs than in the Wistar Kyoto (WKY) rat control strain[68]. Bacteria from the phylum Actinobacteria

were also enriched in SHRs compared with WKY rats. Moreover, the SHRs had lower bacterial load, richness, evenness, and diversity. The changes in the gut microbiota in SHRs were consistent with a reduced number of acetate-producing and butyrate-producing bacteria, and an increased number of lactate-producing bacteria[68]. In another study, faecal samples transplanted from stroke-prone SHRs (SHRSP) into antibiotic-treated WKY rats led to an increase in systolic BP, whereas faecal samples transplanted from WKY rats to SHRSP lowered systolic BP (albeit not significantly)[67]. WKY rats transplanted with faeces from SHRSP had an increased Firmicutes-to-Bacteroidetes ratio compared with WKY rats that were transplanted with faeces from other WKY rats[67]. This observation is consistent with a report showing that chow-fed normotensive rats with obstructive sleep apnoea developed hypertension after being transplanted with faeces from hypertensive rats with obstructive sleep apnoea fed a high-fat diet[69]. The high-fat diet was also associated with a decrease in bacterial richness and the prevalence of types of bacteria that produce the SCFA butyrate[69]. Notably, 4 weeks of exercise training modulated the composition and diversity of gut bacteria in SHRs and WKY rats, but not in obese rats[70]. However, in contradiction with other studies[67,68], the basal and post-exercise gut microbiome between SHRs and WKY rats were not different[70]. This discrepancy might be due to the small sample size analyzed ($n = 3$ per group) or the use of SHR substrains from different sources[71].

Ang II and DOCA–salt mice. Reduced microbial richness in the gut was also evident in two other models of hypertension: angiotensin II (Ang II)-induced hypertensive mice[68] and deoxycorticosterone acetate (DOCA)–salt mice[8]. Our group has shown that DOCA–salt mice had lower levels of bacteria from the phylum Bacteroidetes than sham–salt mice, but higher prevalence of Proteobacteria and Cyanobacteria, especially bacteria from the YS2 genus[8]. DOCA–salt mice fed a high-fibre diet showed a significant reduction in the Firmicutes-to-Bacteroidetes ratio compared with DOCA–salt mice fed a normal diet, alongside an increase in the Actinobacteria phylum and in *Bacteroides acidifaciens*[8]. These changes were accompanied by a reduction in systolic and diastolic BP, cardiac weight index, cardiac fibrosis, systolic and diastolic left ventricular internal dimension, and left ventricular posterior wall dimension, and an improvement in cardiac function, as evidenced by increased fractional shortening[8]. Although we did not treat DOCA–salt mice with *Bacteroides acidifaciens*, other research teams have found that treatment with *Bacteroides acidifaciens* improved insulin sensitivity and prevented obesity in mice[72]. Taken together, these studies suggest a complex interaction whereby the gut microbiota influences BP levels, but might also be influenced by the presence of hypertension.

Investigators using an antibiotic ablation approach were able to reduce 24-h mean arterial pressure with the use of the anti-inflammatory antibiotic minocycline in an Ang II mouse model[68]. Furthermore, the investigators also showed that intracerebroventricular infusion of minocycline lowered BP, probably by increasing the levels of anti-inflammatory cytokines such as IL-10 (REF. 73). Minocycline treatment did not change bacterial load or richness in the Ang II mice, but significantly altered the composition of the gut microbiota, exemplified by a reduced Firmicutes-to-Bacteroidetes ratio[68]. According to the Chao1 and Shannon diversity indices, quantitative measures of species richness, no changes were observed in α diversity (BOX 1). A subsequent study assessed the role of the gut microbiota in the pathogenesis of Ang II-induced dysfunction and hypertension with the use of germ-free mice[74]. Germ-free mice infused with Ang II had lower BP levels and less cardiac fibrosis than sham germ-free or Ang II germ-free mice that received a faecal transplant 2 weeks before the experiment, and were protected from vascular oxidative stress and inflammation[74]. Together, these findings suggest a critical role for the gut microbiota in the development of Ang II-induced hypertension and hypertension-induced end-organ damage.

Box 1 | **A guide to gut microbiota for clinicians**

- 16S ribosomal RNA (rRNA) gene: a part of the 30S small subunit of the prokaryotic ribosome. 16S rRNA contains ~1,500 nucleotides and nine hypervariable regions (V1–V9) that present diversity between different taxa. Owing to slow rates of evolution, 16S rRNA is used for the construction of phylogenies and serves as a proxy for metagenomics
- α Diversity: a measure of microorganism diversity within one sample. Rarefaction curves are used to determine whether sampling depth was sufficient to characterize the gut microbiota of the samples being analyzed. Several types of α diversity indices exist, including:
  - Richness: how many species are present in one sample, measured as operational taxonomic units (for example, the Chao1 index)
  - Evenness: how evenly the operational taxonomic units from the microbial community of one sample are distributed
  - Diversity: a measurement of diversity that combines evenness and richness (for example, the Shannon index)
- β Diversity: a measure of microorganism diversity between samples. β Diversity calculates the distance between samples, which can be visualized as a principal coordinate analysis
- Bacterial load: the faecal biomass DNA per mg of faeces
- Gnotobiotic (germ-free) mice: mice born in germ-free conditions and raised within isolators, being free from any microorganisms during their lifetime. Germ-free mice are an important model to determine the role of the gut microbiota in health and disease
- Gut microbiome: the genome of the gut microbiota which is used for identification through sequencing
- Gut microbiota: the microorganisms (bacteria, eurkaryotes, and archaea) and viruses that live within the colon
- Metagenomics: the study of genetic material from the environment. In the context of the microbiome, metagenomics often refers to sequencing of long DNA strands, called shotgun sequencing, which covers the entire gut microbiome
- Operational taxonomic units: operational definition used to group closely related individuals on the basis of the similarity of their DNA sequence (usually set at 97%), and used as proxy for species at different taxonomic levels
- Prebiotics: some types of fibre, such as resistant starches, that pass through the upper gastrointestinal tract mostly undigested. Once these fibres reach the colon, they feed commensal bacteria to stimulate their growth
- Probiotics: live microorganisms that can be ingested to aid health benefits

Gut dysbiosis. Both SHRs and Ang II hypertensive mice have a leaky intestinal barrier with increased permeability and fibrotic and inflammatory markers, combined with altered tight junction proteins and decreased blood flow[75,76]. In the SHR model, this leaky intestinal barrier was restored when BP was lowered with an angiotensin-converting enzyme (ACE) inhibitor[75]. Notably, prehypertensive SHRs did not show changes in gut microbiota or in the Firmicutes-to-Bacteroidetes ratio compared with control rats, which lends support to the hypothesis that the development of gut dysbiosis coincides with the increase in BP[75]. SHRs also had increased splanchnic sympathetic nerve activity and higher tyrosine hydroxylase levels in the small intestine compared with control rats[75]. Moreover, green fluorescence protein (GFP)-labelled neurons in the hypothalamic paraventricular nucleus were detected in the SHRs and Ang II-treated WKY rats (but not in the normotensive WKY rats) 4 days after the application of GFP-labelled pseudorabies virus to the proximal colon and small intestine, indicative of enhanced sympathetic neuronal communication between the gut and the hypothalamic paraventricular nucleus[75]. Considering the essential role of the sympathetic nervous system in hypertension[77] and the gut microbiota–brain axis reported in the context of several other diseases[78], a gut–sympathetic nervous system axis is likely to be involved in the regulation of BP. However, the causal relationship between the two systems remains unclear. Combined, these studies support the concept that gut microbiota and dysbiosis are essential for the development of hypertension (FIG. 2), and that gut dysbiosis is not merely a consequence of increased BP, but a direct cause of increased BP. However, whether hypertension further exacerbates gut dysbiosis is still yet to be explored.

Link between salt and the gut microbiota. The proximal colon is an important site for dietary sodium absorption, and might have important roles in BP regulation[79,80]. Indeed, inhibition of the gut sodium–hydrogen exchanger 3 has been shown to reduce BP levels and increase sodium excretion in disease models[81]. Gut-derived hormones, such as glucagon-like peptide 1 and gastrin, in addition to other hormones such as glucocorticoids and aldosterone, participate in sodium

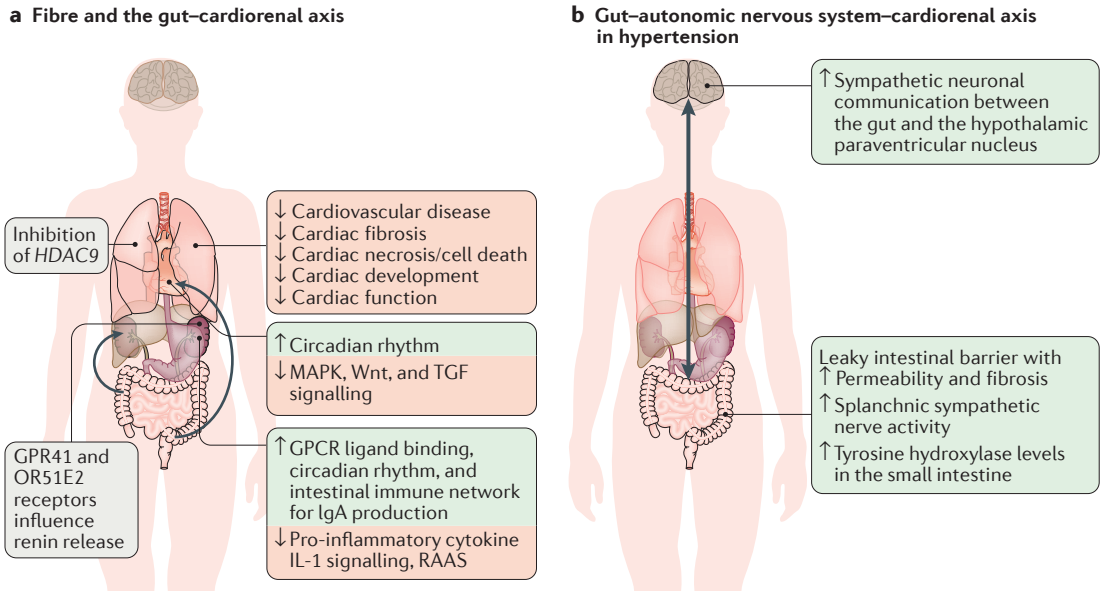

Figure 2 | **Mechanisms of the gut–autonomic nervous system–cardiorenal axis that regulate blood pressure.** **a** | A diet rich in resistant starches, which results in increased release of short-chain fatty acids (SCFAs), has several systemic effects that lead to a decrease in blood-pressure levels and cardiovascular disease in general. In the lung, SCFAs downregulate signalling pathways related to cardiac fibrosis, cardiac necrosis and cell death, and cardiovascular system development and function. Fibre and the SCFA acetate function independently of the G-coupled protein receptor (GPCR) GPR43, but act through histone deacetylase (HDAC9) in the lung. In the heart, fibre and acetate upregulate circadian rhythm pathways and downregulate mitogen-activated protein kinase (MAPK), Wnt, and transforming growth factor (TGF)-β signalling. In the kidney, fibre and acetate upregulate pathways related to GPCR ligand binding, circadian rhythm, and intestinal immune network for immunoglobulin (Ig) A production, and downregulate pro-inflammatory IL-1 signalling and the renin–angiotensin–aldosterone system (RAAS). In the kidney, the SCFA propionate seems to act through the olfactory receptor (Olfr78 in mice or OR51E2 in humans) and GPR41 (TABLE 1). **b** | Experimental models of hypertension, such as the spontaneously hypertensive rat (SHR) and the angiotensin II mouse model, have higher sympathetic neuronal communication between the gut and the hypothalamic paraventricular nucleus. Both animal models have a leaky intestinal barrier with increased permeability, fibrosis, and inflammatory markers, combined with altered tight junction proteins and decreased blood flow. The SHRs also have increased splanchnic sympathetic nerve activity and higher tyrosine hydroxylase levels in the small intestine. Together, these studies suggest the presence of a gut–sympathetic nervous system–cardiorenal axis in the regulation of blood pressure.

homeostasis in the gut and contribute to the modulation of BP[82]. The role of the gut microbiota has been investigated in rats genetically bred to be either sensitive (that is, hypertensive) or resistant (that is, normotensive) to the BP effects of a high-salt diet (2%)[66]. The composition of the gut microbiota was different between these Dahl-salt sensitive and salt-resistant rats[66]. Compared with the salt-resistant controls, the salt-sensitive strain had higher prevalence of bacteria from the Bacteroidetes phylum, but no change in the Firmicutes phylum[66]. After administration of antibiotics to eliminate the existing microbiota, faecal samples were transplanted between the two strains. Whereas salt-resistant rats that received a transplant from salt-sensitive rats showed no change in BP or sodium excretion levels, faecal transplants from the salt-resistant to the salt-sensitive strain resulted in exacerbation of hypertension and reduction in lifespan[66]. This elevation in BP only occurred when rats were fed a high-salt diet and was independent of the treatment with antibiotics[66]. As these rat strains had been bred and housed in a similar environment since the 1970s, the authors suggested that the genetic variations between strains were responsible for the differences in the gut microbiota[66]. Dahl salt-sensitive and salt-resistant rats have polymorphisms in the genes encoding proposed SCFA receptors, such as the genes coding for GPCRs *Gpr37l1*, *Gpr89b*, *Gpr110*, *Gpr155*, and *Gpr161* (REF. 66). In addition to the genomic differences, we propose that the phenotype selection that occurred over several generations to produce the two independent strains could have bred rats with different gut microbiota compositions. As the gut microbiota is usually passed from the mother to the newborn litter, once established, the gut microbiota would be kept consistent through the next generations.

Gut microbiota in human hypertension

The role of dysbiosis of the gut microbiota in the development of human hypertension has received increased attention in the past 2 years. An analysis of the 16S gene in a small cohort of seven patients with hypertension and 10 control individuals, a subset of patients from ongoing clinical trials[83,84], showed that the human gut microbiome in patients with hypertension harboured lower microbial diversity than the gut microbiome from a healthy individual[68]. However, both BP-lowering medication and a history of hypertension among the healthy individuals could be confounding factors in this setting. To address some of the issues related to small sample size and use of BP-lowering medication, Li and colleagues compared gut microbiota composition between 56 patients with prehypertension, 99 patients with newly diagnosed or untreated essential hypertension, and 41 healthy controls[85]. Faecal samples from all participants were subject to shot-gun metagenomic sequencing, functional annotation, and metabolomics analysis[85]. The gut microbiota of both patients with prehypertension and patients with hypertension had lower gene richness and α diversity than healthy controls[85]. Two main enterotypes were identified; the gut microbiota in patients with prehypertension and in patients with hypertension had a higher percentage of an enterotype rich in bacteria from the genus *Prevotella*, whereas the gut microbiota of healthy controls were mostly made up of an enterotype rich in bacteria from the genus *Bacteroidetes*[85]. This observation is consistent with our findings in a DOCA-salt mouse model of hypertension, in which the prevalence of *Prevotella* was significantly lower in mice fed a high-fibre diet[8]. *Prevotella*, in addition to other bacteria associated with prehypertension and hypertension including *Klebsiella*, *Porphyromonas*, and *Actinomyces*, are associated with infections and periodontal diseases[86–88]. Compared with healthy controls, patients with prehypertension or hypertension have lower levels of bacteria such as *Faecalibacterium*, *Roseburia*, and *Bifidobacterium* that are usually associated with intestinal microbial homeostasis and production of the SCFA butyrate[85]. This finding was also reflected in co-abundance groups composed of >50 genes[85]. In a smaller subset of patients, the study investigators identified 26 serum metabolites that differed between healthy controls and patients with prehypertension or hypertension[85]. Although these differences in metabolites and some of the most prevalent microbiota genera between the disease groups do not implicate causation, these findings suggest that the metabolic profile of hypertension is likely to be associated with composition of the gut microbiota and the systemic distribution of its products. By combining gut microbiota co-abundance groups and metabolites, the study investigators were able to identify patients with prehypertension or hypertension with high accuracy (0.89 and 0.91 area under the receiver operating curve, respectively)[85]. The use of this method to identify individuals at risk of developing hypertension should be assessed in future. Finally, the study investigators transplanted germ-free mice with faecal samples from one healthy individual or from two patients with hypertension (five mice per donor). Consistent with the findings in humans, mice that received a faecal transplant from a patient with hypertension had higher systolic and diastolic BP, lower microbial diversity (as measured by the Shannon index), and an overall different gut microbiome profile compared with mice receiving a faecal transplant from a healthy individual[85]. These findings lend further support for the role of the gut microbiota in the development of hypertension.

In another Chinese study to investigate the link between serum metabolites and hypertension, the levels of lyxose (a byproduct of gut bacterial fermentation) were found to be higher in patients with newly diagnosed hypertension ($n = 29$) than in healthy controls ($n = 29$), all of whom were part of a larger ($n = 1{,}133$), population-based, nested case-control study[89]. Although these findings are preliminary, gut microbiota metabolites might be associated with future development of hypertension. The findings from these early studies need to be replicated in independent, large cohorts from multiple ethnic backgrounds, ideally with 24-h BP measurements and characterization of other environmental factors (such as diet) that might affect the gut microbiota.

Table 1 | **Receptors for short-chain fatty acids and their effect on blood pressure**

Short-chain fatty acid	Effect on blood pressure	Receptors
Acetate	↓Blood pressure[8]	GPR41, GPR43, Olfr78, HDAC9
Propionate	↓Blood pressure (through GPR41)[93,97] or ↑blood pressure (Olfr78)[93]	GPR41*, GPR43, Olfr78*
Butyrate	Unknown	GPR41, GPR43, GPR109A

*Receptors shown to have an effect in blood-pressure levels *in vivo*. Besides GPR41 and Olfr78, other receptors have not been characterized in hypertension. GPR41, G-protein-coupled receptor 41 (also known as free fatty acid receptor 3); GPR43, G-protein-coupled receptor 43 (also known as free fatty acid receptor 2); GPR109A, G-protein-coupled receptor 109A (also known as hydroxycarboxylic acid receptor 2); HDAC9, histone deacetylase 9; Olfr78, olfactory receptor 51E2.

SCFAs: the missing link?

Early studies published >20 years ago have shown that the three most common SCFAs — acetate, propionate, and butyrate — mediate concentration-dependent dilatation of rat tail arteries[90,91] and human colonic resistance arteries[92]. These observations are consistent with more recent findings that acetate[8] and propionate[93] reduce BP levels in mouse models, and that two independent models of hypertension, SHRs and Ang II mice, have less SCFA-producing bacteria than their normotensive counterparts[68] (TABLE 1). Propionate increased the release of renin, the rate-limiting enzyme of the renin–angiotensin–aldosterone system (RAAS) in an *ex vivo* preparation of isolated renal juxtaglomerular cells[93]. However, administration of propionate in wild-type mice resulted in an acute, dose-dependent, but a short-lived ~20 mmHg reduction in BP, indicating that propionate acts on different renal mechanisms[93]. In our study, we assessed the effect of chronic intake of acetate in the drinking water of sham and DOCA–salt mice[8]. DOCA-treated mice that received acetate had reduced systolic and diastolic BP (both reduced by 21 mmHg), lower cardiorenal weight index, less fibrosis, reduced left ventricular wall thickness, and improved cardiac function compared with sham mice[8]. Surprisingly, the beneficial cardiovascular effects of acetate were even more pronounced than a diet rich in resistant starch itself[8]. However, this discrepancy might be related to pharmacological dosing, as acetate in the drinking water is absorbed rapidly in the small intestine, potentially allowing for greater exposure to peripheral tissues. In a model of ischaemia–reperfusion injury in the kidney, intraperitoneal administration of the SCFAs acetate, propionate, and butyrate significantly reduced inflammation and tissue injury, and improved renal function[94]. The efficacy of SCFA administration in the treatment of human hypertension has not yet been explored.

Mechanisms of BP regulation. The role of metabolite-sensing GPCRs in the development of hypertension and other CVDs has been reviewed previously[95]. To date, 12 metabolite-sensing GPCRs have been identified, but others are likely to emerge with the deorphanization of more members of the GPCR family. Metabolite-sensing GPCRs bind metabolites from fibre (SCFAs), tryptophan catabolites (such as kynurenic acid), medium-chain fatty acids (found in milk), and long-chain fatty acids (such as omega-3 fatty acids found in fish). All metabolite-sensing GPCRs might be important for gut homeostasis, metabolism, or the regulation of immune responses[95]. Our group has hypothesized that insufficient signalling through one or more of these receptors can lead to compromised gut integrity (leaky gut), dysregulated inflammation, and passage of substances such as lipopolysaccharides into blood and tissues. Excess fat or sugar in the diet might contribute to poor metabolite-sensing GPCR engagement[95,96]. At least three metabolite-sensing GPCRs have been shown to bind SCFAs, GPR41 (also known as free fatty acid receptor 3), GPR43, and GPR109A, and these GPCRs control gut homeostasis, host metabolism, and immune response. In addition, numerous histone deacetylases (which control gene expression through regulation of chromatin structure) have evolved to be regulated by butyrate and other SCFAs. As a result, microbiota composition might affect gene expression in many cell types.

The GPCR olfactory receptor 51E2 (also known as Olfr78 in mice, or OR51E2 in humans), has also been identified as one of the receptors for acetate and propionate[93]. Olfr78 is localized in the smooth muscle cells of arteries, in autonomic nerves in the heart and gut, and in the renal juxtaglomerular complex[93]. Given that secreted renin is also localized in these regions, Olfr78 might be involved in renin regulation. Indeed, when treated with propionate, lower amounts of renin were released from *ex vivo* renal juxtaglomerular cells from *Olfr78*$^{-/-}$mice compared with those from wild-type mice[93]. *Olfr78*$^{-/-}$ mice have lower plasma renin and slightly lower mean arterial pressure than wild-type mice (81.4 mmHg versus 94.5 mmHg)[93]. Treatment with antibiotics resulted in a modest increase in BP in *Olfr78*$^{-/-}$mice compared with wild-type mice, suggesting a gut microbiota-dependent effect[93]. However, *Olfr78*$^{-/-}$mice still showed a drop in BP upon acute administration of propionate[93], suggesting that propionate also acts through other receptors to regulate BP. The effect of propionate on BP in *Gpr41*$^{-/-}$ mice was also investigated. In the absence of GPR41, propionate administration did not lower BP levels[93]. GPR41 is localized to the vascular endothelium, and is essential for SCFA-mediated vasodilation[97]. *Gpr41*$^{-/-}$ mice had isolated systolic hypertension, and, as mice aged, showed thickening of the aorta and higher collagen deposition, both markers of arterial stiffening[97]. Together, these findings suggest that propionate can have opposite physiological effects through Olfr78 and GPR41; propionate can increase the release of renal renin through Olfr78, but can also mediate a hypotensive effect that is dependent on GPR41 (FIG. 2; TABLE 1).

Most metabolite-sensing GPCRs, particularly the SCFA receptors, have very low affinity for their metabolite agonists, indicating that their main site of action is in the gut[84]. However, this notion is at odds with the phenotypes that we and others have observed in animal models, for instance, the GPR109a-knockout mouse model[98,99]. The complex effect that metabolite-sensing GPCRs might have outside of the gut needs to be explored further.

SCFA-mediated transcriptome changes. A study by Thorburn and colleagues in 2015 showed that mice fed a diet high in fibre or acetate during pregnancy produced offspring that were protected from developing asthma[13]. Although these findings are not directly relevant to hypertension, maternal fibre and acetate can lead to the downregulation of pathways related to development of CVD and cardiac hypertrophy, including the differential expression of genes encoding proteins involved in BP regulation, such as atrial and brain natriuretic peptides[13].

In our study, we assessed by RNA sequencing the cardiorenal transcriptome of mice fed a standard-fibre diet, high-fibre diet, or acetate for 3 weeks[8]. The analysis was performed before the induction of any adverse cardiovascular phenotype to exclude differences linked to cell populations. In the kidney, 77% of the genes differentially expressed as a consequence of fibre intake were also dysregulated with acetate intake[8]. Such genes included *Rasal1* (previously associated with renal fibrosis)[100], *Cyp4a14* (encoding a protein with a role in fluid absorption through sodium channel regulation)[101], and *Cck* (which is associated with anti-inflammatory properties)[102]. Expression of genes related to pathways involved in BP regulation, such as the RAAS, and those modulating circadian rhythm was downregulated[8]. High-fibre intake was associated with downregulation of pro-inflammatory IL-1 signalling, and upregulated GPCR ligand binding and intestinal immune signalling for immunoglobulin A production[8]. In the heart, 42% of genes that were differentially expressed were in common between fibre and acetate intake, including genes coding for proteins thought to have a preventive role for heart disease, such as *Tcap*[103] and *Timp4* (REF. 104). Genes related to circadian rhythm pathways were upregulated, whereas those related to mitogen-activated protein kinase signalling pathways were downregulated in response to fibre[8]. The gene coding for early growth response protein 1, considered to be a master regulator of cardiovascular pathology[105–107], was downregulated in both kidney and heart with fibre intake or supplementation with acetate[8]. Combined with the protective cardiovascular changes observed with intake of fibre or acetate, these transcriptome changes point to the presence of a gut–cardiorenal axis (FIG. 2).

SCFA-independent determinants of BP. Although variations in fibre content might directly influence the gut microbiota and its metabolites, leading to changes in BP levels as described above, other investigators have proposed an alternative relationship between diet and hypertension. Diets low in fibre are often rich in other nutrients such as fat, protein, and refined sugars, which might also influence the gut microbiota and BP. Tang and colleagues have shown that a gut metabolite of dietary choline and phosphatidylcholine, known as trimethylamine N-oxide (TMAO), is associated with development of atherosclerosis[108]. L-carnitine was subsequently described as another source of TMAO[109]. Choline, phosphatidylcholine, and L-carnitine are essential dietary nutrients found in foods rich in cholesterol, such as red meat[6]. An intact gut microbiota is needed for the conversion of choline, phosphatidylcholine, or L-carnitine to TMAO[108,109]. Plasma levels of TMAO have been associated with short-term (30 days) and long-term (6 months) risk of major adverse cardiovascular events and death in patients with acute coronary syndrome[110]. Although TMAO seems to be highly relevant to the development of atherosclerosis and major cardiovascular events[6,110], the contribution of TMAO to BP is less clear. On its own, TMAO infusion did not increase BP in Sprague–Dawley rats, but when combined with a low dose of Ang II, TMAO infusion influenced the haemodynamic response, prolonging the effect of Ang II-induced hypertension[111]. However, whether this response is a direct effect of TMAO on BP levels or a consequence of its effect on atherosclerotic markers needs to be determined.

Given their effect on the innate immune system and inflammation, lipopolysaccharides might also be involved in the pathogenesis of hypertension. Lipopolysaccharides are major components of the outer membrane of most Gram-negative (pathogenic) bacteria, contributing to their structural integrity[112]. In the blood, lipopolysaccharides are transferred from circulating lipopolysaccharide-binding proteins to CD14 molecules on the surface of leukocytes, which presents the lipopolysaccharide to a complex formed by TLR4 and lymphocyte antigen 96 (MD-2). This complex activates a strong innate immune response, leading to the release of pro-inflammatory cytokines such as IL-1 and IL-18 (REF. 113). In experimental models, lipopolysaccharides have been shown to accelerate the formation of unstable plaques[114], and are associated with human atherosclerosis[115]. Pathway analyses from metagenome sequencing of faecal samples linked elevated lipopolysaccharide biosynthesis by gut bacteria and lipopolysaccharide export with prehypertension and hypertension in the host[85], effects that are likely related to gut dysbiosis and increased gut permeability. Lipopolysaccharide levels in the blood have been inversely correlated with adherence to the Mediterranean diet, but no association has been found with hypertension[116]. The relationship between lipopolysaccharides and BP, however, is likely to be complex, given that high levels of circulating lipopolysaccharides, as a consequence of an infection, can lead to septic shock and hypotension[117]. Further studies are needed to determine if lipopolysaccharides indeed have a role in hypertension, and to pinpoint the effects of different lipopolysaccharide doses in BP levels.

Probiotics, milk peptides, and BP

Probiotics can be selectively used to introduce and expand new types of microorganisms. The intake of milk peptides and different strains of *Lactobacillus* was shown to lower BP levels in experimental models, including WKY rats and SHRs[118–121]. For example, the fermentation of milk with *Lactobacillus helveticus* released peptides with the amino acid sequences isoleucine–proline–proline and valine–proline–proline, which have the capacity to inhibit ACE, resulting in lower BP levels in experimental models such as the SHR[121–123].

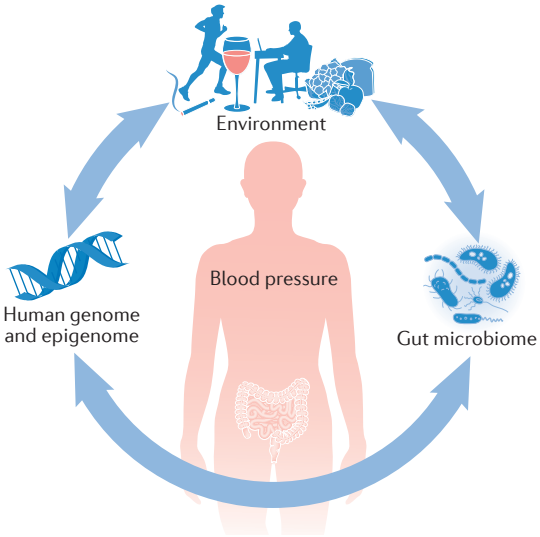

Figure 3 | **Hypertension as a multifactorial disease.** Future studies should address the interaction between the human genome and epigenome, the environment (nutrition), and the gut microbiome, and how they together determine blood-pressure levels.

A meta-analysis of 14 randomized, placebo-controlled clinical trials involving a total of 702 participants who ingested probiotic fermented milk reported a modest, albeit statistically significant decrease in systolic (−3 mmHg) and diastolic (−1 mmHg) BP[124]. The BP-lowering effect was more pronounced in patients who were hypertensive (−3.98 mmHg systolic BP) than in participants who were normotensive (−2.09 mmHg), and was more pronounced in the six Japanese studies (−6.12 mmHg) than in the eight European studies (−2.08 mmHg)[124], suggesting that other genetic or environmental factors might be involved.

A separate meta-analysis assessed the effect of probiotics on BP levels in nine randomized, controlled trials with a total of 543 participants[125]. Overall, a significant reduction in systolic BP (−3.56 mmHg; 95% CI −6.46 to −0.66) and diastolic BP (−2.38 mmHg; 95% CI −3.84 to −0.93) was observed, but individually the effect was highly variable and mostly neutral for diastolic BP[125]. Yoghurt was the main source of probiotics in these studies (4 out of the 9), but other studies included sour milk, milk, cheese, and a capsule containing specific bacterial strains[125]. The type of probiotics used differed between each study, with combinations that included *Enterococcus faecium*, *Streptococcus thermophiles*, *Lactobacillus acidophilus*, *Lactobacillus helveticus*, *Lactobacillus reuteri*, *Lactobacillus casei*, *Lactobacillus plantarum*, *Bifidobacteria infantis*, *Saccharomyces cerevisiae*, and *Bifidobacterium animalis*[125]. The discrepancies between the probiotic source and type, combined with different study designs in terms of dosage, patient population, and length of treatment can account for the differences in the findings between individual studies. Therefore, these results need to be interpreted with caution until further trials with more standardized probiotics and design are available.

Gut microbiota and antihypertensive drugs

Numerous studies have shown that the human gut microbiota can both directly and indirectly influence the metabolism of >40 known agents, such as those used to treat cancer[126] and CVD[127], by influencing both their efficacy and toxicity. For example, the cardiac drug digoxin, used to treat heart failure and arrhythmias, can be inactivated by the strain DSM2243 of the Actinobacterium *Eggerthella lenta* (*E. lenta*)[127]. Although arginine from dietary protein can stimulate the growth of *E. lenta*, arginine also prevented the inactivation of digoxin by decreasing the expression of the cardiac glycoside reductase operon gene[127]. Importantly, this finding highlighted that drug metabolism can be influenced by interactions between specific bacterial strains and the host diet.

The data available on the effects of gut microbiota and metabolism of antihypertensive agents are still very preliminary. The metabolism of commonly used antihypertensive drugs such as ACE inhibitors, β-blockers, and Ang II-receptor antagonists have been associated with interindividual variation of the human gut microbiome in 1,135 Dutch individuals from population-based cohorts[16]. Approximately 13.7% of patients with hypertension are estimated to be treatment-resistant[128]. A case-study published in 2015 described an improvement in BP control after intensive antibiotic therapy in a patients with treatment-resistant hypertension[129]. Two ongoing clinical trials will assess the effect of the anti-inflammatory antibiotic minocycline in patients with treatment-resistant hypertension[83,84]. If the gut microbiota is indeed involved in drug responses in hypertension, pinpointing the specific strains involved is essential for understanding the underlying mechanisms, and to develop novel therapeutic targets.

Pregnancy, gut microbiota, and BP

During birth, the newborn baby is exposed to a wide variety of microorganisms from the environment and the mother. Therefore, the mode of delivery has a substantial influence on the newly formed microbiota. Infants who are delivered vaginally have a microbiome that resembles their own mother's vaginal microbiota, whereas those delivered by caesarean section have predominantly bacterial communities found on the skin surface[34]. A pilot study reported that exposing newborn babies delivered by caesarean section to maternal vaginal fluids at birth could influence the bacterial communities in the baby[130]. Gestational age also seems to have a profound effect on the composition of the neonatal microbiome[131]. Although the long-term effects of this early colonization on health is not yet understood, the individual human gut microbiome is thought to be mostly (60%) stable over a period of at least 5 years after birth and, potentially, for decades[132].

The Developmental Origins of Health and Disease (DOHaD) hypothesis proposed that environmental conditions during fetal and early life shape an individual's susceptibility to disease later in life[133], but the effect of the gut microbiota and its metabolites during pregnancy is still to be elucidated. The intergenerational effect, and consequent epigenetic modifications

of dietary macronutrients such as protein and protein-to-carbohydrate ratio have been studied previously in the context of BP[134–138]. An interesting notion stipulates that excessive intake of sugar, fat, or protein might be at the expense of sufficient intake of dietary fibre. Indeed, as mentioned previously, a high-fibre diet or supplementation with the SCFA acetate during pregnancy prevented the development of asthma in the offspring through an epigenetic mechanism involving histone deacetylase 9 (FIG. 2; TABLE 1), leading to the downregulation of pathways implicated in CVD in the lung[13]. Whether gestational fibre, through the production of SCFAs by the gut microbiota, is cardioprotective remains to be determined. A borderline U-shaped association between intake of fibre during pregnancy and the infants' diastolic BP at 6 months of age has been described[139].

The adult offspring of pregnant Sprague–Dawley rats exposed to late gestational stress through repeated restrain had higher BP and an exaggerated hypothalamic–pituitary–adrenal axis response, but lower innervation in the distal colon[140]. Rats exposed to prenatal stress also showed enhanced colonic sensitivity to stimulation by noradrenaline, which the authors suggest could be related to reduced gut motility[140]. The gut microbiome composition of the prenatally stressed offspring was also distinct from control animals aged 4 months, but no difference in diversity was observed[140]. Prenatally stressed offspring had higher levels of *Oscillibacter*, *Anaerotruncus*, and *Peptococcus*, all part of the Clostridiales order, and lower levels of Lactobacillus and Streptococcaceae families[140]. Consistent with the DOHaD hypothesis, these findings support a long-lasting effect of prenatal stress not only on cardiovascular variables such as BP, but also on the composition of the gut microbiota. Determining the gut microbiome composition of the mother would be important to pinpoint whether the offspring's cardiovascular phenotype was a consequence of epigenetic effects or the transmission of a defective gut microbiota from the mothers to the offspring[13].

The gut microbiota of 205 women who were overweight or obese at 16 weeks of gestation were assessed[141]. Systolic BP was negatively correlated to the bacterial families Odoribacteraceae, Rikenellaceae, Christensenellaceae, and Lachnospiraceae independently of BMI, whereas diastolic BP was negatively correlated with the families Clostridiaceae and Bacteroidaceae[141]. The gene expression related to the production of the SCFA butyrate was also analyzed, because two of the bacterial families associated with BP, Odoribacteraceae and Clostridiaceae, are known to produce butyrate. A negative correlation between BMI and butyrate-producing capacity was identified, especially in the women who were obese[141]. The expression of the gene for butyrate kinase, *BUK*, was negatively correlated with systolic and diastolic BP and the low-grade inflammatory marker plasminogen

Table 2 | **Current limitations and future directions in the field of gut microbiota in hypertension**

Limitations and unanswered questions	Potential solutions
Currently, no data on how genetics can influence the gut microbiota in blood pressure regulation and hypertension are available	Combine gut microbiome with GWAS data to determine mechanisms behind blood pressure regulation and hypertension
Lack of faecal samples from study cohorts already recruited and characterized	Determine if a blood microbiome exists and whether it is a proxy for the gut microbiome
The bacterial species that protect against or contribute to hypertension and CVD are unknown	Large and well-characterized human studies to describe the gut microbiome in normotension and hypertension, combined with animal studies to test the role of specific bacterial strains
Can bacteria be used individually to treat hypertension, or do they need a microbial community to have an effect?	Use germ-free models of hypertension to inoculate specific bacterial strains or communities
Are bacterial strains and their metabolites involved in the activation of the sympathetic nervous system?	Measure sympathetic activity in central nervous system and innervation in tissues such as in the kidney in models before and after treatment with different bacterial strains and metabolites
Which receptors are involved in SCFA sensing in BP regulation besides GPR41 and Olfr78?	Use knockout models of SCFA ligands, such as GPR43, GPR109A, and HDAC9, to determine the receptors involved
What are the specific strains of bacteria and SCFAs that might be protective or contribute to transgenerational CVD?	Determine using translational studies, starting with models of hypertension
Inconclusive results on the use of probiotics to lower blood-pressure levels	Perform large, multicentre, clinical trials that consistently use the same types of bacteria as probiotics
Current lack of trials to assess the use of prebiotics (such as resistant starches) to lower blood-pressure levels	Perform large, multicentre, clinical trials that consistently use the same type of resistant starches as prebiotics
How does salt intake modify the human gut microbiota?	Perform clinical trials to determine if the human gut microbiota has a role in salt-sensitive hypertension
How can we manipulate the gut microbiota to prevent CVD?	Perform animal and clinical studies that use prebiotics, probiotics with specific strains, antibiotics, and faecal transplants
Can the gut microbiota and its metabolites be used as early markers for the development of hypertension?	Use stool and plasma samples from longitudinal studies with cohorts of patients with well-characterized blood pressure

BP, blood pressure; CVD, cardiovascular disease; GPR41, G-protein-coupled receptor 41 (also known as free fatty acid receptor 3); GPR43, G-protein-coupled receptor 43 (also known as free fatty acid receptor 2); GPR109A, G-protein-coupled receptor 109A (also known as hydroxycarboxylic acid receptor 2); GWAS, genome-wide association studies; HDAC9, histone deacetylase 9; Olfr78, olfactory receptor 51E2; SCFA, short-chain fatty acids.

activator inhibitor type 1, but was independent of fibre ingestion[141]. Although this study cannot prove causality between the gut microbiota, butyrate, and its effects on inflammation and BP levels, the findings suggest that other bacterial metabolites besides acetate and propionate might be beneficial for lowering BP levels through their anti-inflammatory properties. Indeed, a prospective study in 33,399 primiparous women showed that daily or weekly intake of probiotics from milk-based products was associated with lower risk of pre-eclampsia[142].

Future directions

Given that data from numerous studies have supported a role for the gut microbiota in the development and maintenance of hypertension, we suggest that future genome-wide association studies should be combined with gut microbiome analyses. Calculations of personalized risk should take into account not only the gut microbiota, genetic factors, and environment (nutrition), but how these factors interact together (FIG. 3), because the gut microbiota itself can be influenced by both genetic and environmental factors[38]. However, the lack of faecal sample data in the study cohorts for which genomic data is currently available might necessitate new studies to include information on nutrition and metabolites (TABLE 2). An important question remains: how important is host genetics versus nutrition/antibiotic use and the composition of the gut microbiota?

Similar to other molecular studies, human studies should also be validated in independent cohorts and populations, because the gut microbiota of individuals living in different geographical areas can vary[143]. Proper characterization of participants' BP levels with methods other than office BP are needed to ensure that cases of white-coat and masked hypertension are identified and properly grouped. Owing to the possible influence of BP-lowering medication on the gut microbiota[16], study cohorts need to be either untreated or grouped according to medications being taken. Moreover, the gut microbiota of patients with resistant hypertension needs to be characterized (TABLE 2).

If successful, these studies might lead to personalized gut microbiota therapies for hypertension, or prebiotics that modify microbiota composition to improve BP. Although faecal microbiota transplants are being used to treat *Clostridium difficile* infections that are resistant to traditional therapy[144], adverse effects such as unexpected weight gain are possible[145]. The FDA has consequently released statements of caution against the use of stool banks[146]. Going forward, the identification and characterization of the specific bacterial strains that could lower BP levels, and the use of these strains in personalized probiotic supplements are pertinent. Moreover, whereas the hypertensive gut microbiome has received much attention in the past few years, studies reporting a role for the oral microbiome in hypertension are starting to emerge and deserves further investigation[147]. If nutrition and dietary fibre, working through the gut microbiota, do explain elements of hypertension and CVD, will this new knowledge provide an impetus for human populations to consume higher amounts of fibre?

Future studies should also explore the use of gut microbiota or related metabolites as early markers for the development of hypertension and CVD. For example, lower gut microbiota richness and the presence of six genera (*Alloprevotella*, *Catenibacterium, Prevotella 2*, *Prevotella 7*, *Tyzzerella*, and *Tyzzerella 4*) were consistently associated with lifetime risk of CVD in an extreme phenotype design, which had a higher statistical power than cohorts with milder phenotypes[148]. Methodological concerns are also of importance: for example, although microbiota can be detected in blood samples[149], how closely it correlates with the gut microbiota in humans remains to be determined.

Conclusions

Current experimental and clinical studies strongly support a close but complex inter-relationship between nutrition, the gut microbiota and its metabolites, and hypertension. At the population level, these findings further strengthen the importance of diet and lifestyle modifications as a means to improve global BP control and, consequently, cardiovascular health. At a more pragmatic level, further work is required to determine the precise cellular and molecular mechanisms responsible for gut–cardiovascular interactions. We believe that medicinal foods and the gut microbiota and its metabolites offer a highly promising and effective means to counter hypertension, as well as other diseases associated with the Western lifestyle.

1. Surendran, P. *et al.* Trans-ancestry meta-analyses identify rare and common variants associated with blood pressure and hypertension. *Nat. Genet.* **48**, 1151–1161 (2016).
2. Liu, C. *et al.* Meta-analysis identifies common and rare variants influencing blood pressure and overlapping with metabolic trait loci. *Nat. Genet.* **48**, 1162–1170 (2016).
3. Ehret, G. B. *et al.* The genetics of blood pressure regulation and its target organs from association studies in 342,415 individuals. *Nat. Genet.* **48**, 1171–1184 (2016).
4. Ezzati, M. & Riboli, E. Behavioral and dietary risk factors for noncommunicable diseases. *N. Engl. J. Med.* **369**, 954–964 (2013).
5. Hu, G. *et al.* Relationship of physical activity and body mass index to the risk of hypertension: a prospective study in Finland. *Hypertension* **43**, 25–30 (2004).
6. Tang, W. H. & Hazen, S. L. The contributory role of gut microbiota in cardiovascular disease. *J. Clin. Invest.* **124**, 4204–4211 (2014).
7. Maslowski, K. M. *et al.* Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* **461**, 1282–1286 (2009).
8. Marques, F. Z. *et al.* High fibre diet and acetate supplementation change the gut microbiota and prevent the development of hypertension and heart failure in DOCA-salt hypertensive mice. *Circulation* http://dx.doi.org/10.1161/CIRCULATIONAHA.116.024545 (2017).
9. Forslund, K. *et al.* Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature* **528**, 262–266 (2015).
10. Turnbaugh, P. J. *et al.* An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* **444**, 1027–1031 (2006).
11. Foster, J. A. & McVey Neufeld, K. A. Gut–brain axis: how the microbiome influences anxiety and depression. *Trends Neurosci.* **36**, 305–312 (2013).
12. Dinan, T. G., Borre, Y. E. & Cryan, J. F. Genomics of schizophrenia: time to consider the gut microbiome? *Mol. Psychiatry* **19**, 1252–1257 (2014).
13. Thorburn, A. N. *et al.* Evidence that asthma is a developmental origin disease influenced by maternal diet and bacterial metabolites. *Nat. Commun.* **6**, 7320 (2015).
14. Thorburn, A. N., Macia, L. & Mackay, C. R. Diet, metabolites, and "western-lifestyle" inflammatory diseases. *Immunity* **40**, 833–842 (2014).
15. Kamada, N., Seo, S. U., Chen, G. Y. & Nunez, G. Role of the gut microbiota in immunity and inflammatory disease. *Nat. Rev. Immunol.* **13**, 321–335 (2013).

16. Zhernakova, A. *et al.* Population-based metagenomics analysis reveals markers for gut microbiome composition and diversity. *Science* **352**, 565–569 (2016).
17. Sender, R., Fuchs, S. & Milo, R. Revised estimates for the number of human and bacteria cells in the body. *PLoS Biol.* **14**, e1002533 (2016).
18. Yang, X., Xie, L., Li, Y. & Wei, C. More than 9,000,000 unique genes in human gut bacterial community: estimating gene numbers inside a human body. *PLoS ONE* **4**, e6074 (2009).
19. Li, J. *et al.* An integrated catalog of reference genes in the human gut microbiome. *Nat. Biotechnol.* **32**, 834–841 (2014).
20. Qin, J. *et al.* A human gut microbial gene catalogue established by metagenomic sequencing. *Nature* **464**, 59–65 (2010).
21. Tringe, S. G. *et al.* Comparative metagenomics of microbial communities. *Science* **308**, 554–557 (2005).
22. Maslowski, K. M. & Mackay, C. R. Diet, gut microbiota and immune responses. *Nat. Immunol.* **12**, 5–9 (2011).
23. Kau, A. L., Ahern, P. P., Griffin, N. W., Goodman, A. L. & Gordon, J. I. Human nutrition, the gut microbiome and the immune system. *Nature* **474**, 327–336 (2011).
24. De Filippo, C. *et al.* Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc. Natl Acad. Sci. USA* **107**, 14691–14696 (2010).
25. David, L. A. *et al.* Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**, 559–563 (2014).
26. Ley, R. E. *et al.* Obesity alters gut microbial ecology. *Proc. Natl Acad. Sci. USA* **102**, 11070–11075 (2005).
27. Sze, M. A. & Schloss, P. D. Looking for a signal in the noise: revisiting obesity and the microbiome. *mBio* **7**, e01018-16 (2016).
28. Cummings, J. H., Pomare, E. W., Branch, W. J., Naylor, C. P. & Macfarlane, G. T. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* **28**, 1221–1227 (1987).
29. Tan, J. *et al.* The role of short-chain fatty acids in health and disease. *Adv. Immunol.* **121**, 91–119 (2014).
30. Clausen, M. R. & Mortensen, P. B. Kinetic studies on colonocyte metabolism of short chain fatty acids and glucose in ulcerative colitis. *Gut* **37**, 684–689 (1995).
31. Xu, Z. & Knight, R. Dietary effects on human gut microbiome diversity. *Br. J. Nutr.* **113** (Suppl.), S1–S5 (2015).
32. Chassaing, B. *et al.* Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. *Nature* **519**, 92–96 (2015).
33. Suez, J. *et al.* Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature* **514**, 181–186 (2014).
34. Dominguez-Bello, M. G. *et al.* Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc. Natl Acad. Sci. USA* **107**, 11971–11975 (2010).
35. Smith, D. L., Harris, A. D., Johnson, J. A., Silbergeld, E. K. & Morris, J. G. Jr. Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. *Proc. Natl Acad. Sci. USA* **99**, 6434–6439 (2002).
36. Clarke, S. F. *et al.* Exercise and associated dietary extremes impact on gut microbial diversity. *Gut* **63**, 1913–1920 (2014).
37. Ridaura, V. K. *et al.* Gut microbiota from twins discordant for obesity modulate metabolism in mice. *Science* **341**, 1241214 (2013).
38. Goodrich, J. K. *et al.* Human genetics shape the gut microbiome. *Cell* **159**, 789–799 (2014).
39. Xie, H. *et al.* Shotgun metagenomics of 250 adult twins reveals genetic and environmental impacts on the gut microbiome. *Cell Syst.* **3**, 572–584.e3 (2016).
40. Zhang, C. *et al.* Interactions between gut microbiota, host genetics and diet relevant to development of metabolic syndromes in mice. *ISME J.* **4**, 232–241 (2010).
41. Wenzel, U. *et al.* Immune mechanisms in arterial hypertension. *J. Am. Soc. Nephrol.* **27**, 677–686 (2016).
42. Schiffrin, E. L. T lymphocytes: a role in hypertension? *Curr. Opin. Nephrol. Hypertens.* **19**, 181–186 (2010).
43. Ivanov, I. I. *et al.* Specific microbiota direct the differentiation of IL-17-producing T-helper cells in the mucosa of the small intestine. *Cell Host Microbe* **4**, 337–349 (2008).
44. Strauch, U. G. *et al.* Influence of intestinal bacteria on induction of regulatory T cells: lessons from a transfer model of colitis. *Gut* **54**, 1546–1552 (2005).
45. Mazmanian, S. K., Liu, C. H., Tzianabos, A. O. & Kasper, D. L. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* **122**, 107–118 (2005).
46. Gaboriau-Routhiau, V. *et al.* The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity* **31**, 677–689 (2009).
47. Wang, X. *et al.* Fruit and vegetable consumption and mortality from all causes, cardiovascular disease, and cancer: systematic review and dose-response meta-analysis of prospective cohort studies. *BMJ* **349**, g4490 (2014).
48. Miura, K. *et al.* Relation of vegetable, fruit, and meat intake to 7-year blood pressure change in middle-aged men: the Chicago Western Electric Study. *Am. J. Epidemiol.* **159**, 572–580 (2004).
49. Ascherio, A. *et al.* Prospective study of nutritional factors, blood pressure, and hypertension among US women. *Hypertension* **27**, 1065–1072 (1996).
50. Appel, L. J. *et al.* A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *N. Engl. J. Med.* **336**, 1117–1124 (1997).
51. Nissensohn, M., Roman-Vinas, B., Sanchez-Villegas, A., Piscopo, S. & Serra-Majem, L. The effect of the Mediterranean diet on hypertension: a systematic review and meta-analysis. *J. Nutr. Educ. Behav.* **48**, 42–53.e1 (2016).
52. Estruch, R. *et al.* Primary prevention of cardiovascular disease with a Mediterranean diet. *N. Engl. J. Med.* **368**, 1279–1290 (2013).
53. Alonso, A. *et al.* Fruit and vegetable consumption is inversely associated with blood pressure in a Mediterranean population with a high vegetable-fat intake: the Seguimiento Universidad de Navarra (SUN) Study. *Br. J. Nutr.* **92**, 311–319 (2004).
54. Threapleton, D. E. *et al.* Dietary fibre intake and risk of cardiovascular disease: systematic review and meta-analysis. *BMJ* **347**, f6879 (2013).
55. Australian Bureau of Statistics. 4364.0.55.007 – Australian Health Survey: Nutrition First Results – Food and Nutrients, 2011–12. http://www.abs.gov.au/ausstats/abs@.nsf/Lookup/4364.0.55.007main+features12011-12 (2014).
56. King, D. E., Mainous, A. G. III & Lambourne, C. A. Trends in dietary fiber intake in the United States, 1999–2008. *J. Acad. Nutr. Diet.* **112**, 642–648 (2012).
57. Park, Y., Subar, A. F., Hollenbeck, A. & Schatzkin, A. Dietary fiber intake and mortality in the NIH-AARP diet and health study. *Arch. Intern. Med.* **171**, 1061–1068 (2011).
58. Whelton, S. P. *et al.* Effect of dietary fiber intake on blood pressure: a meta-analysis of randomized, controlled clinical trials. *J. Hypertens.* **23**, 475–481 (2005).
59. Streppel, M. T., Arends, L. R., van 't Veer, P., Grobbee, D. E. & Geleijnse, J. M. Dietary fiber and blood pressure: a meta-analysis of randomized placebo-controlled trials. *Arch. Intern. Med.* **165**, 150–156 (2005).
60. Aljuraiban, G. S. *et al.* Total, insoluble and soluble dietary fibre intake in relation to blood pressure: the INTERMAP Study. *Br. J. Nutr.* **114**, 1480–1486 (2015).
61. Lattimer, J. M. & Haub, M. D. Effects of dietary fiber and its components on metabolic health. *Nutrients* **2**, 1266–1289 (2010).
62. Erkkila, A. T. & Lichtenstein, A. H. Fiber and cardiovascular disease risk: how strong is the evidence? *J. Cardiovasc. Nurs.* **21**, 3–8 (2006).
63. Baez, S. & Gordon, H. A. Tone and reactivity of vascular smooth muscle in germfree rat mesentery. *J. Exp. Med.* **134**, 846–856 (1971).
64. Gordon, H. A., Wostmann, B. S. & Bruckner-Kardoss, E. Effects of microbial flora on cardiac output and other elements of blood circulation. *Proc. Soc. Exp. Biol. Med.* **114**, 301–304 (1963).
65. Heneghan, J. B. Response of germfree animals to shock. *J. Med.* **21**, 51–66 (1990).
66. Mell, B. *et al.* Evidence for a link between gut microbiota and hypertension in the Dahl rat. *Physiol. Genomics* **47**, 187–197 (2015).
67. Adnan, S. *et al.* Alterations in the gut microbiota can elicit hypertension in rats. *Physiol. Genomics* **49**, 96–104 (2017).
68. Yang, T. *et al.* Gut dysbiosis is linked to hypertension. *Hypertension* **65**, 1331–1340 (2015).
69. Durgan, D. J. *et al.* Role of the gut microbiome in obstructive sleep apnea-induced hypertension. *Hypertension* **67**, 469–474 (2016).
70. Petriz, B. A. *et al.* Exercise induction of gut microbiota modifications in obese, non-obese and hypertensive rats. *BMC Genomics* **15**, 511 (2014).
71. Zhang-James, Y., Middleton, F. A. & Faraone, S. V. Genetic architecture of Wistar-Kyoto rat and spontaneously hypertensive rat substrains from different sources. *Physiol. Genomics* **45**, 528–538 (2013).
72. Yang, J. Y. *et al.* Gut commensal Bacteroides acidifaciens prevents obesity and improves insulin sensitivity in mice. *Mucosal Immunol.* **10**, 104–116 (2017).
73. Shi, P. *et al.* Brain microglial cytokines in neurogenic hypertension. *Hypertension* **56**, 297–303 (2010).
74. Karbach, S. H. *et al.* Gut microbiota promote angiotensin II-induced arterial hypertension and vascular dysfunction. *J. Am. Heart Assoc.* **5**, e003698 (2016).
75. Santisteban, M. M. *et al.* Hypertension-linked pathophysiological alterations in the gut. *Circ. Res.* **120**, 312–323 (2017).
76. Stewart, D. C. *et al.* Hypertension-linked mechanical changes of rat gut. *Acta Biomater.* **45**, 296–302 (2016).
77. Grassi, G., Mark, A. & Esler, M. The sympathetic nervous system alterations in human hypertension. *Circ. Res.* **116**, 976–990 (2015).
78. Carabotti, M., Scirocco, A., Maselli, M. A. & Severi, C. The gut–brain axis: interactions between enteric microbiota, central and enteric nervous systems. *Ann. Gastroenterol.* **28**, 203–209 (2015).
79. He, F. J. & MacGregor, G. A. A comprehensive review on salt and health and current experience of worldwide salt reduction programmes. *J. Hum. Hypertens.* **23**, 363–384 (2009).
80. Sandle, G. I. Salt and water absorption in the human colon: a modern appraisal. *Gut* **43**, 294–299 (1998).
81. Linz, D. *et al.* Antihypertensive and laxative effects by pharmacological inhibition of sodium-proton-exchanger subtype 3-mediated sodium absorption in the gut. *Hypertension* **60**, 1560–1567 (2012).
82. Afsar, B., Vaziri, N. D., Aslan, G., Tarim, K. & Kanbay, M. Gut hormones and gut microbiota: implications for kidney function and hypertension. *J. Am. Soc. Hypertens.* **10**, 954–961 (2016).
83. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT02133872 (2016).
84. US National Library of Medicine. *ClinicalTrials.gov* https://clinicaltrials.gov/ct2/show/NCT02133885 (2017).
85. Li, J. *et al.* Gut microbiota dysbiosis contributes to the development of hypertension. *Microbiome* **5**, 14 (2017).
86. How, K. Y., Song, K. P. & Chan, K. G. Porphyromonas gingivalis: an overview of periodontopathic pathogen below the gum line. *Front. Microbiol.* **7**, 53 (2016).
87. Valour, F. *et al.* Actinomycosis: etiology, clinical features, diagnosis, treatment, and management. *Infect. Drug Resist.* **7**, 183–197 (2014).
88. Munoz-Price, L. S. *et al.* Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. *Lancet Infect. Dis.* **13**, 785–796 (2013).
89. Hao, Y. *et al.* A nested case-control study of association between metabolome and hypertension risk. *Biomed Res. Int.* **2016**, 7646979 (2016).
90. Daugirdas, J. T. & Nawab, Z. M. Acetate relaxation of isolated vascular smooth muscle. *Kidney Int.* **32**, 39–46 (1987).
91. Nutting, C. W., Islam, S. & Daugirdas, J. T. Vasorelaxant effects of short chain fatty acid salts in rat caudal artery. *Am. J. Physiol.* **261**, H561–H567 (1991).
92. Mortensen, F. V., Nielsen, H., Mulvany, M. J. & Hessov, I. Short chain fatty acids dilate isolated human colonic resistance arteries. *Gut* **31**, 1391–1394 (1990).
93. Pluznick, J. L. *et al.* Olfactory receptor responding to gut microbiota-derived signals plays a role in renin secretion and blood pressure regulation. *Proc. Natl Acad. Sci. USA* **110**, 4410–4415 (2013).
94. Andrade-Oliveira, V. *et al.* Gut bacteria products prevent AKI induced by ischemia-reperfusion. *J. Am. Soc. Nephrol.* **26**, 1877–1888 (2015).

95. Tan, J. K., McKenzie, C., Marino, E., Macia, L. & Mackay, C. R. Metabolite-sensing G protein-coupled receptors-facilitators of diet-related immune regulation. *Annu. Rev. Immunol.* **35**, 371–402 (2017).
96. Macia, L. *et al.* Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflammasome. *Nat. Commun.* **6**, 6734 (2015).
97. Natarajan, N. *et al.* Microbial short chain fatty acid metabolites lower blood pressure via endothelial G-protein coupled receptor 41. *Physiol. Genomics* **48**, 826–834 (2016).
98. Marino, E. *et al.* Gut microbial metabolites limit the frequency of autoimmune T cells and protect against type 1 diabetes. *Nat. Immunol.* **18**, 552–562 (2017).
99. Chai, J. T., Digby, J. E. & Choudhury, R. P. GPR109A and vascular inflammation. *Curr. Atheroscler. Rep.* **15**, 325 (2013).
100. Bechtel, W. *et al.* Methylation determines fibroblast activation and fibrogenesis in the kidney. *Nat. Med.* **16**, 544–550 (2010).
101. Nakagawa, K. *et al.* Salt-sensitive hypertension is associated with dysfunctional Cyp4a10 gene and kidney epithelial sodium channel. *J. Clin. Invest.* **116**, 1696–1702 (2006).
102. Miyamoto, S. *et al.* Cholecystokinin plays a novel protective role in diabetic kidney through anti-inflammatory actions on macrophage: anti-inflammatory effect of cholecystokinin. *Diabetes* **61**, 897–907 (2012).
103. Knoll, R. *et al.* Telethonin deficiency is associated with maladaptation to biomechanical stress in the mammalian heart. *Circ. Res.* **109**, 758–769 (2011).
104. Koskivirta, I. *et al.* Mice with tissue inhibitor of metalloproteinases 4 (Timp4) deletion succumb to induced myocardial infarction but not to cardiac pressure overload. *J. Biol. Chem.* **285**, 24487–24493 (2010).
105. Khachigian, L. M. Early growth response-1 in cardiovascular pathobiology. *Circ. Res.* **98**, 186–191 (2006).
106. Ho, L. C. *et al.* Egr-1 deficiency protects from renal inflammation and fibrosis. *J. Mol. Med. (Berl.)* **94**, 933–942 (2016).
107. Wang, N. P. *et al.* Attenuation of inflammatory response and reduction in infarct size by postconditioning are associated with downregulation of early growth response 1 during reperfusion in rat heart. *Shock* **41**, 346–354 (2014).
108. Wang, Z. *et al.* Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* **472**, 57–63 (2011).
109. Koeth, R. A. *et al.* Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat. Med.* **19**, 576–585 (2013).
110. Li, X. S. *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.* **38**, 814–824 (2017).
111. Ufnal, M. *et al.* Trimethylamine-N-oxide: a carnitine-derived metabolite that prolongs the hypertensive effect of angiotensin II in rats. *Can. J. Cardiol.* **30**, 1700–1705 (2014).
112. Caroff, M. & Karibian, D. Structure of bacterial lipopolysaccharides. *Carbohydr. Res.* **338**, 2431–2447 (2003).
113. Lu, Y. C., Yeh, W. C. & Ohashi, P. S. LPS/TLR4 signal transduction pathway. *Cytokine* **42**, 145–151 (2008).
114. Jaw, J. E. *et al.* Lung exposure to lipopolysaccharide causes atherosclerotic plaque destabilisation. *Eur. Respir. J.* **48**, 205–215 (2016).
115. Serrano, M. *et al.* Serum lipopolysaccharide-binding protein as a marker of atherosclerosis. *Atherosclerosis* **230**, 223–227 (2013).
116. Pastori, D. *et al.* Gut-derived serum lipopolysaccharide is associated with enhanced risk of major adverse cardiovascular events in atrial fibrillation: effect of adherence to Mediterranean diet. *J. Am. Heart Assoc.* **6**, e005784 (2017).
117. Downey, J. S. & Han, J. Cellular activation mechanisms in septic shock. *Front. Biosci.* **3**, d468–d476 (1998).
118. Tanida, M. *et al.* Effects of intraduodenal injection of *Lactobacillus* johnsonii La1 on renal sympathetic nerve activity and blood pressure in urethane-anesthetized rats. *Neurosci. Lett.* **389**, 109–114 (2005).
119. Gomez-Guzman, M. *et al.* Antihypertensive effects of probiotics *Lactobacillus* strains in spontaneously hypertensive rats. *Mol. Nutr. Food Res.* **59**, 2326–2336 (2015).
120. Sipola, M. *et al.* Long-term intake of milk peptides attenuates development of hypertension in spontaneously hypertensive rats. *J. Physiol. Pharmacol.* **52**, 745–754 (2001).
121. Nakamura, Y., Yamamoto, N., Sakai, K. & Takano, T. Antihypertensive effect of sour milk and peptides isolated from it that are inhibitors to angiotensin I-converting enzyme. *J. Dairy Sci.* **78**, 1253–1257 (1995).
122. Nakamura, Y. *et al.* Purification and characterization of angiotensin I-converting enzyme inhibitors from sour milk. *J. Dairy Sci.* **78**, 777–783 (1995).
123. Yamamoto, N., Akino, A. & Takano, T. Antihypertensive effect of the peptides derived from casein by an extracellular proteinase from *Lactobacillus* helveticus CP790. *J. Dairy Sci.* **77**, 917–922 (1994).
124. Dong, J. Y. *et al.* Effect of probiotic fermented milk on blood pressure: a meta-analysis of randomised controlled trials. *Br. J. Nutr.* **110**, 1188–1194 (2013).
125. Khalesi, S., Sun, J., Buys, N. & Jayasinghe, R. Effect of probiotics on blood pressure: a systematic review and meta-analysis of randomized, controlled trials. *Hypertension* **64**, 897–903 (2014).
126. Wallace, B. D. *et al.* Alleviating cancer drug toxicity by inhibiting a bacterial enzyme. *Science* **330**, 831–835 (2010).
127. Haiser, H. J. *et al.* Predicting and manipulating cardiac drug inactivation by the human gut bacterium Eggerthella lenta. *Science* **341**, 295–298 (2013).
128. Achelrod, D., Wenzel, U. & Frey, S. Systematic review and meta-analysis of the prevalence of resistant hypertension in treated hypertensive populations. *Am. J. Hypertens.* **28**, 355–361 (2015).
129. Qi, Y., Aranda, J. M., Rodriguez, V., Raizada, M. K. & Pepine, C. J. Impact of antibiotics on arterial blood pressure in a patient with resistant hypertension — a case report. *Int. J. Cardiol.* **201**, 157–158 (2015).
130. Dominguez-Bello, M. G. *et al.* Partial restoration of the microbiota of cesarean-born infants via vaginal microbial transfer. *Nat. Med.* **22**, 250–253 (2016).
131. Hill, C. J. *et al.* Evolution of gut microbiota composition from birth to 24 weeks in the INFANTMET Cohort. *Microbiome* **5**, 4 (2017).
132. Faith, J. J. *et al.* The long-term stability of the human gut microbiota. *Science* **341**, 1237439 (2013).
133. Blackmore, H. L. & Ozanne, S. E. Maternal diet-induced obesity and offspring cardiovascular health. *J. Dev. Orig. Health Dis.* **4**, 338–347 (2013).
134. Langley-Evans, S. C. Critical differences between two low protein diet protocols in the programming of hypertension in the rat. *Int. J. Food Sci. Nutr.* **51**, 11–17 (2000).
135. Bogdarina, I., Welham, S., King, P. J., Burns, S. P. & Clark, A. J. Epigenetic modification of the renin-angiotensin system in the fetal programming of hypertension. *Circ. Res.* **100**, 520–526 (2007).
136. Harrison, M. & Langley-Evans, S. C. Intergenerational programming of impaired nephrogenesis and hypertension in rats following maternal protein restriction during pregnancy. *Br. J. Nutr.* **101**, 1020–1030 (2009).
137. Geurts, A. M. *et al.* Maternal diet during gestation and lactation modifies the severity of salt-induced hypertension and renal injury in Dahl salt-sensitive rats. *Hypertension* **65**, 447–455 (2015).
138. Blumfield, M. L. *et al.* Lower protein-to-carbohydrate ratio in maternal diet is associated with higher childhood systolic blood pressure up to age four years. *Nutrients* **7**, 3078–3093 (2015).
139. Aaltonen, J. *et al.* Evidence of infant blood pressure programming by maternal nutrition during pregnancy: a prospective randomized controlled intervention study. *J. Pediatr.* **152**, 79–84.e2 (2008).
140. Golubeva, A. V. *et al.* Prenatal stress-induced alterations in major physiological systems correlate with gut microbiota composition in adulthood. *Psychoneuroendocrinology* **60**, 58–74 (2015).
141. Gomez-Arango, L. F. *et al.* Increased systolic and diastolic blood pressure is associated with altered gut microbiota composition and butyrate production in early pregnancy. *Hypertension* **68**, 974–981 (2016).
142. Brantsaeter, A. L. *et al.* Intake of probiotic food and risk of preeclampsia in primiparous women: the Norwegian Mother and Child Cohort Study. *Am. J. Epidemiol.* **174**, 807–815 (2011).
143. Yatsunenko, T. *et al.* Human gut microbiome viewed across age and geography. *Nature* **486**, 222–227 (2012).
144. Li, Y. T., Cai, H. F., Wang, Z. H., Xu, J. & Fang, J. Y. Systematic review with meta-analysis: long-term outcomes of faecal microbiota transplantation for *Clostridium difficile* infection. *Aliment. Pharmacol. Ther.* **43**, 445–457 (2016).
145. Alang, N. & Kelly, C. R. Weight gain after fecal microbiota transplantation. *Open Forum Infect. Dis.* **2**, ofv004 (2015).
146. US Food & Drug Administration. Draft guidance for industry: enforcement policy regarding investigational new drug requirements for use of fecal microbiota for transplantation to treat Clostridium difficile infection not responsive to standard therapies. https://www.fda.gov/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/vaccines/ucm387023.htm (2016).
147. Bryan, N. S., Tribble, G. & Angelov, N. Oral microbiome and nitric oxide: the missing link in the management of blood pressure. *Curr. Hypertens. Rep.* **19**, 33 (2017).
148. Kelly, T. N. *et al.* Gut microbiome associates with lifetime cardiovascular disease risk profile among Bogalusa Heart Study participants. *Circ. Res.* **119**, 956–964 (2016).
149. Amar, J. *et al.* Blood microbiota dysbiosis is associated with the onset of cardiovascular events in a large general population: the D.E.S.I.R. study. *PLoS ONE* **8**, e54461 (2013).

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The authors declare no competing interests.