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SPOTLIGHT REVIEW

The effect of diet on hypertensive pathology: is there a link via gut microbiota-driven immunometabolism?

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Abstract

Over the past decade, the immune system has emerged as an important component in the aetiology of hypertension. There has been a blooming interest in the contribution of the gut microbiota, the microbes that inhabit our small and large intestine, to blood pressure (BP) regulation. The gastrointestinal tract houses the largest number of immune cells in our body, thus, it is no surprise that its microbiota plays an important functional role in the appropriate development of the immune system through a co-ordinated sequence of events leading to immune tolerance of commensal bacteria. Importantly, recent evidence supports that the gut microbiota can protect or promote the development of experimental hypertension and is likely to have a role in human hypertension. One of the major modulators of the gut microbiota is diet: diets that emphasize high intake of fermentable fibre, such as the Mediterranean diet and the Dietary Approaches to Stop Hypertension, promote expansion of protective microbes that release gut metabolites such as short-chain fatty acids, which are immune-, BP-, and cardio-protective, likely acting through G-coupled protein receptors. In contrast, diets lacking fibre or high in salt and fat, such as the Western diet, reduce prevalence of commensal microbial species and support a pathogenic and pro-inflammatory environment, including the release of the pro-atherosclerotic trimethylamine N-oxide. Here, we review the current understanding of the gut microbiota-driven immune dysfunction in both experimental and clinical hypertension, and how these changes may be addressed through dietary interventions.

Keywords Hypertensive • Essential hypertension • Metabolites • Immune dysfunction • Gut microbiota • Metabolism • Tregs • Th17 • GPCRs • GPR41 • GPR43 • GPR109A

This article is part of the Spotlight Issue on Immunometabolism.

1. Introduction

A growing body of evidence has implicated the immune system as an important driver of hypertension.[1,2] Both innate and adaptive components of the immune system drive hypertension via monocytes, T cell activation and inflammatory cytokine production.[2] One of the current hypotheses for the development of hypertension involves an underlying inflammatory state which promotes up-regulated sympathetic activity and alterations to the systemic vasculature and renal function.[2] Consequently, vascular and renal infiltration of immune cells such as leucocytes, coupled with reductions in circulating nitric oxide and increased vascular superoxide production are observed. These cellular events, in addition to increased renal salt and water retention, amount to increases in blood pressure (BP) which may result in collagen deposition, and ultimately lead to tissue injury and fibrosis.

The microbiota is a complex and dynamic network composed of commensal bacteria, viruses, fungi, and archaea. The number of bacterial cells (3.8×10^{13}) is similar to the calculated number of human cells in the average healthy body.[3] Compared to the human genome (~20 000 genes), the human microbiome contains about 450 times the number of genes (~9 million genes).[4] The gastrointestinal tract houses the largest number of immune and microbial cells in our body. There has been a blooming interest in the contribution of the gut microbiota, the microbes that inhabit our small and large intestine, to BP regulation. Gut microbes directly influence physiological function via a number of processes including priming immunological responses, energy and vitamin extraction from food, and susceptibility to pathogenic colonic colonization.[5]

An important role of the gut microbiota is to digest dietary fibre as a source of nutrition and energy for their host. Resistant starch, which is

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made up of dozens of polysaccharides, is a type of fibre considered to be prebiotic; i.e. it escapes digestion in the upper gastrointestinal tract and reaches the large intestine mostly undigested, where it is fermented and metabolized by colonic commensal bacteria as their energy source. This results in the production of gut metabolites called short-chain fatty acids (SCFAs), especially acetate, propionate, and butyrate, which have been the subject of intense investigation due to their clear connection with not only the gut microbiota and epithelium but also the immune system.[6]

The gut epithelium, mucus lining, and tight junction proteins act as a physical barrier to restrict passage of pathogens and unwanted molecules into the systemic circulation. The loss of epithelial integrity, sometimes due to changes in the gut microbiota composition that might, for example, consume mucus instead of fibre, allows unregulated permeability between the lumen and the neighbouring blood vessels.[7] These structural alterations to gut permeability, combined with changes in the prevalence of difference microbes, is termed 'gut dysbiosis', which has been associated with inflammatory diseases that affect the gut.[8] When comparing the microbiome of African children, whose diet was rich in plant polysaccharides, to Italian children, who consumed a Western diet, there was higher abundance of species belonging to the phylum Bacteroidetes, while there were lower levels of the phylum Firmicutes. Since then, the ratio between the prevalence of Firmicutes to Bacteroidetes has been used as an indication of gut dysbiosis, with a high score reflecting higher prevalence of Firmicutes, and, hence, a dysbiotic state. However, not all bacteria from the phylum Firmicutes are detrimental, as some are important for production of SCFAs.

Given the intimate interplay between gut microbiota and the host's immune system, it is critical to understand the link between the two and the role they play in the pathogenesis of hypertension. Here, we review the current understanding of interactions between the gut microbiota, diet, the immune system and hypertension, and propose novel mechanisms that might be involved in the regulation of BP (*Figure 1*).

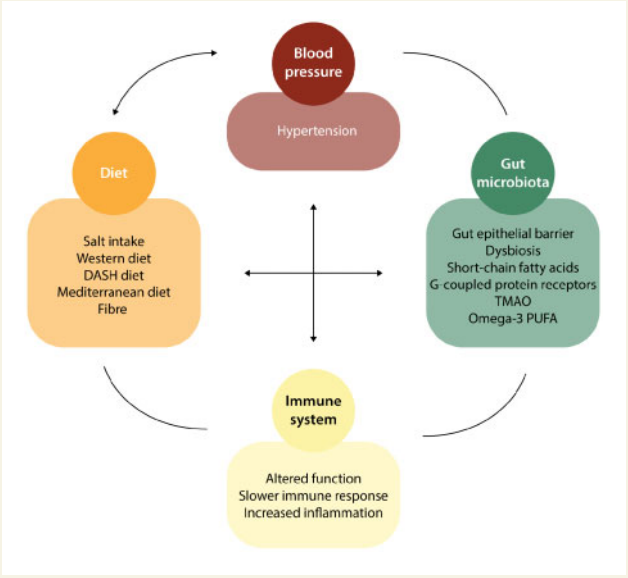

Figure 1 Summary of the review topic. Diet, immune system, and gut microbiota play a role in the pathogenesis of hypertension. Solid lines with arrows represent strong interaction, solid lines represent weaker or emerging interactions.

2. Hypertension and the immune system

A growing body of evidence from the past decade supports that the immune system is involved in the regulation of BP. In a seminal paper, Guzik and colleagues[9] demonstrated that recombinase activating gene-1 deficient (RAG-1$^{-/-}$) mice, which are deficient in T and B cells, exhibited blunted hypertensive responses to angiotensin II (Ang II)- and deoxycorticosterone acetate (DOCA) salt-induced hypertension. However, when T but not B cells were adoptively transferred into the RAG-1$^{-/-}$ mice, hypertension was restored, observed by an increase in BP, endothelial dysfunction and vascular reactive oxygen species production.[9] Pharmacological inhibition of T cell activation not only prevents hypertension, but also reverses it in mice.[10] Experimental hypertension is associated with increases in circulating T cells expressing surface markers CD69, CD44^{high}, and CCR5, which represent an activated T cell phenotype.[9–11] This is also associated with increased infiltration of leucocytes (CD45^{+}) and T cells (CD3^{+}) into BP-controlling organs such as the kidney and the aorta.[10–13]

Whilst it is understood that T cells are predominantly localized to the perivascular fat surrounding the aorta, it is not known how their presence promotes elevated BP. It has been proposed that vascular infiltrating T cells are a source of chemokines and cytokines, which drive the inflammatory response in part by inducing the recruitment and maturation of other immune cells.[2] CD4+ T-helper (Th) cells are the predominant source of T cell-derived cytokines, however, cytotoxic CD8+ T cells have also emerged as significant players.[2] Indeed, untreated hypertensive subjects have higher levels of immunosenescent CD8+ T cells, which are pro-inflammatory.[14] Th cells can be categorized into Th1, Th2, and Th17 subsets based on the types of cytokines released and these can govern the subsequent immune response. Th1 and Th17 subsets release pro-inflammatory cytokines such as interferon-γ (IFN-γ), tumour necrosis factor-alpha (TNFα), and interleukin (IL)-17A, which are predominant in models of hypertension.[15,16] Th17 subsets have been implicated in hypertension-induced inflammation, where Ang II-induced hypertension was not sustained after 1 week in IL-17A$^{-/-}$ mice relative to wild-type mice.[15] In addition, Ang II-induced aortic T cell infiltration and subsequent increases in vascular oxidative stress and dysfunction were not observed in IL-17A$^{-/-}$ mice compared with wild-type mice, highlighting the inflammatory phenotype of Th17 cells.[15] Indeed, hypertensive subjects have higher numbers of Th17 cells and levels of IL-17A compared with normotensive subjects.[17,18] A further subset of T cells that opposes the inflammatory effects of T-effector lymphocytes and protects against autoimmunity are T regulatory (Treg) cells, which are characterized by the surface marker Forkhead box P3. Treg cells carry out their protective effects through the release of anti-inflammatory cytokines such as IL-10 and transforming growth factor-β (TGFβ). Endothelial dysfunction and superoxide production are only observed in the carotid arteries of IL-10$^{-/-}$ mice following Ang II incubation, suggesting a protective role of Treg cells.[19] Indeed, mice that received adoptive transfers of Treg cells had a blunt response to Ang II and vascular injury, with an increase in Treg cells accumulation in the renal cortex.[20] Together, these data support a role for Th1 and Th17 T cells in the development and maintenance of hypertension, while Treg cells offer protection against increased BP. More recent evidence also support a role for cells of the innate immune system, including macrophages, monocytes, and dendritic cells, in the development of hypertension

(reviewed in Ref.[2]). Similarly to Treg cells, myeloid-derived suppressor cells are protective against hypertension in the context of Ang II.[21]

3. The gut microbiota plays an essential role in the development of the immune system

Several studies have highlighted how the gut microbiota is essential for shaping the immediate local gut environment by curbing the growth of opportunistic enteric pathogens. The modulation of the immune system by gut microbiota is not, however, limited to the gut itself. Strikingly, gut microbes shape systemic immune responses through direct interaction with immune cells or the production of metabolites that travel to distal sites and activate or inhibit the inflammatory cascade.

3.1 Gut microbiota and the development of gut immunity

Under normal physiological conditions, the commensal bacteria shape gut immunity and maintain homeostasis. This begins within the intestinal lumen, where constant secretion of mucins (such as the mucin protein MUC2) and antimicrobial peptides (such as REG3γ and REG3β) secreted by Paneth cells are maintained by exposure to microbial peptides.[22] This provides a chemical and physical barrier preventing penetrance and adherence of pathobionts to gut epithelial cells. Breakdown or inability to maintain a barrier, for example in the case of lack of dietary fibre or *Muc2* deficiency, results in the adherence of pathobionts, the breakdown of the gut epithelial barrier and subsequent immune activation.[22,23] Gnotobiotic (germ-free) mice are a powerful tool to study the effects of microbial communities in health and disease because they are bred in a microbe free environment.[24] Comparison of colonic permeability in gnotobiotic and conventionally housed mice revealed that colonization of gut microbes is necessary for the maturation of the intestinal barrier and the induction of transient IL-18 expression,[25] believed to initiate tight junctional protein expression and, thus, the establishment of the physical gut barrier.[25,26] Moreover, thickness and biochemical nature of the mucus layer support microbial abundance, which is associated with more mucin production.[22] Intestinal epithelial cells have a dual function as both a physical barrier and microbial sensor through expression of various receptors such as Toll-like receptors (TLRs), nucleotide oligomerization domain (NOD) and peroxisome proliferator-activated receptor-γ (PPAR-γ).[27,28] The length of the gastrointestinal tract is also lined by a specialized immune structure called gut-associated lymphoid organs that house a full complement of immune cells including resident naive T cells and B cells.[27] The primary function of these structures is to mediate the interaction between the surveying immune cells and potential immunogenic peptides or pathogens.[29] However, without the full complement of gut microbiota, these intestinal T and B cells fail to develop or differentiate appropriately. Commensal microbiota, such as those belonging to Bacteroidetes, have been implicated in the development and maturation of a specific subset Treg cells that express RORγ$^+$, a cell population believed to control intestinal inflammation through modulation of colonic Th17 differentiation and activity.[30] Furthermore, the SCFA butyrate alone induced the differentiation of Treg cells even in Th1 and Th17 polarizing conditions.[31] Butyrate also inhibits histone deacetylase 3 (HDAC3) in monocytes during macrophage differentiation, inducing metabolic changes that enhance their antimicrobial function.[32] Together these data support the differentiation and activity of colonic immune cells is strongly linked to the composition of gut microbiota.

3.2 Gut microbiota and the development of systemic immunity

The immune-modulatory effect of gut microbes extends beyond the gut. Monocolonization of gnotobiotic mice with *Bacteroides fragilis*, but not with a mutant strain, was sufficient to restore splenic T and B cell numbers to levels comparable with conventionally housed mice and promote lymphoid organogenesis.[33] Furthermore, the presence of TLRs at the gut epithelial barrier modulate the intestinal immune response and prevent overstimulation of the immune system. Colonization with *B. fragilis* exposes polysaccharide A (PSA), activating TLR2 and favouring a cascade of anti-inflammatory events.[34] This leads to the suppression of pro-inflammatory IL-17 production, induces Treg cells and increases tolerance to commensal microbes.[34] Purified PSA alone could increase CD4+ T cell numbers and Th1 cytokines, thus, demonstrating a role for the gut microbiota in the immune maturation at extra-intestinal sites.[30,31,33]

3.3 Importance of the anaerobic environment for immune regulation

Low availability of oxygen in the gastrointestinal tract favours the growth of strictly anaerobic microbial species. As gut microbes and the host have co-evolved, host metabolic processes also contribute to keeping the colonic environment devoid of oxygen.[35] The PPAR-γ signalling pathway facilitates this process by supporting metabolism, transcriptional activation, and immune function.[28] Microbial metabolites, such as the SCFA butyrate, can initiate PPAR-γ-mediated reduction of oxygen and nitrate, thus, maintaining an anaerobic environment.[35] However, loss of PPAR-γ signalling or depletion of butyrate-producing bacteria increase oxygen availability, reducing Tregs and leading to gut dysbiosis.[35]

3.4 Gut microbiota and the immune system: a double-edged sword

NOD2 is an important intracellular innate immune receptor that contributes to intestinal homeostasis by promoting the secretion of mucus and bactericidal proteins such as defensins, which inhibit microbial growth. NOD2 knockout (KO) mice have distinct microbial composition when compared with wild-type mice, with significant expansion of *Rikenella and Alistipes* (*Rikenellaceae*) and a reduction of the proportion of the phyla Firmicutes to Bacteroidetes, and lower diversity.[36,37] NOD2 KO mice have increased CD4+ T cells, interferon-gamma (IFNγ) and TNFα activity, a phenotype rescued with use of anti-CD4+ therapy.[37] Moreover, oral antibiotic treatment decreases the number of CD4+ T cells and reduced inflammatory markers IFNγ and TNFα within the ileum, demonstrating that the intestinal inflammatory phenotype and associated dysfunction seen in NOD2 KO mice is driven by the presence of (certain) gut microbiota.[37] The immune dysfunction in these mice also extends to their ability to clear pathogen infection and is necessary for the development of normal gut microbial colonization.[36] Mutations in the human *NOD2* gene are associated with increased susceptibility to the development of several inflammatory bowel diseases such as Crohn's disease.[38,39] Combined, these findings stress the importance of NOD2 in the maintenance of intestinal homeostasis and microbial composition.

The absence of gut microbiota colonization has a profound impact on the development of the immune system and proper cardiovascular development: gnotobiotic rats have significantly smaller hearts and reduced cardiac output when compared with conventionally raised mice.[40,41]

Gnotobiotic mice also lack mature gut-associated lymphoid organs, secretory defences such as IgA and antimicrobial peptides such as Reg3γ.[42] Following colonization, the intestinal immune system undergoes a rapid transformation that involves the stimulation of both pro- and anti-inflammatory processes, providing evidence for a synchronized process directed by commensal microbiota.[43] Gut microbiota composition is also implicated in controlling the organization of immune cells within the intestinal tissue. Two independent studies were able to link the colonization of segmented filamentous bacteria with increased aggregation of Th17 cells within the lamina propria.[43,44] Thus, the gut microbiota is essential for the priming of the immune system but the immune system is also important for gut homeostasis. The interplay between the immune system and the gut microbiota is undeniable, particularly when considering the mountain of evidence showing immunosuppressive and pro-inflammatory phenotypes observed as a consequence of the lack of microbial colonization or absence of key immune receptors.

4. Gut microbiota and BP

With the advent of high-throughput sequencing, it is possible to determine the bacterial component of gut microbiota by sequencing the 16S rRNA gene, while the whole microbiome can only be assessed by shot-gun metagenomics sequencing. Inter-individual differences in the composition of microbial species are high and frequently reported in studies. Important features of these differences arising from 16S sequencing include α-diversity, which shows the number of species within a sample, and β-diversity, which shows types and prevalence of species between individuals, both of which can be modulated by the host's environment.[45]

4.1 Gut dysbiosis is present in experimental hypertension

Compared with their respective normotensive strains and models, all experimental models of hypertension studied to date, including the spontaneously hypertensive rat (SHR), Dahl salt-sensitive (SS), Ang II and DOCA-salt, have an altered gut microbiota.[46–49] We have reviewed the role of the gut microbiota in experimental hypertension in detail elsewhere,[50] so here we will focus in the findings relevant to immune regulation and inflammation. Adult, but not pre-hypertensive, SHR had a two-fold increase in fluorescein isothiocyanate-dextran in the plasma (used to measure intestinal permeability). However, both pre-hypertensive and hypertensive SHR have lower levels of Goblet cells (which produce mucus) and gut junctional proteins including occludin (Ocln), tight junction protein (Tjp1), and claudin 4 (Cldn4) when compared with age-matched WKY rats, supporting that gut epithelial dysfunction is present before hypertension is established.[51] This increased gut permeability was consistent with findings in the Ang II model.[51] The small and large intestines of the SHR have lower blood flow, but higher collagen deposition and levels of mRNA of inflammatory cells (*Cd68* and *Cd3*), cytokines and their receptors compared with controls.[51] Treatment with the angiotensin-converting enzyme inhibitor captopril decreased gut permeability and fibrosis in the SHR,[51] and the anti-inflammatory antibiotic minocycline decreased proportion of the phyla Firmicutes to Bacteroidetes in Ang II mice.[46]

4.2 Gut microbiota is involved in BP regulation

Gnotobiotic animals have been essential for the determination of the causal link between hypertensive gut microbes and increased BP. A seminal study in the field transplanted faecal samples from one normotensive and two untreated hypertensive subjects into gnotobiotic mice (*Figure* 2A), which developed a similar gut microbiome to their donors after 10 weeks.[52] Strikingly, gnotobiotic mice that received a faecal transplant from hypertensive donors developed reduced microbial diversity, altered microbiome composition (including reduced levels of *Roseburia*) and higher systolic and diastolic BP compared with mice that were transplanted with faeces from the normotensive donor (*Figure* 2A).[52] Moreover, compared with conventional mice, gnotobiotic mice did not develop higher BP when challenged with Ang II (*Figure* 2B) and were protected against vascular dysfunction.[53] In the absence of microbiota, Ang II mice have reduced vascular superoxide formation and infiltration of myelomonocytic cells, as well as a down-regulation of inflammatory genes.[53] Combined, these findings support that (i) the hypertensive gut microbiota leads to higher BP, and (ii) the gut microbiota is essential for the development of hypertension and exaggerated immune and inflammatory responses in the Ang II model. Thus, the gut microbiota may be, at least partially, responsible for the development of hypertension and the activation of the immune system and inflammatory response observed in hypertension (*Figure* 3).

4.3 Essential hypertensive subjects have an altered gut microbiota

Changes in α- (particularly Shannon index) and β-diversity of the gut microbiome have been consistently associated with essential hypertension in independent studies (summarized in Table 1). The main limitations to the current studies are: (i) all of them measured office BP instead of 24 h ambulatory BP (AMBP), thus, a high percentage of participants could have white coat or masked hypertension; (ii) in most studies patients were treated, and in some treated hypertensive subjects with controlled BP were classified as normotensive controls; (iii) most studies included small samples sizes in different populations, and some studies are subsets of the same cohort (and, thus, found similar results); (iv) the largest study included self-reported hypertension, which will most likely underestimate prevalence of high BP; and (v) all studies analysed faecal samples which might not necessarily reflect the composition of the microbiome in the caecum. Combined, however, these studies still identified some common bacterial species and mechanisms that are dysregulated in essential hypertension. This includes higher prevalence of *Klebsiella* and *Parabacteroides*, and lower prevalence of *Faecalibacterium* and *Roseburia* in faecal samples of hypertensive subjects (*Figure* 2B). This was usually accompanied by an increase in lipopolysaccharide (LPS) or LPS-producing bacteria, which is considered pro-inflammatory, and a decrease in SCFAs or SCFA-producing bacteria, which are anti-inflammatory. Indeed, *Faecalibacterium* and *Roseburia* are usually associated with gut homeostasis and production of the SCFA butyrate.[52] Some of these studies also reported that the gut microbiota and its metabolites were able to predict existence of hypertension,[18,52,54] which may be promising for early identification of those at risk.

The relationship between the gut microbiota, inflammation and BP may be relevant when considering sex differences in the development of hypertension. Women's BP and arterial stiffness is lower than men's during reproductive years, but it increases sharply following menopause, and surpasses that of men.[55,56] There are also substantial differences in the immune system between men and women.[57] Indeed, a large study of female twins established a relationship between intestinal microbial diversity and arterial stiffness as measured by pulse wave velocity,[58] which remained significant after adjusting for C-reactive protein level as a single marker of inflammation.

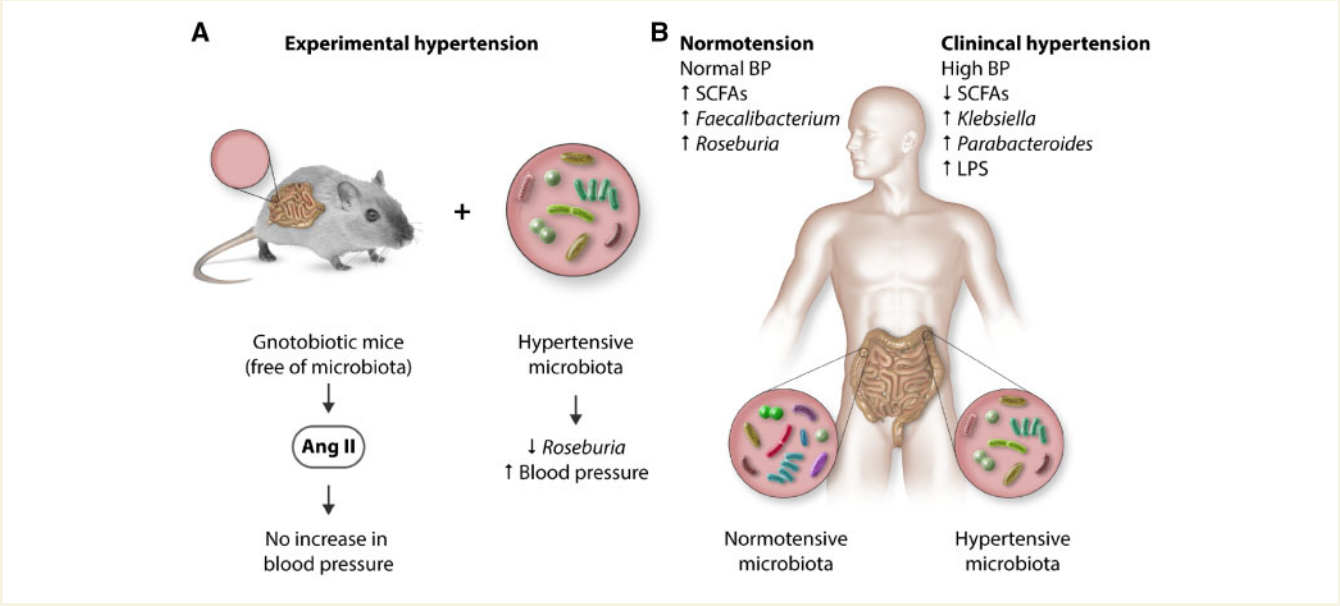

Figure 2 Differences in microbial composition and metabolites of (A) Studies using gnotobiotic (germ-free) mice have been fundamental to determine how gut microbiota is involved in BP regulation. Gnotobiotic mice that received a faecal transplant from hypertensive donors developed significantly higher systolic and diastolic BP than mice that received a faecal transplant from a normotensive subject.[52] Compared with conventional C57BL/6 mice gnotobiotic C57BL/6 mice are protected from developing angiotensin II (Ang II)-induced hypertension.[53] (B) clinical normotension and essential hypertension (summarized from Table 1).

5. Gut-derived metabolites that lower BP and their pathways

5.1 SCFAs: key for gut homeostasis and links to immune regulation

Humans only produce about a dozen enzymes to digest starch, while the gut microbiome contains more than 1600 enzymes to ferment and digest these complex starches.[23] Certain microbes of the phyla Bacteriodetes, Firmicutes, and Actinobacteria are the main fibrolytic microbes.[59] These include the species *Bifidobacterium adolescentis*, *Eubacterium rectale*, *Eubacterium hallii*, *Faecalibacterium prausnitzii*, and *Ruminococcus bromii*, which are able to synthesize the necessary enzymes to breakdown dietary fibre and produce SCFAs.[60] They can alter the local colonic environment by changing the composition of mucus, acting as an energy source for intestinal epithelial cells and entering the systemic circulation to initiate signalling cascade in distal organs, as we have recently shown to happen in hypertension (discussed in detail below).[60] The beneficial role of SCFAs in inflammatory diseases may be explained, at least in part, by the direct effect of SCFAs on cells of the immune system, particularly Tregs and inflammatory cells such as neutrophils.[61,62]

5.2 Dietary interventions modify gut microbes and the immune response in experimental hypertension

SCFAs are essential for gut homeostasis, but their absence or lack of SCFA-sensing mechanisms might be involved in the development of hypertension. Indeed, the abundance of acetate- and butyrate-producing bacteria are blunted in both experimental models of hypertension and in human hypertensive subjects.[18,46,49,54] Both fibre and acetate (supplemented in the water) modulated the gut microbiota and lowered BP in the DOCA-salt mouse model, protecting the mice from the development of cardiac hypertrophy and heart failure.[49] Acetate also protected against the development of obstructive sleep apnoea-induced hypertension in a model fed a high fat diet.[63] In this model, fibre also prevented the activation of microglia as well as increased the prevalence of Treg cells in the brain.[63] This is relevant as the SHR was reported to have increased sympathetic neuronal communication between the gut and the hypothalamic paraventricular nucleus,[51] and perhaps fibre or SCFAs might be able to decrease it. Indeed, intracerabroventricular administration of chemically modified tetracycline-3, an anti-inflammatory agent, abrogated Ang II-induced microglia proliferation and activation, decreased BP and cardiac hypertrophy, and improved gut dysbiosis,[64] highlighting the possible gut-central nervous system-BP axis. Moreover, a diet high in fibre or intake of acetate produced transcriptome-wide changes in the kidney and the heart, supporting a gut-cardiorenal axis.[49] Down-regulation of pathways relevant to this review include pro-inflammatory renal IL-1 signalling and cardiac TGFβ signalling.[49] Combined, these findings suggest that the central nervous system might increase sympathetic activation in the gut, but it is also possible that the gut microbiota regulates sympathetic activation through the immune system.

Another SCFA that has received increasing interest in hypertension is butyrate. When delivered intraperitoneally or intramedullary, butyrate reduced BP, cardiac hypertrophy, renal injury and fibrosis, and improved cardiac function.[18,65] It also decreased levels of inflammatory markers (IL-6 and TNFα), and improved intestinal hypoxia.[65] In the gut, butyrate modulated the microbial composition and increased species known to restore gut function and reduce inflammation.[18] One of the possible mechanisms is through the attenuation of Ang II-induced expression of soluble (pro)renin receptor.[65]

Similarly, acute infusion[66] or chronic supplementation[67] with propionate has hypotensive effects in mouse models. In the Ang II model, one

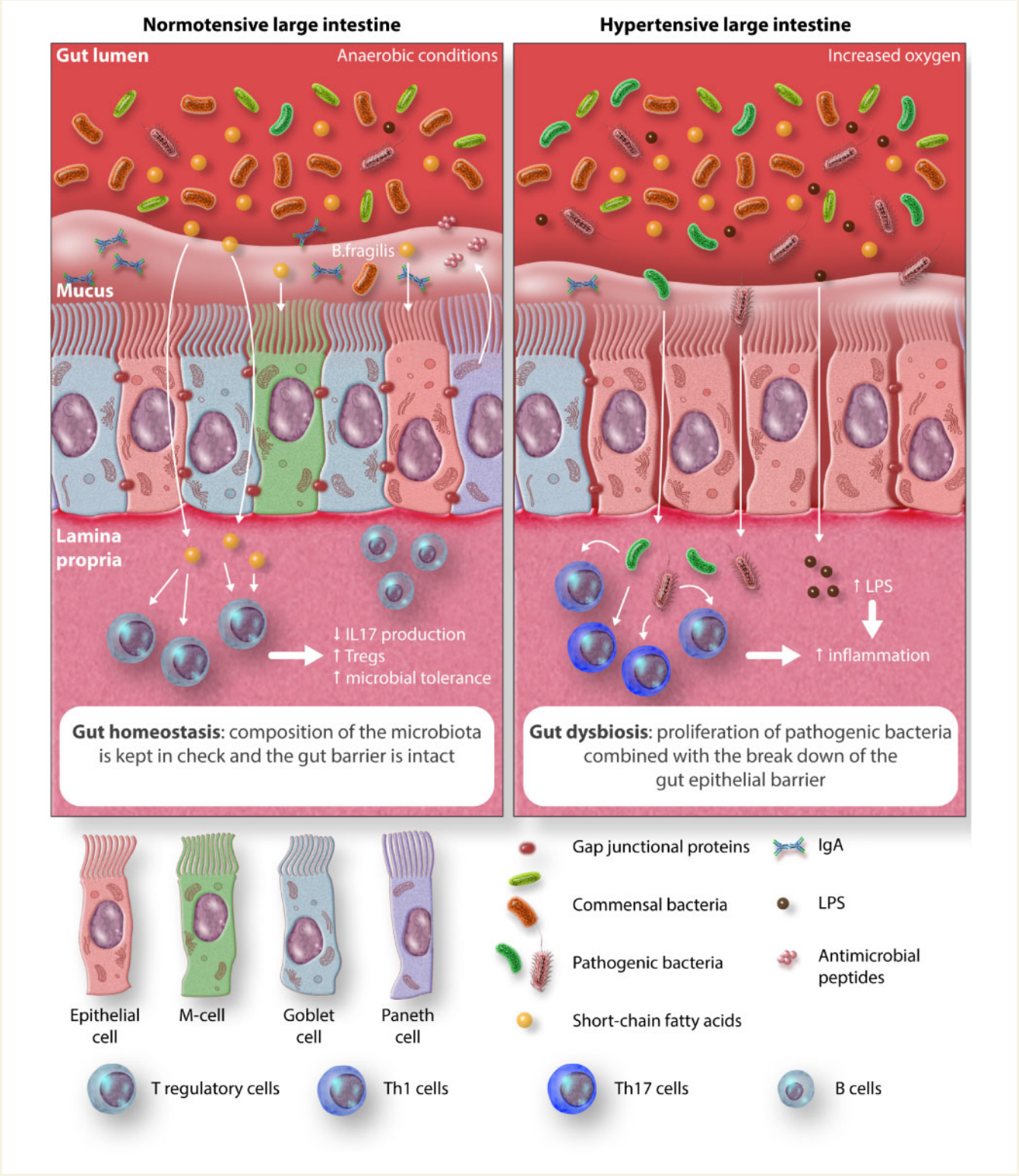

Figure 3 Functional and structural changes that occur in the epithelial barrier during normal and high BP in the large intestine. During normotension, the gut barrier is intact, regulated by a complex cross-talk between specialized cells of the epithelium including M-cells, goblet cells, Paneth cells and epithelial cells, immune cells and gut microbiota through secretion of antimicrobial peptides, IgA and mucin proteins. In hypertension, the gut barrier integrity is loss due to disturbances in microbial composition, allowing degradation of mucins, passage and adherence of pathobionts and pro-inflammatory LPS and subsequent activation of resident immune cells initiating an inflammatory cascade.

Table 1 Main findings related to gut microbiota and essential hypertension

Reference	Cohort size	Cohort background	BP-lowering medication	How BP was measured	Methodology	Gut microbiota changes in hypertension	Immune system
Yang *et al.*[46,a]	10 controls and 7 patients	American	Yes (including controls)	Office BP	16S	↓ **α-diversity** (Chao richness and Shannon diversity) and **different β-diversity**	N/A
Li *et al.*[52]	41 controls, 56 pre-hypertensives, 99 hypertensives	Chinese	No	Office BP	Metagenomics	↓ **α-diversity** (Shannon diversity), ↑ *Prevotella*, ***Klebsiella***, *Porphyromonas* and *Actinomyces*, ↓ ***Faecalibacterium***, *Oscillibacter*, ***Roseburia***, *Bifidobacterium*, *Coprococcus* and *Butyrivibrio*	↑ **genes for LPS production**
Yan *et al.*[54]	60 controls, 60 hypertensives	Chinese	N/A	Office BP	Metagenomics	↓ gene count and **α-diversity** (Shannon diversity), ↑ ***Klebsiella***, *Clostridium*, *Streptococcus*, ***Parabacteroides***, *Eggerthella* and *Salmonella*, ↓ ***Roseburia***, ***Faecalibacterium*** and *Synergistetes*	↑ **genes for LPS and** TMA production, ↓ **SCFA-producing enzymes**
Kim *et al.*[18,a]	18 controls and 22 patients	Caucasians and African Americans	Yes (including controls)	Office BP	Metagenomics	**Different β-diversity,** ↓ *E. rectale*, ↑ correlation with *Ruminococcus torques*, ***Parabacteroides*** *johnsonii*, ***Klebsiella* spp.**, *Anaerotruncus* spp., *Eubacterium siraeum*, *Alistipes indistinctus*, *Alistipes finegoldii*, ↓ correlation with *Bacteroides thetaiotaomicron*, *Paraprevotella* spp.	↑ **LPS** and Th17 cells, ↓ **butyrate-producing bacterium**,
Walejko *et al.*[116,a]	30 controls and 22 patients	Caucasians and African Americans	N/A	N/A	Metagenomics	**Shift of β-diversity** according to BP and race	N/A
Jackson *et al.*[117]	1790 controls and 756 patients	British	Yes (self-reported)	Self-reported	16S	↓ **α-diversity** (Shannon index)	N/A

Highlighted in bold microbes or mechanisms that were identified by more than one study.
BP, blood pressure; LPS, lipopolysaccharide; N/A, information not available; SCFA, short-chain fatty acids.
[a]Indicates studies that analysed increasing numbers of the same cohort.

of the possible mechanisms of chronic propionate supplementation is an expansion of splenic Treg cell population.[67] This was proposed by the use of an anti-CD25 antibody, which depletes Treg cells, in combination with propionate treatment. However, depletion of Treg cells alone might have cancelled the protective effect offered by propionate, as transfer of Treg cells from wild-type mice lower BP in the Ang II model.[20]

In conclusion, mechanisms involved in SCFA cardio-protection are complex. A comprehensive study comparing the effect of the three main SCFAs is needed, in conjunction with determination of a wide array of mechanisms involved that engulf the complexity of hypertension (i.e. immune, sodium/potassium, vascular, sympathetic, etc.).

5.3 G-coupled protein receptors: the link between diet, gut microbes, and immune system in hypertension?

Currently there are a dozen 'metabolite-sensing' G-coupled protein receptors (GPCRs), and it is likely that others will emerge. Metabolite-sensing GPCRs bind metabolites such as SCFAs from common foodstuffs (reviewed in Ref.[6]). All metabolite-sensing GPCRs appear to be important for gut homeostasis, metabolism or the regulation of immune responses.[6] Perhaps insufficient signalling through these receptors may lead to compromise of the gut epithelial barrier, which allows the passage of substances such as LPSs to blood and tissues, increasing low-grade chronic inflammation (such as observed in essential hypertension in Ref.18). Many of these receptors play a dual role in both gut homeostasis and immune regulation, as well as host metabolism.[6,68] There are at least three metabolite-sensing GPCRs that bind SCFAs, namely GPR41, GPR43, and GPR109A, and these control gut homeostasis, host metabolism, and immune responses. These three GPCRs are highly expressed in immune tissues such as the appendix, the spleen, and bone marrow.[69] Another receptor, called olfactory receptor *Olfr78*, was identified as a GPCR for acetate and propionate, and is localized in the smooth muscle cells of arteries, autonomic nerves in the heart and gut, and the renal juxtaglomerular complex.[66] While studies are yet to show a role of GPR43 and GPR109A in BP regulation and cardiovascular disease (CVD), Gpr41 KO mice have higher systolic BP and, as they aged, thickening of the aorta and higher collagen deposition, a sign of arterial stifenning.[70] Propionate, however, may have opposite physiological effects on BP through *Olfr78* and *Gpr41*: propionate can increase the release of renal renin through *Olfr78*,[66] while it can cause a hypotensive effect which is dependent on *Gpr41*.[70]

5.4 Other relevant metabolites: trimethylamine N-oxide and long-chain fatty acids

While a large body of evidence to date has focused on SCFAs as the main gut metabolites involved in hypertension, other metabolites and dietary nutrients should not be ignored. By far the microbial-derived metabolite trimethylamine N-oxide (TMAO) has garnered the most traction as a predictor of poor cardiovascular health and a potential target for therapy. Eggs, dairy products and red meat contain choline and L-carnitine, which are metabolised by gut microbiota into an intermediate compound, called trimethylamine (TMA).[71,72] In the liver, TMA is rapidly is metabolised into TMAO by the enzyme flavin monooxygenase 3 (FMO3).[71] In animal models, TMAO up-regulated macrophage scavenger receptors and promoted atherosclerosis.[71] Similarly, in humans increased plasma concentration of TMAO has been consistently associated with risk of cardiovascular events and mortality.[71–73] Non-lethal inhibition of microbial production of TMA using 3, 3-dimethyl-1-butanol (DMB)[74] or targeting TMA-generating enzyme pair choline trimethylamine-lyase C and D (CutC/D)[75] reduced the production of TMAO, and rescued atherosclerotic lesion and formation of thrombus, respectively. The effect of DMB and CutC/D has not been studied in the context of high BP. In experimental hypertension, SHR were found to have significantly elevated plasma TMA levels when compared to normotensive WKY rats.[76] Treatment with the antihypertensive agent enalapril markedly reduced the TMA levels,[76] but it is unclear if this association was causal.

Long-chain polyunsaturated fatty acids (PUFAs), such as omega-3 (also referred to n-3), are found predominately in fish and include predominantly eicosapentaenoic acid and docosahexanoic acid (DHA). Omega-3 PUFAs have anti-inflammatory properties inhibiting the activation of the NLRP3 inflammasome,[77] acting through the GPR120 receptor.[78] A meta-analysis containing almost 78 thousand participants from large randomised trials did not report a decrease in the risk of fatal coronary heart disease, nonfatal myocardial infarction, stroke or major vascular events, during a mean period of 4.4 years.[79] However, in 29 high-risk hypertensive patients, omega-3 was able to lower pulse wave velocity and increase flow-mediated dilation, suggesting it might improve vascular ageing.[80] Dosage, for example, might account for the difference in the findings. Moreover, in a randomised cross-over trial involving 22 healthy volunteers, omega-3 PUFA did not result in changes in α- or β-diversity, but it increased butyrate-producing bacteria including *Bifidobacterium*, *Roseburia* and *Lactobacillus*.[81] In 876 middle-age and elderly females from the TwinsUK cohort, DHA and total omega-3 levels had a small correlation with α-diversity (Shannon index) and bacteria from the *Lachnospiraceae* family.[82]

6. Nutritional interventions: Diet strongly modulates BP and gut microbiota

Short- and long-term dietary choices are the most important modifiers of the balance between pathogenic and beneficial intestinal microbiota.[83,84] Due to the connection between diet and BP, we discuss below the impact of the pro-hypertensinogenic diets, such as the Western diet, and anti-hypertensinogenic diets, such as the Mediterranean and the Dietary Approaches to Stop Hypertension (DASH) diets, on the gut microbiome, inflammation and BP levels.

6.1 The Western diet is associated with increased BP

Dietary factors that modify microbiome composition include presence of emulsifiers[85] and artificial sweeteners,[86] intake of high fat and low fibre[87] and high salt,[88,89] and direct or indirect use of antibiotics.[90] All of these are characteristic of the Western diet. Increased BP is usually associated with poor dietary choices, which are also characteristics of the Western diet, including high intake of saturated fats, refined carbohydrates and sodium.[91] Perhaps the greatest impact of the Western diet is observed on the immune system, with significant increase in inflammation.[92,93] Compared to a plant-rich diet, the Western diet is strongly associated with elevated plasma acute phase proteins such as C-reactive protein, indicative of systemic inflammation.[94] Even short-term exposure to a Western diet primes innate immune cells for an inflammatory

response: as little as 4-weeks of a Western diet induced functional, transcriptional and epigenetic reprograming of mouse monocyte precursor cells.[95] A Western-style diet was also positively correlated with low-grade inflammation in a large cross-sectional study.[96] Moreover, lower dietary inflammatory index was associated with decreased incidence of hypertension.[96] Together, these data support the notion that eating a pro-inflammatory diet, such as the Western diet, is likely to support the development of hypertension.

6.2 Salt and hypertension

Sodium consumption is considered an independent risk factor for the development of hypertension and other CVD. A recent (and controversial) prospective study in 18 countries including more than 80, 000 subjects followed-up for a median of 8.1 years found that only intakes higher than 5 g of sodium per day were associated with increased risk of CVD.[97] Although the method to determine sodium intake used in this study (i.e., measured in a once-off urine sample) might account for the lack of association in those consuming less than 5 g of sodium per day, there was a 2.86 mmHg increase in systolic BP per 1 g of sodium intake.[97]

It is likely that the gut microbiota is involved in salt-sensitivity in hypertension since the colon is an important site for sodium absorption. Excessive salt intake also significantly alters the gut microbiome of Dahl-SS rats when compared to that of animals on a control diet.[88] High salt diet contributes to the expansion of several tax including *Corynebacteriaceae*, previously linked to chronic kidney disease,[88] as well as a decrease in *Lactobacillus* species, leading to the exacerbation of intestinal inflammation and colitis.[98] In a high profile study, salt intake immediately decreased the prevalence of rodent-specific bacteria *Lactobacillus murinus* and increased the number of pro-inflammatory splenic Th17 cells.[89] Daily treatment with *L. murinus* as a probiotic was able to reduce Th17 cells, as well as ameliorated BP.[89] Most of the *Lactobacillus* spp. (originally present in ~40% of subjects) was lost with a 14 day salt challenge in a small cohort of 12 subjects.[89] Some strains of *Lactobacillus* spp. help us digest lactose present in milk, but they are usually absent in adults. Given the heterogeneity of BP responses to salt it will be important to determine the relative abundances of *Lactobacillus* spp. in large cohorts of essential hypertensive subjects and particularly in salt-sensitive individuals. Salt intake and decreases in *Lactobacillus* spp. will likely represent just one of several mechanisms that disrupt gut homeostasis, increase Th17 cell numbers and cause hypertension. Regardless, salt intake and its effect on the gut microbiome represent an additional lifestyle factor that may explain salt-sensitive hypertension.

6.3 Mediterranean and DASH diets lower BP

In contrast to the Western diet, a reduction in BP in hypertensive subjects has been observed with the use of both the DASH and Mediterranean diets, which value low intake of dietary fat and sodium and high intake of fibre.[99,100] The findings from these studies have led to investigations of several micronutrients, including potassium, nitric oxide and, highly relevant to the gut microbiota, fibre, to BP regulation. This is in accordance with epidemiological studies that show lower rates of cardiovascular mortality[101] and reduced BP[102,103] in populations with a high dietary intake of fruit and vegetables. Even combined with a diet high in fat, a diet rich in fruit and vegetables is inversely associated with BP.[104]

The Mediterranean diet gained popularity following publication of a seminal study showing evidence of decreased mortality and risk of cardiovascular events in Cretan farmers.[105] Since this time, it has been widely prescribed as a dietary intervention for those at risk of developing CVD.[106] Several clinical trials supported that the Mediterranean diet reduces overall cardiovascular mortality, BP, total cholesterol, and risk of future cardiovascular events,[107,108] while also decreasing systemic inflammation.[109,110] Together, these data suggest that the Mediterranean diet is in fact anti-inflammatory and may be used to enhance the effects of pharmacological therapies. The DASH diet shares many similarities with the Mediterranean diet, with the exception of intake of olive oil. Indeed, the DASH diet also reduced BP, plasma cholesterol levels, and risk of cardiovascular events even with a 10% increment in protein or unsaturated fats.[111] Similar to the Mediterranean diet, the DASH diet has been reported to decrease inflammatory markers.[112,113]

Compared with a control diet, the DASH diet leads to a different metabolite profile in pre-hypertensive and hypertensive subjects.[114] Similarly, adherence to the Mediterranean diet promoted the expansion SCFA-producing bacteria such as *Roseburia*, which was accompanied by increased faecal levels of SCFAs.[115] In contrast, adherence to the Western diet resulted in increased levels of *Streptococcus* and *L-Ruminococcus*, which was correlated with urine levels of TMAO levels.[115] These data would suggest dietary fibre directly impacts gut microbial composition, circulating levels of metabolites and the inflammatory response.

7. Future directions

There is substantial evidence that, individually, diet, the gut microbiota, and the immune system have a role in the pathogenesis of hypertension, but the complex interplay between these potentially protective (i.e. fibre) or hypertensinogenic (i.e. fat and sodium) factors is still poorly understood. Future intervention studies need to address the utility of combining dietary elements that may be additive—for instance, reduced sodium with increased intake of resistant starches or its beneficial gut metabolites, and their impact on inflammatory, immune and microbial profile. We currently lack evidence of the microbial signature between hypertensive phenotypes, such as white coat, essential and masked hypertension, and how BP-lowering therapy and gender might impact the gut microbiota in this context, but large cohorts would be required for such conclusions. Similarly to genome-wide association studies, a joint global effort to recruit and properly characterize hypertensive subjects and their gut microbiome is needed to properly assert the contribution of the microbiota to BP regulation and the microbiota role in immune regulation in this setting. Finally, we are only scratching the surface of the gut microbiota in hypertension as most studies analyse only the bacterial 16S rRNA gene. No doubt future studies will identify a role not only for specific bacterial strains and communities, but also for archaea, viruses (particularly bacteriophages) and fungi in the development of hypertension.

8. Conclusions

Recent advances in experimental and clinical hypertension have revealed that the gut microbiota is likely to be an important component of the multifactorial aetiology of high BP. Our understanding of the complex and interconnected nature of the microbial-host immune interactions in hypertension, however, is still rudimentary (summarized in *Figure 4*). Understanding the role of the gut microbiota in the development and progression of hypertension will require careful consideration on how the immune system, gut microbes and diet interact, and their potential as

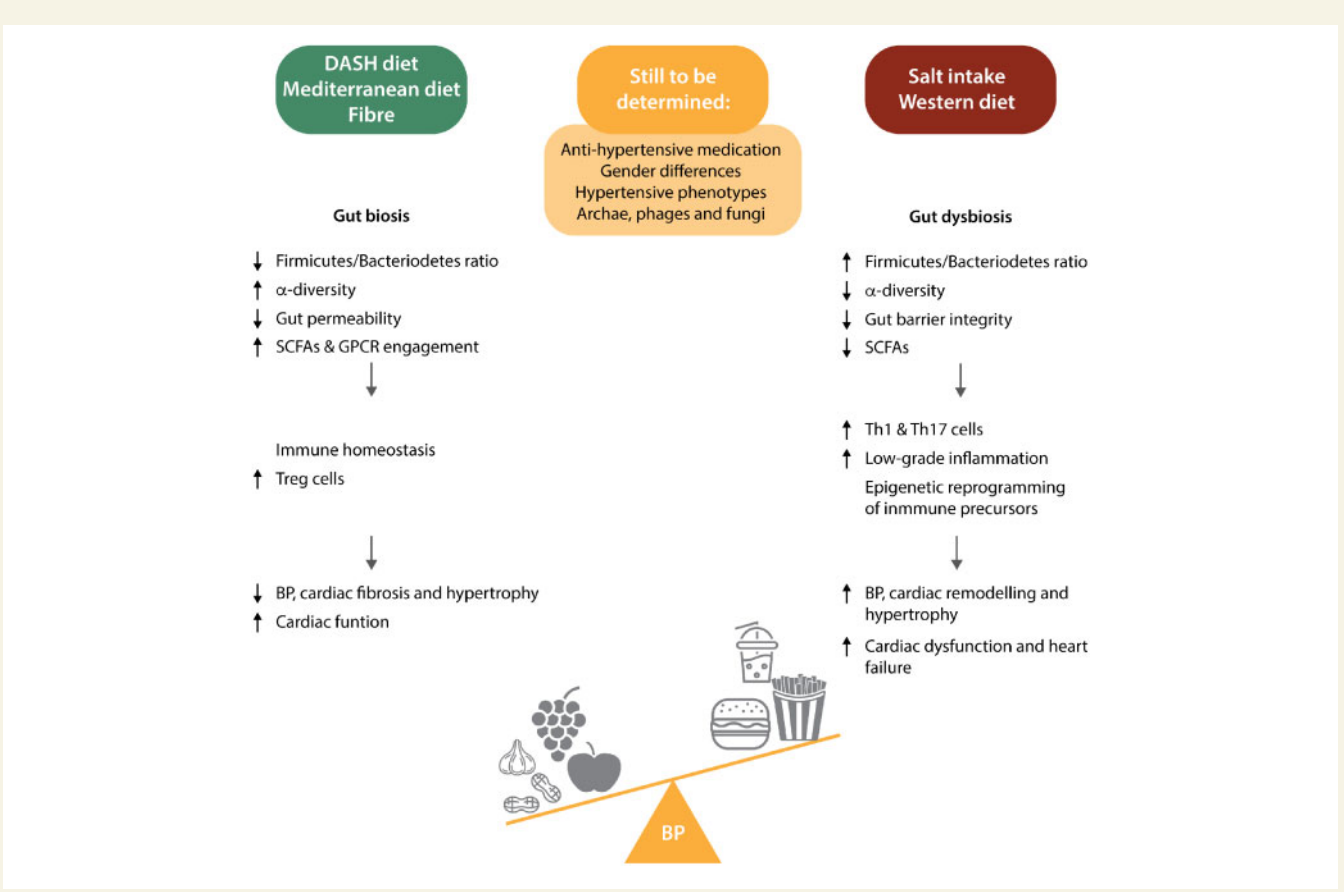

Figure 4 Summary of findings to date. High fibre intake, DASH, and Mediterranean diets are linked to increased gut health (improved gut epithelial barrier and α-diversity) and production of SCFAs, immune homeostasis and higher number of anti-inflammatory Treg cells, decrease BP and reduced incidence of CVD. In contrast, Western diet and high salt intake is associated to gut dysfunction with decreased microbial diversity, high number of pro-inflammatory Th1 and Th17 T cells, low-grade inflammation, high BP, and adverse cardiac function.

markers or therapeutic targets for high BP. Translational studies are needed to ascertain the BP-lowering capacity of gut metabolites such as acetate and butyrate that have shown promising results in experimental models of hypertension and immune diseases. Until then, the DASH and Mediterranean diet, both high in fibre, are the best dietary interventions that support cardiovascular, gut microbial, and immune health.

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References

1. Oparil S, Acelajado MC, Bakris GL, Berlowitz DR, Cifkova R, Dominiczak AF, Grassi G, Jordan J, Poulter NR, Rodgers A, Whelton PK. Hypertension. *Nat Rev Dis Primers* 2018;**4**:18014.
2. Norlander AE, Madhur MS, Harrison DG. The immunology of hypertension. *J Exp Med* 2018;**215**:21–33.
3. Sender R, Fuchs S, Milo R. Revised estimates for the number of human and bacteria cells in the body. *PLoS Biol* 2016;**14**:e1002533.
4. Li J, Jia H, Cai X, Zhong H, Feng Q, Sunagawa S, Arumugam M, Kultima JR, Prifti E, Nielsen T, Juncker AS, Manichanh C, Chen B, Zhang W, Levenez F, Wang J, Xu X, Xiao L, Liang S, Zhang D, Zhang Z, Chen W, Zhao H, Al-Aama JY, Edris S, Yang H, Wang J, Hansen T, Nielsen HB, Brunak S, Kristiansen K, Guarner F, Pedersen O, Dore J, Ehrlich SD, Meta H, Bork P, Wang J, Meta H. An integrated catalog of reference genes in the human gut microbiome. *Nat Biotechnol* 2014;**32**:834–841.
5. Tremaroli V, Bäckhed F. Functional interactions between the gut microbiota and host metabolism. *Nature* 2012;**489**:242.
6. Tan JK, McKenzie C, Marino E, Macia L, Mackay CR. Metabolite-sensing G protein-coupled receptors-facilitators of diet-related immune regulation. *Annu Rev Immunol* 2017;**35**:371–402.
7. Vancamelbeke M, Vermeire S. The intestinal barrier: a fundamental role in health and disease. *Expert Rev Gastroenterol Hepatol* 2017;**11**:821–834.
8. Levy M, Kolodziejczyk AA, Thaiss CA, Elinav E. Dysbiosis and the immune system. *Nat Rev Immunol* 2017;**17**:219–232.
9. Guzik TJ, Hoch NE, Brown KA, McCann LA, Rahman A, Dikalov S, Goronzy J, Weyand C, Harrison DG. Role of the T cell in the genesis of angiotensin II–induced hypertension and vascular dysfunction. *J Exp Med* 2007;**204**:2449–2460.
10. Vinh A, Chen W, Blinder Y, Weiss D, Robert Taylor W, Goronzy J, Weyand C, Harrison DG, Guzik TJ. Inhibition and genetic ablation of the B7/CD28 T cell costimulation axis prevents experimental hypertension. *Circulation* 2010;**122**:2529–2537.
11. Marvar PJ, Thabet SR, Guzik TJ, Lob HE, McCann LA, Weyand C, Gordon FJ, Harrison DG. Central and peripheral mechanisms of T lymphocyte activation and vascular inflammation produced by angiotensin II-induced hypertension. *Circ Res* 2010;**107**:263–270.
12. Guzik T, Mangalat D, Korbut R. Adipocytokines novel link between inflammation. *J Physiol Pharmacol* 2006;**4**:505–528.
13. Lob HE, Marvar PJ, Guzik TJ, Sharma S, McCann LA, Weyand C, Gordon FJ, Harrison DG. Induction of hypertension and peripheral inflammation by reduction

of extracellular superoxide dismutase in the central nervous system. *Hypertension* 2010;**55**:277–283.
14. Youn JC, Yu HT, Lim BJ, Koh MJ, Lee J, Chang DY, Choi YS, Lee SH, Kang SM, Jang Y, Yoo OJ, Shin EC, Park S. Immunosenescent CD8+ T cells and C-X-C chemokine receptor type 3 chemokines are increased in human hypertension. *Hypertension* 2013;**62**:126–133.
15. Madhur MS, Lob HE, McCann LA, Iwakura Y, Blinder Y, Guzik TJ, Harrison DG. Interleukin 17 promotes angiotensin II–induced hypertension and vascular dysfunction. *Hypertension* 2010;**55**:500–507.
16. Mazzolai L, Duchosal MA, Korber M, Bouzourene K, Aubert JF, Hao H, Vallet V, Brunner HR, Nussberger J, Gabbiani G, Hayoz D. Endogenous angiotensin II induces atherosclerotic plaque vulnerability and elicits a Th1 response in ApoE-/- mice. *Hypertension* 2004;**44**:277–282.
17. Itani HA, McMaster WG Jr, Saleh MA, Nazarewicz RR, Mikolajczyk TP, Kaszuba AM, Konior A, Prejbisz A, Januszewicz A, Norlander AE, Chen W, Bonami RH, Marshall AF, Poffenberger G, Weyand CM, Madhur MS, Moore DJ, Harrison DG, Guzik TJ. Activation of human T cells in hypertension: studies of humanized mice and hypertensive humans. *Hypertension* 2016;**68**:123–132.
18. Kim S, Goel R, Kumar A, Qi Y, Lobaton G, Hosaka K, Mohammed M, Handberg EM, Richards EM, Pepine CJ, Raizada MK. Imbalance of gut microbiome and intestinal epithelial barrier dysfunction in patients with high blood pressure. *Clin Sci* 2018; **132**:701–718.
19. Didion SP, Kinzenbaw DA, Schrader LI, Chu Y, Faraci FM. Endogenous interleukin-10 inhibits angiotensin II-induced vascular dysfunction. *Hypertension* 2009;**54**:619–624.
20. Barhoumi T, Kasal DA, Li MW, Shbat L, Laurant P, Neves MF, Paradis P, Schiffrin EL. T regulatory lymphocytes prevent angiotensin II-induced hypertension and vascular injury. *Hypertension* 2011;**57**:469–476.
21. Shah KH, Shi P, Giani JF, Janjulia T, Bernstein EA, Li Y, Zhao T, Harrison DG, Bernstein KE, Shen XZ. Myeloid suppressor cells accumulate and regulate blood pressure in hypertension. *Circ Res* 2015;**117**:858–869.
22. Jakobsson HE, Rodríguez-Piñeiro AM, Schütte A, Ermund A, Boysen P, Bemark M, Sommer F, Bäckhed F, Hansson GC, Johansson M. The composition of the gut microbiota shapes the colon mucus barrier. *EMBO Rep* 2015;**16**:164–167.
23. Desai MS, Seekatz AM, Koropatkin NM, Kamada N, Hickey CA, Wolter M, Pudlo NA, Kitamoto S, Terrapon N, Muller A, Young VB, Henrissat B, Wilmes P, Stappenbeck TS, Nunez G, Martens EC. A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell* 2016; **167**:1339–1353.e21.
24. Tlaskalová-Hogenová H, Štěrzl J, Štěpánková R, Dlabač V, Větvička V, Rossmann P, Mandel L, Rejnek J. Development of immunological capacity under germfree and conventional conditions. *Ann NY Acad Sci* 1983;**409**:96–113.
25. Hayes CL, Dong J, Galipeau HJ, Jury J, McCarville J, Huang X, Wang X-Y, Naidoo A, Anbazhagan AN, Libertucci J, Sheridan C, Dudeja PK, Bowdish DME, Surette MG, Verdu EF. Commensal microbiota induces colonic barrier structure and functions that contribute to homeostasis. *Sci Rep* 2018;**8**:14184.
26. Nowarski R, Jackson R, Gagliani N, de Zoete MR, Palm NW, Bailis W, Low JS, Harman CCD, Graham M, Elinav E, Flavell RA. Epithelial IL-18 equilibrium controls barrier function in colitis. *Cell* 2015;**163**:1444–1456.
27. Cerf-Bensussan N, Gaboriau-Routhiau V. The immune system and the gut microbiota: friends or foes? *Nat Rev Immunol* 2010;**10**:735.
28. Dubuquoy L, Jansson EÖ, Deeb S, Rakotobe S, Karoui M, Colombel J-F, Auwerx J, Pettersson S, Desreumaux P. Impaired expression of peroxisome proliferator-activated receptor γ in ulcerative colitis. *Gastroenterology* 2003;**124**:1265–1276.
29. Min YW, Rhee PL. The role of microbiota on the gut immunology. *Clin Ther* 2015; **37**:968–975.
30. Sefik E, Geva-Zatorsky N, Oh S, Konnikova L, Zemmour D, McGuire AM, Burzyn D, Ortiz-Lopez A, Lobera M, Yang J, Ghosh S, Earl A, Snapper SB, Jupp R, Kasper D, Mathis D, Benoist C. Individual intestinal symbionts induce a distinct population of RORγ+regulatory T cells. *Science* 2015;**349**:993–997.
31. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, Nakanishi Y, Uetake C, Kato K, Kato T, Takahashi M, Fukuda NN, Murakami S, Miyauchi E, Hino S, Atarashi K, Onawa S, Fujimura Y, Lockett T, Clarke JM, Topping DL, Tomita M, Hori S, Ohara O, Morita T, Koseki H, Kikuchi J, Honda K, Hase K, Ohno H. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 2013;**504**:446.
32. Schulthess J, Pandey S, Capitani M, Rue-Albrecht KC, Arnold I, Franchini F, Chomka A, Ilott NE, Johnston DGW, Pires E, McCullagh J, Sansom SN, Arancibia-Carcamo CV, Uhlig HH, Powrie F. The short chain fatty acid butyrate imprints an antimicrobial program in macrophages. *Immunity* 2019;**50**:432–445.e7.
33. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. *Cell* 2005; **122**:107–118.
34. Round JL, Lee SM, Li J, Tran G, Jabri B, Chatila TA, Mazmanian SK. The Toll-like receptor 2 pathway establishes colonization by a commensal of the human microbiota. *Science* 2011;**332**:974–977.
35. Byndloss MX, Olsan EE, Rivera-Chávez F, Tiffany CR, Cevallos SA, Lokken KL, Torres TP, Byndloss AJ, Faber F, Gao Y, Litvak Y, Lopez CA, Xu G, Napoli E, Giulivi C, Tsolis RM, Revzin A, Lebrilla CB, Bäumler AJ. Microbiota-activated PPAR-gamma signaling inhibits dysbiotic Enterobacteriaceae expansion. *Science* 2017;**357**:570–575.
36. Rehman A, Sina C, Gavrilova O, Häsler R, Ott S, Baines JF, Schreiber S, Rosenstiel P. Nod2 is essential for temporal development of intestinal microbial communities. *Gut* 2011;**60**:1354.
37. Barreau F, Madre C, Meinzer U, Berrebi D, Dussaillant M, Merlin F, Eckmann L, Karin M, Sterkers G, Bonacorsi S, Lesuffleur T, Hugot JP. Nod2 regulates the host response towards microflora by modulating T cell function and epithelial permeability in mouse Peyer's patches. *Gut* 2010;**59**:207–217.
38. Barrett JC, Hansoul S, Nicolae DL, Cho JH, Duerr RH, Rioux JD, Brant SR, Silverberg MS, Taylor KD, Barmada MM, Bitton A, Dassopoulos T, Datta LW, Green T, Griffiths AM, Kistner EO, Murtha MT, Regueiro MD, Rotter JI, Schumm LP, Steinhart AH, Targan SR, Xavier RJ, Libioulle C, Sandor C, Lathrop M, Belaiche J, Dewit O, Gut I, Heath S, Laukens D, Mni M, Rutgeerts P, Van Gossum A, Zelenika D, Franchimont D, Hugot JP, de Vos M, Vermeire S, Louis E, Cardon LR, Anderson CA, Drummond H, Nimmo E, Ahmad T, Prescott NJ, Onnie CM, Fisher SA, Marchini J, Ghori J, Bumpstead S, Gwilliam R, Tremelling M, Deloukas P, Mansfield J, Jewell D, Satsangi J, Mathew CG, Parkes M, Georges M, Daly MJ. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat Genet* 2008;**40**:955–962.
39. Strober W, Watanabe T. NOD2, an intracellular innate immune sensor involved in host defense and Crohn's disease. *Mucosal Immunol* 2011;**4**:484–495.
40. Shinoda M, Kurosawa T, Maejima K, Watarai S. [A study on heart weight and electrocardiogram of germfree, ex-germfree and conventional ICR mice (author's transl)]. *Jikken Dobutsu* 1981;**30**:481–485.
41. Wostmann BS, Bruckner-Kardoss E, Knight PL. Jr., Cecal enlargement, cardiac output, and O2 consumption in germfree rats. *Proc Soc Exp Biol Med* 1968;**128**:137–141.
42. Sommer F, Bäckhed F. The gut microbiota—masters of host development and physiology. *Nat Rev Microbiol* 2013;**11**:227.
43. Gaboriau-Routhiau V, Rakotobe S, Lecuyer E, Mulder I, Lan A, Bridonneau C, Rochet V, Pisi A, De Paepe M, Brandi G, Eberl G, Snel J, Kelly D, Cerf-Bensussan N. The key role of segmented filamentous bacteria in the coordinated maturation of gut helper T cell responses. *Immunity* 2009;**31**:677–689.
44. Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K, Littman DR. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell* 2009;**139**:485–498.
45. Gilbert JA, Blaser MJ, Caporaso JG, Jansson JK, Lynch SV, Knight R. Current understanding of the human microbiome. *Nat Med* 2018;**24**:392–400.
46. Yang T, Santisteban MM, Rodriguez V, Li E, Ahmari N, Carvajal JM, Zadeh M, Gong M, Qi Y, Zubcevic J, Sahay B, Pepine CJ, Raizada MK, Mohamadzadeh M. Gut dysbiosis is linked to hypertension. *Hypertension* 2015;**65**:1331–1340.
47. Adnan S, Nelson JW, Ajami NJ, Venna VR, Petrosino JF, Bryan RM Jr, Durgan DJ. Alterations in the gut microbiota can elicit hypertension in rats. *Physiol Genomics* 2017;**49**:96–104.
48. Mell B, Jala VR, Mathew AV, Byun J, Waghulde H, Zhang Y, Haribabu B, Vijay-Kumar M, Pennathur S, Joe B. Evidence for a link between gut microbiota and hypertension in the Dahl rat. *Physiol Genomics* 2015;**47**:187–197.
49. Marques FZ, Nelson E, Chu PY, Horlock D, Fiedler A, Ziemann M, Tan JK, Kuruppu S, Rajapakse NW, El-Osta A, Mackay CR, Kaye DM. High-fiber diet and acetate supplementation change the gut microbiota and prevent the development of hypertension and heart failure in hypertensive mice. *Circulation* 2017;**135**:964–977.
50. Jama H, Kaye DM, Marques FZ. The gut microbiota and blood pressure in experimental models. *Curr Opin Nephrol Hypertens* 2019;**28**:97–104.
51. Santisteban MM, Qi Y, Zubcevic J, Kim S, Yang T, Shenoy V, Cole-Jeffrey CT, Lobaton GO, Stewart DC, Rubiano A, Simmons CS, Garcia-Pereira F, Johnson RD, Pepine CJ, Raizada MK. Hypertension-linked pathophysiological alterations in the gut. *Circ Res* 2017;**120**:312–323.
52. Li J, Zhao F, Wang Y, Chen J, Tao J, Tian G, Wu S, Liu W, Cui Q, Geng B, Zhang W, Weldon R, Auguste K, Yang L, Liu X, Chen L, Yang X, Zhu B, Cai J. Gut microbiota dysbiosis contributes to the development of hypertension. *Microbiome* 2017;**5**:14.
53. Karbach SH, Schönfelder T, Brandão I, Wilms E, Hörmann N, Jäckel S, Schüler R, Finger S, Knorr M, Lagrange J, Brandt M, Waisman A, Kossmann S, Schäfer K, Münzel T, Reinhardt C, Wenzel P. Gut microbiota promote angiotensin II–induced arterial hypertension and vascular dysfunction. *J Am Heart Assoc* 2016;**5**:e003698.
54. Yan Q, Gu Y, Li X, Yang W, Jia L, Chen C, Han X, Huang Y, Zhao L, Li P, Fang Z, Zhou J, Guan X, Ding Y, Wang S, Khan M, Xin Y, Li S, Ma Y. Alterations of the gut microbiome in hypertension. *Front Cell Infect Microbiol* 2017;**7**:381.
55. Colafella KMM, Denton KM. Sex-specific differences in hypertension and associated cardiovascular disease. *Nat Rev Nephrol* 2018;**14**:185–201.
56. Ogola BO, Zimmerman MA, Clark GL, Abshire CM, Gentry KM, Miller KS, Lindsey SH. New insights into arterial stiffening: does sex matter? *Am J Physiol Heart Circ Physiol* 2018;**315**:H1073–H1087.
57. Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol* 2016; **16**:626–638.
58. Menni C, Lin C, Cecelja M, Mangino M, Matey-Hernandez ML, Keehn L, Mohney RP, Steves CJ, Spector TD, Kuo CF, Chowienczyk P, Valdes AM. Gut microbial diversity is associated with lower arterial stiffness in women. *Eur Heart J* 2018;**39**: 2390–2397.
59. Flint HJ, Duncan SH, Scott KP, Louis P. Links between diet, gut microbiota composition and gut metabolism. *Proc Nutr Soc* 2015;**74**:13–22.

60. Gill PA, Zelm MC, Muir JG, Gibson PR. Review article: short chain fatty acids as potential therapeutic agents in human gastrointestinal and inflammatory disorders. *Aliment Pharmacol Ther* 2018;**48**:15–34.
61. Thorburn AN, McKenzie CI, Shen S, Stanley D, Macia L, Mason LJ, Roberts LK, Wong CH, Shim R, Robert R, Chevalier N, Tan JK, Marino E, Moore RJ, Wong L, McConville MJ, Tull DL, Wood LG, Murphy VE, Mattes J, Gibson PG, Mackay CR. Evidence that asthma is a developmental origin disease influenced by maternal diet and bacterial metabolites. *Nat Commun* 2015;**6**:7320.
62. Thorburn AN, Macia L, Mackay CR. Diet, metabolites, and "western-lifestyle" inflammatory diseases. *Immunity* 2014;**40**:833–842.
63. Ganesh BP, Nelson JW, Eskew JR, Ganesan A, Ajami NJ, Petrosino JF, Bryan RM Jr, Durgan DJ. Prebiotics, probiotics, and acetate supplementation prevent hypertension in a model of obstructive sleep apnea. *Hypertension* 2018;**72**:1174–1150.
64. Sharma RK, Yang T, Oliveira AC, Lobaton GO, Aquino V, Kim S, Richards EM, Pepine CJ, Sumners C, Raizada MK. Microglial cells impact gut microbiota and gut pathology in angiotensin II-induced hypertension. *Circ Res* 2019;**124**:727–736.
65. Wang L, Zhu Q, Lu A, Liu X, Zhang L, Xu C, Liu X, Li H, Yang T. Sodium butyrate suppresses angiotensin II-induced hypertension by inhibition of renal (pro)renin receptor and intrarenal renin-angiotensin system. *J Hypertens* 2017;**35**:1899–1908.
66. Pluznick JL, Protzko RJ, Gevorgyan H, Peterlin Z, Sipos A, Han J, Brunet I, Wan LX, Rey F, Wang T, Firestein SJ, Yanagisawa M, Gordon JI, Eichmann A, Peti-Peterdi J, Caplan MJ. Olfactory receptor responding to gut microbiota-derived signals plays a role in renin secretion and blood pressure regulation. *Proc Natl Acad Sci USA* 2013;**110**:4410–4415.
67. Bartolomaeus H, Balogh A, Yakoub M, Homann S, Marko L, Hoges S, Tsvetkov D, Krannich A, Wundersitz S, Avery EG, Haase N, Kraker K, Hering L, Maase M, Kusche-Vihrog K, Grandoch M, Fielitz J, Kempa S, Gollasch M, Zhumadilov Z, Kozhakhmetov S, Kushugulova A, Eckardt KU, Dechend R, Rump LC, Forslund SK, Muller DN, Stegbauer J, Wilck N. The short-chain fatty acid propionate protects from hypertensive cardiovascular damage. *Circulation* 2019;**139**:1407–1421
68. Macia L, Tan J, Vieira AT, Leach K, Stanley D, Luong S, Maruya M, Ian McKenzie C, Hijikata A, Wong C, Binge L, Thorburn AN, Chevalier N, Ang C, Marino E, Robert R, Offermanns S, Teixeira MM, Moore RJ, Flavell RA, Fagarasan S, Mackay CR. Metabolite-sensing receptors GPR43 and GPR109A facilitate dietary fibre-induced gut homeostasis through regulation of the inflammasome. *Nat Commun* 2015;**6**:6734.
69. Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson Ö, Kampf C, Sjöstedt E, Asplund A, Olsson I, Edlund K, Lundberg E, Navani S, Szigyarto CA-K, Odeberg J, Djureinovic D, Takanen JO, Hober S, Alm T, Edqvist P-H, Berling H, Tegel H, Mulder J, Rockberg J, Nilsson P, Schwenk JM, Hamsten M, von Feilitzen K, Forsberg M, Persson L, Johansson F, Zwahlen M, von Heijne G, Nielsen J, Pontén F. Proteomics. Tissue-based map of the human proteome. *Science* 2015;**347**:1260419.
70. Natarajan N, Hori D, Flavahan S, Steppan J, Flavahan NA, Berkowitz DE, Pluznick JL. Microbial short chain fatty acid metabolites lower blood pressure via endothelial G-protein coupled receptor 41. *Physiol Genomics* 2016;**48**:826–834.
71. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, DuGar B, Feldstein AE, Britt EB, Fu X, Chung Y-M, Wu Y, Schauer P, Smith JD, Allayee H, Tang WHW, DiDonato JA, Lusis AJ, Hazen SL. Gut flora metabolism of phosphatidylcholine promotes cardiovascular disease. *Nature* 2011;**472**:57.
72. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, Britt EB, Fu X, Wu Y, Li L, Smith JD, DiDonato JA, Chen J, Li H, Wu GD, Lewis JD, Warrier M, Brown JM, Krauss RM, Tang WH, Bushman FD, Lusis AJ, Hazen SL. Intestinal microbiota metabolism of L-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nat Med* 2013;**19**:576–585.
73. Tang WH, Wang Z, Fan Y, Levison B, Hazen JE, Donahue LM, Wu Y, Hazen SL. Prognostic value of elevated levels of intestinal microbe-generated metabolite trimethylamine-N-oxide in patients with heart failure: refining the gut hypothesis. *J Am Coll Cardiol* 2014;**64**:1908–1914.
74. Wang Z, Roberts AB, Buffa JA, Levison BS, Zhu W, Org E, Gu X, Huang Y, Zamanian-Daryoush M, Culley MK, DiDonato AJ, Fu X, Hazen JE, Krajcik D, DiDonato JA, Lusis AJ, Hazen SL. Non-lethal inhibition of gut microbial trimethylamine production for the treatment of atherosclerosis. *Cell* 2015;**163**:1585–1595.
75. Roberts AB, Gu X, Buffa JA, Hurd AG, Wang Z, Zhu W, Gupta N, Skye SM, Cody DB, Levison BS, Barrington WT, Russell MW, Reed JM, Duzan A, Lang JM, Fu X, Li L, Myers AJ, Rachakonda S, DiDonato JA, Brown JM, Gogonea V, Lusis AJ, Garcia-Garcia JC, Hazen SL. Development of a gut microbe–targeted nonlethal therapeutic to inhibit thrombosis potential. *Nat Med* 2018;**24**:1407–1417.
76. Jaworska K, Huc T, Samborowska E, Dobrowolski L, Bielinska K, Gawlak M, Ufnal M. Hypertension in rats is associated with an increased permeability of the colon to TMA, a gut bacteria metabolite. *PLoS One* 2017;**12**:e0189310.
77. Yan Y, Jiang W, Spinetti T, Tardivel A, Castillo R, Bourquin C, Guarda G, Tian Z, Tschopp J, Zhou R. Omega-3 fatty acids prevent inflammation and metabolic disorder through inhibition of NLRP3 inflammasome activation. *Immunity* 2013;**38**:1154–1163.
78. Oh DY, Talukdar S, Bae EJ, Imamura T, Morinaga H, Fan W, Li P, Lu WJ, Watkins SM, Olefsky JM. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. *Cell* 2010;**142**:687–698.
79. Aung T, Halsey J, Kromhout D, Gerstein HC, Marchioli R, Tavazzi L, Geleijnse JM, Rauch B, Ness A, Galan P, Chew EY, Bosch J, Collins R, Lewington S, Armitage J, Clarke R, Omega 3, Treatment Trialists C. Associations of omega-3 fatty acid supplement use with cardiovascular disease risks: meta-analysis of 10 trials involving 77917 individuals. *JAMA Cardiol* 2018;**3**:225–234.
80. Casanova MA, Medeiros F, Trindade M, Cohen C, Oigman W, Neves MF. Omega-3 fatty acids supplementation improves endothelial function and arterial stiffness in hypertensive patients with hypertriglyceridemia and high cardiovascular risk. *J Am Soc Hypertens* 2017;**11**:10–19.
81. Watson H, Mitra S, Croden FC, Taylor M, Wood HM, Perry SL, Spencer JA, Quirke P, Toogood GJ, Lawton CL, Dye L, Loadman PM, Hull MA. A randomised trial of the effect of omega-3 polyunsaturated fatty acid supplements on the human intestinal microbiota. *Gut* 2018;**67**:1974–1983.
82. Menni C, Zierer J, Pallister T, Jackson MA, Long T, Mohney RP, Steves CJ, Spector TD, Valdes AM. Omega-3 fatty acids correlate with gut microbiome diversity and production of N-carbamylglutamate in middle aged and elderly women. *Sci Rep* 2017;**7**:11079.
83. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G, Lionetti P. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci USA* 2010;**107**:14691–14696.
84. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling AV, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, Turnbaugh PJ. Diet rapidly and reproducibly alters the human gut microbiome. *Nature* 2014;**505**:559–563.
85. Chassaing B, Koren O, Goodrich JK, Poole AC, Srinivasan S, Ley RE, Gewirtz AT. Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. *Nature* 2015;**519**:92–96.
86. Suez J, Korem T, Zeevi D, Zilberman-Schapira G, Thaiss CA, Maza O, Israeli D, Zmora N, Gilad S, Weinberger A, Kuperman Y, Harmelin A, Kolodkin-Gal I, Shapiro H, Halpern Z, Segal E, Elinav E. Artificial sweeteners induce glucose intolerance by altering the gut microbiota. *Nature* 2014;**514**:181–186.
87. Xu Z, Knight R. Dietary effects on human gut microbiome diversity. *Br J Nutr* 2015;**113(Suppl.)**:S1–S5.
88. Bier A, Braun T, Khasbab R, Di Segni A, Grossman E, Haberman Y, Leibowitz A. A high salt diet modulates the gut microbiota and short chain fatty acids production in a salt-sensitive hypertension rat model. *Nutrients* 2018;**10**:1154.
89. Wilck N, Matus MG, Kearney SM, Olesen SW, Forslund K, Bartolomaeus H, Haase S, Mähler A, Balogh A, Markó L, Vvedenskaya O, Kleiner FH, Tsvetkov D, Klug L, Costea PI, Sunagawa S, Maier L, Rakova N, Schatz V, Neubert P, Frätzer C, Krannich A, Gollasch M, Grohme DA, Côrte-Real BF, Gerlach RG, Basic M, Typas A, Wu C, Titze JM, Jantsch J, Boschmann M, Dechend R, Kleinewietfeld M, Kempa S, Bork P, Linker RA, Alm EJ, Müller DN. Salt-responsive gut commensal modulates TH17 axis and disease. *Nature* 2017;**551**:585–589.
90. Smith DL, Harris AD, Johnson JA, Silbergeld EK, Morris JG 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* 2002;**99**:6434–6439.
91. Cordain L, Eaton SB, Sebastian A, Mann N, Lindeberg S, Watkins BA, O'Keefe JH, Brand-Miller J. Origins and evolution of the Western diet: health implications for the 21st century. *Am J Clin Nutr* 2005;**81**:341–354.
92. Myles IA. Fast food fever: reviewing the impacts of the Western diet on immunity. *Nutr J* 2014;**13**:61.
93. Milner JJ, Beck MA. The impact of obesity on the immune response to infection. *Proc Nutr Soc* 2012;**71**:298–306.
94. Fung TT, Rimm EB, Spiegelman D, Rifai N, Tofler GH, Willett WC, Hu FB. Association between dietary patterns and plasma biomarkers of obesity and cardiovascular disease risk. *Am J Clin Nutr* 2001;**73**:61–67.
95. Christ A, Günther P, Lauterbach MAR, Duewell P, Biswas D, Pelka K, Scholz CJ, Oosting M, Haendler K, Baßler K, Klee K, Schulte-Schrepping J, Ulas T, Moorlag SJCFM, Kumar V, Park MH, Joosten LAB, Groh LA, Riksen NP, Espevik T, Schlitzer A, Li Y, Fitzgerald ML, Netea MG, Schultze JL, Latz E. Western diet triggers NLRP3-dependent innate immune reprogramming. *Cell* 2018;**172**:162–175.e14.
96. Shivappa N, Bonaccio M, Hebert JR, Di Castelnuovo A, Costanzo S, Ruggiero E, Pounis G, Donati MB, de Gaetano G, Iacoviello L; Moli-sani study Investigators. Association of proinflammatory diet with low-grade inflammation: results from the Moli-sani study. *Nutrition* 2018;**54**:182–188.
97. Mente A, O'Donnell M, Rangarajan S, McQueen M, Dagenais G, Wielgosz A, Lear S, Ah STL, Wei L, Diaz R, Avezum A, Lopez-Jaramillo P, Lanas F, Mony P, Szuba A, Iqbal R, Yusuf R, Mohammadifard N, Khatib R, Yusoff K, Ismail N, Gulec S, Rosengren A, Yusufali A, Kruger L, Tsolekile LP, Chifamba J, Dans A, Alhabib KF, Yeates K, Teo K, Yusuf S. Urinary sodium excretion, blood pressure, cardiovascular disease, and mortality: a community-level prospective epidemiological cohort study. *Lancet* 2018;**392**:496–506.
98. Miranda PM, De Palma G, Serkis V, Lu J, Louis-Auguste MP, McCarville JL, Verdu EF, Collins SM, Bercik P. High salt diet exacerbates colitis in mice by decreasing Lactobacillus levels and butyrate production. *Microbiome* 2018;**6**:57–57.
99. Appel LJ, Moore TJ, Obarzanek E, Vollmer WM, Svetkey LP, Sacks FM, Bray GA, Vogt TM, Cutler JA, Windhauser MM, Lin PH, Karanja N. A clinical trial of the effects of dietary patterns on blood pressure. DASH Collaborative Research Group. *N Engl J Med* 1997;**336**:1117–1124.

100. Estruch R, Ros E, Salas-Salvado J, Covas MI, Corella D, Aros F, Gomez-Gracia E, Ruiz-Gutierrez V, Fiol M, Lapetra J, Lamuela-Raventos RM, Serra-Majem L, Pinto X, Basora J, Munoz MA, Sorli JV, Martinez JA, Martinez GM; PREDIMED Study Investigators. Primary prevention of cardiovascular disease with a Mediterranean diet. *N Engl J Med* 2013;**368**:1279–1290.
101. Wang X, Ouyang Y, Liu J, Zhu M, Zhao G, Bao W, Hu FB. 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* 2014;**349**:g4490.
102. Miura K, Greenland P, Stamler J, Liu K, Daviglus ML, Nakagawa H. 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* 2004;**159**:572–580.
103. Ascherio A, Hennekens C, Willett WC, Sacks F, Rosner B, Manson J, Witteman J, Stampfer MJ. Prospective study of nutritional factors, blood pressure, and hypertension among US women. *Hypertension* 1996;**27**:1065–1072.
104. Alonso A, de la Fuente C, Martín-Arnau AM, de Irala J, Alfredo Martínez J, Martínez-González MÁ. 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. *BJN* 2004;**92**:311–319.
105. Kandle RP. Crete, a case study of an underdeveloped area. *Am J Public Health Nations Health* 1953;**43**:928–929.
106. Trichopoulou A, Costacou T, Bamia C, Trichopoulos D. Adherence to a Mediterranean diet and survival in a Greek population. *N Engl J Med* 2003;**348**:2599–2608.
107. de Lorgeril M, Salen P, Martin JL, Monjaud I, Delaye J, Mamelle N. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. *Circulation* 1999;**99**:779–785.
108. Ndanuko RN, Tapsell LC, Charlton KE, Neale EP, Batterham MJ. Dietary patterns and blood pressure in adults: a systematic review and meta-analysis of randomized controlled trials. *Adv Nutr* 2016;**7**:76–89.
109. Dai J, Miller AH, Bremner JD, Goldberg J, Jones L, Shallenberger L, Buckham R, Murrah NV, Veledar E, Wilson PW, Vaccarino V. Adherence to the Mediterranean diet is inversely associated with circulating interleukin-6 among middle-aged men: a twin study. *Circulation* 2008;**117**:169–175.
110. Sureda A, Bibiloni MDM, Julibert A, Bouzas C, Argelich E, Llompart I, Pons A, Tur JA. Adherence to the Mediterranean diet and inflammatory markers. *Nutrients* 2018;**10**:62.
111. Swain JF, McCarron PB, Hamilton EF, Sacks FM, Appel LJ. Characteristics of the diet patterns tested in the optimal macronutrient intake trial to prevent heart disease (OmniHeart): options for a heart-healthy diet. *J Am Diet Assoc* 2008;**108**:257–265.
112. Saneei P, Hashemipour M, Kelishadi R, Esmaillzadeh A. The Dietary Approaches to Stop Hypertension (DASH) diet affects inflammation in childhood metabolic syndrome: a randomized cross-over clinical trial. *Ann Nutr Metab* 2014;**64**:20–27.
113. Phillips CM, Harrington JM, Perry IJ. Relationship between dietary quality, determined by DASH score, and cardiometabolic health biomarkers: a cross-sectional analysis in adults. *Clin Nutr* 2018;doi:10.1016/j.clnu.2018.08.028.
114. Rebholz CM, Lichtenstein AH, Zheng Z, Appel LJ, Coresh J. Serum untargeted metabolomic profile of the Dietary Approaches to Stop Hypertension (DASH) dietary pattern. *Am J Clin Nutr* 2018;**108**:243–255
115. De Filippis F, Pellegrini N, Vannini L, Jeffery IB, La Storia A, Laghi L, Serrazanetti DI, Di Cagno R, Ferrocino I, Lazzi C, Turroni S, Cocolin L, Brigidi P, Neviani E, Gobbetti M, O'Toole PW, Ercolini D. High-level adherence to a Mediterranean diet beneficially impacts the gut microbiota and associated metabolome. *Gut* 2016;**65**:1812–1821.
116. Walejko JM, Kim S, Goel R, Handberg EM, Richards EM, Pepine CJ, Raizada MK. Gut microbiota and serum metabolite differences in African Americans and White Americans with high blood pressure. *Int J Cardiol* 2018;**271**:336–339.
117. Jackson MA, Verdi S, Maxan ME, Shin CM, Zierer J, Bowyer RCE, Martin T, Williams FMK, Menni C, Bell JT, Spector TD, Steves CJ. Gut microbiota associations with common diseases and prescription medications in a population-based cohort. *Nat Commun* 2018;**9**:2655.