The gut microbiome in hypertension: recent advances and future perspectives

Avery E.G., Bartolomaeus H., Maifeld A., Marko L., Wiig H., Wilck N., Rosshart S.P., Forslund S.K., Muller D.N.

This is the final version of the accepted manuscript. The original article has been published in final edited form in:

Circulation Research
2021 APR 02 ; 128(7): 934-950
2021 APR 01 (first published online)
doi: 10.1161/CIRCRESAHA.121.318065

Publisher: American Heart Association

Copyright © 2021 American Heart Association, Inc.
The gut microbiome in hypertension: recent advances and future perspectives

Running head: Microbiome and blood pressure

Ellen Avery1,3,4,5, Hendrik Bartolomaeus1,2,3,4,5, Andras Maifeld1,2,4,5, Lajos Marko1,2,4,5, Helge Wiig7,
Nicola Wilck1,2,4,5, Stephan P. Rosshart8, Sofia K. Forslund1,2,3,4,5, and Dominik N. Müller1,2,3,4,5

Affiliations
1 Experimental and Clinical Research Center, a cooperation of Charité-Universitätsmedizin Berlin and Max Delbruck Center for Molecular Medicine, Berlin, Germany.
2 Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, Berlin, Germany.
3 Max Delbruck Center for Molecular Medicine in the Helmholtz Association, Berlin, Germany.
4 DZHK (German Centre for Cardiovascular Research), partner site Berlin, Germany.
5 Berlin Institute of Health (BIH), Berlin, Germany.
6 Freie Universität Berlin, Berlin, Germany.
7 Department of Biomedicine, University of Bergen, Norway.
8 Medical Center - University of Freiburg, Department of Medicine II, Gastroenterology, Hepatology, Endocrinology, and Infectious Diseases, Freiburg, Germany.

Corresponding author:
Dominik N. Müller, PhD
Experimental and Clinical Research Center & Max Delbruck Center for Molecular Medicine in the Helmholtz Association
Lindenberger Weg 80
13125 Berlin
Germany
Email: dominik.mueller@mdc-berlin.de Phone: +49-30-450540286 Fax: +49-30-450540944

Key Words: Microbiome, blood pressure, host-immune interaction, nutrition, short-chain fatty acids

Disclosures: None directly related to this work.
Abstract

The pathogenesis of hypertension is known to involve a diverse range of contributing factors including genetic, environmental, hormonal, hemodynamic and inflammatory forces, to name a few. There is mounting evidence to suggest that the gut microbiome plays an important role in the development and pathogenesis of hypertension. The gastrointestinal tract, which houses the largest compartment of immune cells in the body, represents the intersection of the environment and the host. Accordingly, lifestyle factors shape and are modulated by the microbiome, modifying the risk for hypertensive disease. One well-studied example is the consumption of dietary fibers, which leads to the production of short-chain fatty acids and can contribute to the expansion of anti-inflammatory immune cells, consequently protecting against the progression of hypertension. Dietary interventions such as fasting have also been shown to impact hypertension via the microbiome. Studying the microbiome in hypertensive disease presents a variety of unique challenges to the use of traditional model systems. Integrating microbiome considerations into preclinical research is crucial, and novel strategies to account for reciprocal host-microbiome interactions, such as the wildling mouse model, may provide new opportunities for translation. The intricacies of the role of the microbiome in hypertensive disease is a matter of ongoing research, and there are several technical considerations which should be accounted for moving forward. In this review we provide insights into the host-microbiome interaction and summarize the evidence of its importance in the regulation of blood pressure (BP). Additionally, we provide recommendations for ongoing and future research, such that important insights from the microbiome field at large can be readily integrated in the context of hypertension.

Non-standard abbreviations and acronyms

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP</td>
<td>Blood pressure</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>SI</td>
<td>Small intestine</td>
</tr>
<tr>
<td>LI</td>
<td>Large intestine</td>
</tr>
<tr>
<td>SCFA</td>
<td>Short chain fatty acid</td>
</tr>
<tr>
<td>GF</td>
<td>Germ-free</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular disease</td>
</tr>
<tr>
<td>FMT</td>
<td>Fecal microbiota transplantation</td>
</tr>
<tr>
<td>NGS</td>
<td>Next-generation sequencing</td>
</tr>
<tr>
<td>SHR</td>
<td>Spontaneously hypertensive rat</td>
</tr>
<tr>
<td>WKY</td>
<td>Wistar-Kyoto rat</td>
</tr>
<tr>
<td>DASH</td>
<td>Dietary Approaches to Stop Hypertension</td>
</tr>
<tr>
<td>Th17</td>
<td>T helper 17 cells</td>
</tr>
<tr>
<td>Th1</td>
<td>Type 1 helper cells</td>
</tr>
<tr>
<td>Treg</td>
<td>T regulatory cells</td>
</tr>
<tr>
<td>GPR41</td>
<td>G protein-coupled receptor 41</td>
</tr>
</tbody>
</table>
Introduction

Microbes are everywhere. They self-organize, creating complex ecosystems within otherwise uninhabitable niches, rapidly adapting to their environment. The human holobiont is the assembly of many different species to form a singular functional unit. Adult human beings contain slightly more than 50% microbial cells, outnumbering the cells of the human host \((3.0 \times 10^{13})\). During pregnancy, the developing fetus is virtually sterile, though upon birth newborns immediately collect up to \(3.8 \times 10^{13}\) bacteria from 500 to 1000 different microbial species at important epithelial barrier sites, reaching a total mass of about 1.5 kg during adulthood, about the same weight as the liver. The microbiome, defined as a catalog of all microbes in a site and their genes, encodes over 40 million distinct gene variants. Half of these gene variants are unique to a single individual, which demonstrates the striking heterogeneity of human microbiome data and perhaps may explain the elusiveness of a universally "healthy" microbiome configuration. The host depends on the microbiome for several fundamental symbiotic functions, such as the priming of the immune system and the production of essential vitamins as well as for the energy harvest from foods. The gut microbiota, defined as the microbial taxa within a human, is now regarded an endocrine organ which generates metabolites that can act as effectors in the host, triggering responses in the local microenvironment or distant target organ systems such as the heart, kidney, vasculature, and brain (Figure 1).

The first description of a living organism in the human gastrointestinal (GI) tract dates back to 1681 when Antonie van Leeuwenhoek reported a number of ‘little animals’ in his stool samples; followed by the isolation of *Escherichia coli* as the first species from the GI tract in 1885. Dramatic technological developments ensued over the proceeding centuries with the establishment of high-throughput sequencing technologies and metabolomics, as well as developments in high-performance computing and artificial intelligence. These advancements have opened a new avenue to decipher the interrelationship between lifestyle, diet, pharmacotherapy, and the gut microbiome.
Each individual’s respective ‘healthy’ gut microbiome is relatively stable over time and coexists in equilibrium with the surrounding environment. Perturbations such as antibiotics, intestinal infections, profound dietary or lifestyle changes, such as moving on or off a ‘Western diet’ can induce transient or persistent changes to this ecosystem. Microbiomes from diseased and non-diseased individuals differ (exhibiting a dysbiotic as opposed to eubiotic state), and this has been demonstrated to hold for several inflammatory (e.g. colitis), cardiovascular, and metabolic disorders. In recent years, the microbiome field has increasingly shifted focus from taxonomic (“Who’s there?”) to functional (“What are they doing?”) analyses with an accompanying emphasis on host-microbiome interactions at the molecular mechanistic level across time and space. Furthermore, the interplay between the host and it’s microbiomes is a critically important consideration. As this reciprocal interaction becomes more evident, novel strategies to account for it in preclinical disease modeling are emerging. A major future challenge is to move beyond biomarker identification to identify bioactive metabolites which could be candidates for therapeutic use. Nevertheless, improved understanding of the host-microbiome interface provides an exciting new avenue for the development of pharmacological and non-pharmacological treatments for hypertensive disease.

**Gut microbiome-host immune interaction**

The “internal environment” of the intestines acts as an interface between the external environment and the host and is constantly challenged by the consumption habits of the host. On the luminal side, microbes are able to attach and colonize this space, while on the host side the GI tract acts as the largest compartment for immune cells in the body. The physiology of the GI tract and accompanying immune cells has been extensively reviewed (by Mowat & Agace). Additionally, the structural and functional comparability of human and mouse GI physiology, and the resultant implications for translational microbiome research, has been outlined elegantly elsewhere (see Nguyen et al.). Therefore, here we will briefly describe aspects necessary to understand GI involvement in hypertensive disease, though our review of this topic is by no means comprehensive.

Anatomically, the intestines consist of different segments. The duodenum, jejunum, and ileum represent the small intestine (SI), which occupies much more physical space than the large intestine (LI), which is made up of the colon and rectum. Rodents have an enlarged cecum compared to humans, which is a blind-ended sac connecting the SI and LI. In mice, the cecum acts as a large reservoir for the commensal microbes that are involved in the fermentation of fibers that cannot be otherwise cleaved by SI enzymes. The role of the cecum in mice is nontrivial, as this acts as the major sight of production for short chain fatty acids (SCFAs), and removal of this compartment led to increased inflammation at distal sites within the GI tract. In humans, the volume of microbes is far smaller than in mice, but this compartment still plays an important role in facultative anaerobic fermentation.
Of note, the composition and abundance of commensal microbes is quite distinct in the different GI regions, for example, abundance of microbes is low in the adult SI (<10^5 microorganisms per ml) and increases to 10^12 in the colon. The SI and LI have distinct physiological functions. While the duodenum and jejunum are involved in the process of digestion, absorption of nutrients, and motility, the LI has 3 primary functions: absorption of water and electrolytes, production and absorption of vitamins, and formation and transport of feces for elimination. Mucus lining the gut lumen represents a physiological barrier against bacterial infections and can bind toxins. In addition, the mucus serves as nutrient source for bacteria, and thus affects colonization by microbes which have the ability to survive and expand in the mucus layer. Akkermansia muciniphila and Citrobacter rodentium are capable of degrading mucin, and the latter blooms during fiber deprivation. Loss of integrity of the colonic mucus layer increases host susceptibility to pathogens. Under healthy conditions, the tight epithelial layer prevents the invasion of pathogenic microbes while certain stimuli like inflammatory disease or a ‘Western Diet’ can lead intestinal permeability and the development of so-called leaky gut syndrome. Altogether, the evidence pinpoints to the pivotal role of the gut microbiota in gut epithelial health.

The intestine is continually exposed to food particles and food antigens, physiological or opportunistic microbiota-derived metabolites, and other immunomodulatory stimuli. Immune cells within the GI tract not only respond to antigenic provocations within the gut but have been shown to egress to distal organs throughout the body, indicating their importance in systems-wide inflammatory homeostasis. We are now just beginning to understand the full breadth of spatial interactions of these stimuli with the respective immune compartment (Figure 2). Gnotobiotic mice are those with a defined community. Germ-free (GF), which are devoid of all bacteria, are an extreme but useful gnotobiotic model system used to elucidate the impact of bacteria on the host immune system and physiology. GF mice have increased gut permeability which could be reversed upon colonization with microbes. GF mice are also severely compromised in immune cell function and lymphoid organ development. Colonization experiments have demonstrated the importance of certain microbes such as segmented filamentous bacterium for the regional specialization of immune cells along the length of the intestine. They have also provide detailed insight as to how human-resident bacterial species may modulate the host immune system. The importance of the microbiota-immune dynamics in human disease is clear as well. For example, fecal microbiota transplantation (FMT) affects the severely perturbated immune system in patients after radiation and chemotherapy during hematopoietic cell transplantation. Furthermore, microbial colonization in early life is critical for the maturation of the human immune system, and perturbations during this phase have been shown to influence susceptibility to allergies and infectious diseases.

Over the last few decades, experimental and clinical studies have demonstrated that cells of the innate and adaptive immune system play pivotal roles in the pathogenesis of hypertension, target
organ damage, and CVD. \textsuperscript{33-35} Pro-inflammatory effector memory T cells (T\textsubscript{EM}), T helper cell subtypes T\textsubscript{H17} (producing IL-17) and T\textsubscript{H1} (producing IFN-\gamma) promote hypertension and cardiovascular target organ damage while regulatory T cells (T\textsubscript{reg}) typically produce high amounts of anti-inflammatory IL-10 and can ameliorate vascular, cardiac and renal damage.\textsuperscript{36-42} In addition, gamma delta (\gamma\delta) T cells\textsuperscript{43} and myeloid derived suppressor cells\textsuperscript{43} play also an important role in the pathogenesis of hypertension. Dendritic cells, which can alter the activation state of several T cell subtypes, have been shown to increase in salt-responsive hypertension and are suggested to play a role in the interplay between microbial dysbiosis and BP.\textsuperscript{44} Bacteria can communicate with different immune cells involved in CVD, either directly or through the metabolites that they produce. For example, segmented filamentous bacteria or \textit{Bifidobacterium adolescentis} induced T\textsubscript{H17} cells\textsuperscript{27, 45-47} while \textit{Lactobacillus murinus} and their tryptophan metabolite indole-3 lactic acid inhibits T\textsubscript{H17} cells.\textsuperscript{48} Further, \textit{Clostridium} spp. and the short-chain fatty acid (SCFA) butyrate are outstanding inducers of T\textsubscript{reg}s in the colon.\textsuperscript{49, 50}

**Gut microbiota and hypertension**

The development of high BP is a complex, multifactorial process that involves both genetic and environmental risk factors. While 901 loci have been identified in the latest genome-wide association study, altogether this can only explain 5.7\% of BP variability.\textsuperscript{51} Additionally, population data from the UK Biobank suggests that lifestyle factors can account for up to 4–5mmHg.\textsuperscript{52} Of note, even a 2 mm Hg BP reduction would decrease overall cardiovascular disease (CVD) mortality by 7\%.\textsuperscript{53} Much of the evidence for the role of the gut microbiome and hypertension has accumulated in the last decade due to the reduction in costs for next generation sequencing (NGS). Several cross-sectional studies in humans show an association between gut microbiome and BP or hypertension.\textsuperscript{54-63} Alpha-diversity describes the microbial variance within a given ecosystem, captured as a biological sample. The majority of published microbiome studies in humans identified a reduced alpha-diversity in hypertensive patients or in patients with higher BP\textsuperscript{55-60, 63} which has also been observed in obesity, hyperinsulinemia, and dyslipidemia. Numerous human gut microbiome studies have reported an association between a higher abundance of Gram-negative microbiota including \textit{Klebsiella}, \textit{Parabacteroides}, \textit{Desulfovibrio}, and \textit{Prevotella} and higher BP, although not all studies were able to identify this pattern.\textsuperscript{54, 56, 59, 61} Data from the cross-sectional HELIUS cohort study\textsuperscript{58} demonstrated that \textit{Klebsiella} spp. and \textit{Streptococccaeae} spp. were positively correlated with BP, as found previously.\textsuperscript{59, 61} Moreover, germ-free mice that received FMT from a hypertensive human donor developed a similar gut microbiota to their donor, as well as elevated systolic and diastolic BP after 8 weeks when compared to germ-free mice which received an FMT from 2 normotensive donors.\textsuperscript{56} In addition, there are several valuable rodent hypertension models which have analyzed the role of the gut microbiome and BP.\textsuperscript{48, 60, 64-67} Adnan et al. demonstrated that the gut microbiota of stroke-prone SHR rats is...
dysbiotic and significantly different than the microbiota of normotensive WKY control rats.\textsuperscript{64} Furthermore, FMT from stroke-prone SHR into WKY controls increased the systolic BP of these otherwise normotensive rats.\textsuperscript{64} Dysbiosis was also described for hypertension models such as angiotensin II-infused mice\textsuperscript{66}, Dahl salt-sensitive rats\textsuperscript{65} as well as high salt-treated mice\textsuperscript{68}, and deoxycorticosterone acetate-salt hypertensive mice.\textsuperscript{67} Additionally, a recent study demonstrated in SHR rats that microbial dysbiosis was linked to pathophysiological changes in the GI tract and diminished intestinal integrity.\textsuperscript{68} Furthermore, intestinal permeability and dysbiosis in SHR could be remedied by treating rats with the anti-hypertensive drug losartan.\textsuperscript{69}

The link between the gut microbiome and hypertension is not species-specific. For example, high salt treatment in both mice and humans reduced \textit{Lactobacillus} spp. abundance and led to an increase in BP.\textsuperscript{48} Of note, a modest reduction in salt in therapy-naïve hypertensive patients was able to reduce BP and improve arterial compliance.\textsuperscript{70} Improved clinical outcomes were accompanied by an increase in 8 circulating SCFA (including 2-methylbutyrate, butyrate, hexanoate, isobutyrate, and valerate).\textsuperscript{70} Furthermore, it was shown that probiotic \textit{Lactobacillus} treatment inhibited Th17 cells and ameliorated salt-sensitive hypertension by restoring indole-3 lactic acid levels, which is product of microbial tryptophan metabolism.\textsuperscript{48} In addition, \textit{Lactobacillus coryniformis} has been shown to improve vascular function and insulin sensitivity.\textsuperscript{71} \textit{Lactobacillus} treatment not only ameliorates cardiovascular diseases (CVD), but also improves experimental autoimmune disease outcomes (detailed in depth in a previous review\textsuperscript{72}). A systematic review of randomized, controlled trials investigating the role of probiotics on high BP showed that \textit{Lactobacillus}-containing probiotics are effective, if used in sufficiently high doses and for at least 8 weeks.\textsuperscript{73} Despite preliminary advances to understand the role of the microbiome in the regulation of BP, it is also essential to elucidate how environmental factors affect this nexus.

\textbf{Lifestyle affects the gut microbiome}

In humans, core microbial communities in the gut are quite stable and change only in response to major perturbations such as enteric infection, global travel or medication, leading to transient or persistent changes in the gut microbiome.\textsuperscript{5,74} In addition, members of the gut microbiota are not only reactive to proportions of certain dietary stimuli, but also may also respond in a spatio-temporal context. Currently, our understanding of the exact mechanisms by which specific dietary changes affect susceptibility to inflammatory, autoimmune, and cardiovascular diseases is rather vague. Using machine learning algorithms trained on microbiome composition and function offers an exciting opportunity to facilitate better predictions of responsiveness to nutritional stimuli. A landmark study by Segal and colleagues investigated a large cohort of healthy individuals and found that they could predict glycemic variability in response to a meal challenge using individual microbiome and nutritional information.\textsuperscript{75}
Emerging research suggests that dietary factors (high salt or high fiber) and lifestyle interventions (salt restriction or caloric restriction) influence microbial community structure and function, and this has major implications for immune cell activation and BP. A western lifestyle typically involves consuming several main meals per day and leads to decreased bacterial diversity, the overgrowth of some commensals, and concomitant suppression of others.\textsuperscript{76, 77} Accordingly, production of metabolites by the bacterial community is shifted\textsuperscript{78}, which promotes inflammation\textsuperscript{79} and ultimately can lead to the development of diseases such as obesity\textsuperscript{80} and atherosclerosis.\textsuperscript{82} While historically meals were often cooked freshly, nowadays humans more frequently consume processed foods, which generally have a higher salt content.\textsuperscript{82} This lifestyle often leads to a higher salt intake than the recommendations of medical guidelines or experts.\textsuperscript{83-85} To reduce the risk of cardiometabolic disease, a healthy diet and exercise are often prescribed. Most recommendations center around changing the ‘Western diet’, which is rich in saturated fat, sugar, salt, and calories but low in fiber, to a healthier ‘Mediterranean-like’ DASH diet\textsuperscript{86} to achieve optimal nutrition, including negative energy balance and lower salt intake, although compliance is a major challenge. Fasting, as an extreme form of caloric restriction, plays an important role in different cultural and religious practices. Dramatic caloric restriction not only affects host health and physiology, but also lowers BP.\textsuperscript{87} Lifestyle and diet-induced perturbations of the microbiota and its metabolites can directly affect epithelial cell and immune cell homeostasis.\textsuperscript{9, 46, 48, 88-90} Our understanding of the connection between nutrition, the microbiota and microbial products, the immune system and host health or disease is still in its infancy.\textsuperscript{6, 91}

**Microbiota-derived short-chain fatty acids**

One of the most well-characterized microbiota-derived metabolite classes is SCFAs, which are produced during the process of fermentation of undigestible fibers. Acetate, propionate, and butyrate are the 3 SCFA of high abundance. Dietary fiber is a hypernym for dietary carbohydrates made up of ≥3 monomers, such as non-starch polysaccharides, resistant starches, inulin, pectins, β-glucan, and oligosaccharides. Most of these fibrous compounds are digested by microbes from the Bacteroidetes, Firmicutes and Actinobacteria phyla. The species *Bifidobacterium adolescentis*, *Eubacterium rectale*, *Eubacterium hallii*, *Faecalibacterium prausnitzii* and *Ruminococcus bromii* typically colonize in the LI and possess enzymes to digest fibers for SCFA production. The LI has about 4-fold higher propionate and butyrate levels compared to SI.\textsuperscript{92} SCFAs are rapidly absorbed in the colon\textsuperscript{93}, and butyrate is utilized to a large extent as fuel to supply energy to colonic epithelial cells.\textsuperscript{94} Of note, intestinal SCFAs are much higher compared to portal blood, whereas SCFA are higher in the portal, then hepatic blood, and least in the peripheral blood, suggesting SCFA are substantially taken up by the liver.\textsuperscript{92} Propionate uptake by the liver serves as precursor for gluconeogenesis, liponeogenesis, and protein synthesis\textsuperscript{95}, while acetate enters the circulation and is metabolized by several tissues and
is a substrate for cholesterol synthesis. SCFA can bind to the G-protein coupled receptors Gpr41, Gpr43, Gpr209a, Olfr558 and Olfr78 in mice, which are also known as free fatty acid receptors (FFAR). FFARs can be found in a variety of tissues, including the vasculature and kidney, and are involved in the regulation of vasoreactivity in response to propionate, acetate, and butyrate. SCFA-induced G-protein coupled receptor signal transduction has been elegantly reviewed by Poll et al.

Of note, Gpr41 and Olfr78 are both involved in the regulation of BP, although they seem to promote opposing actions. Renin secretion is induced upon Olfr78 activation. In line with this, Gpr41 knockout mice are hypertensive, while Olfr78 null animals are hypotensive. Interestingly, acetate was formerly used in hemodialysis buffers, but was largely abandoned, because of its hypotensive action, consistent with the concept that for the most part, SCFAs lower BP. Even though epidemiological and interventional studies have suggested that dietary fiber intake lowers BP, the daily fiber intake in Western societies is often below the recommendations found in nutritional guidelines. Fiber itself has been suggested to shape microbiome composition to some extent. With regards to BP, the stimulatory action of fibers increase the abundance of the SCFA producers Faecalibacterium prausnitzii and Eubacterium rectale as well as Lactobacillus spp., and possess bifidogenic properties. A landmark study showed that European children consuming a Western diet have significantly lower SCFA levels and a high Firmicutes-to-Bacteroidetes (F/B) ratio compared African children, who traditionally had an unprocessed diet high in fiber. Since that study, a high F/B ratio is often used as a surrogate marker of gut dysbiosis, although some Firmicutes bacteria are also known produce microbial metabolites which contribute to a healthy microbiome. Likewise, experimental work often relies on the F/B ratio as a disease marker. Spontaneously hypertensive rats (SHR) and the stroke prone SHR (SHRSP) strain show an increase in F/B ratio, supporting the concept that this can act as a marker for gut dysbiosis.

Blood pressure and SCFA in health and disease

Various experimental or clinical studies have demonstrated the effects of prebiotic higher fiber or postbiotic SCFA treatment on BP. Pluznick et al. reported that propionate induces an acute dose-dependent hypotensive response in anesthetized mice, which was mediated by Gpr41. Kaye and colleagues elegantly showed that prebiotic fiber not only prevents cardiovascular disease, but deficiencies in these nutrients may be a risk factor for the development of hypertension and CVD. The addition of the postbiotics acetate, propionate or butyrate to a low fiber diet was also found to improve BP and reduce target organ damage. Further, FMT in GF mice demonstrated that the gut microbiome resultant of a diet lacking resistant starch compared to the high fiber situation not only resulted in higher BP upon angiotensin II challenge, but also contributed to the pathogenesis of cardiac and renal damage. Similarly, our group recently tested the properties of oral propionate treatment in hypertensive mice, with and without atherosclerosis. Propionate treatment decreased
systemic and local inflammatory responses, BP, and cardiac damage in both models. The therapeutic effects of propionate are mediated by Treg cells. In our study, the BP-lowering potential of propionate was not acute, but occurred over time, suggesting that anti-inflammatory properties of SCFAs indirectly contributed to the improvement of the vascular phenotype. Furthermore, others have shown that Th17 cells and the balance of Th17 to Tregs mediate the role of SCFAs in BP regulation.

Human studies on the role of SCFAs on BP are rather rare. Some studies of microbiota composition and hypertension have indicated SCFA-producers Ruminococcaceae spp., Rothia, or Roseburia spp. were associated with lower BP. In a small intervention trial, the postbiotic butyrate (600 mg/d), the prebiotic inulin (10 g/d), and the combination of these two all reduced diastolic BP in patients with metabolic syndrome. In the HELIUS cohort, machine learning algorithms applied to microbiome data identified Roseburia spp. to account for the largest absolute effect on BP. The highest tertile of Roseburia spp. abundance was associated with a 4.1 mmHg lower systolic BP even after adjusting for confounders, including use of medication. Conversely, fecal SCFA levels were higher in patients with higher BP. This positive correlation is in line with previous studies, but seems to contradict to the negative correlation between BP and microbial SCFA-producers within the GI tract. However, fecal SCFA levels do not necessarily reflect the SCFA levels within the intestine, but rather reflects the SCFA generated in the gut which was not absorbed by the host. This notion is supported by experimental work in SHR rats demonstrating that colonic butyrate absorption into the host is reduced in experimental hypertension. In addition, the AT1 receptor blocker Candesartan, a drug frequently used for the treatment of hypertension, was found to increase Lactobacillus abundance and fecal SCFA levels, improve intestinal integrity, and reduce BP in SHR rats. In the human MetaCardis cohort, depletion of butyrate production genes in the gut of severely obese subjects was ameliorated by Candesartan treatment (Forslund et al., in revision). Altogether in the HELIUS cohort, machine learning models based on gut microbiota composition explained 4.4% and 4.3% of systolic and diastolic BP variability, respectively.

Fiber-derived SCFAs do not solely affect BP, but also play a pivotal role in other cardiovascular diseases and autoimmunity. For instance, postbiotic treatment with acetate, propionate or butyrate ameliorates acute kidney injury. Renoprotection was associated with a reduced local and systemic inflammatory response, oxidative cellular stress, and apoptosis. In an animal model of multiple sclerosis (MS), a T cell-mediated inflammatory disease of the central nervous system (CNS), propionate increased the frequency of anti-inflammatory Tregs in the gut and spleen, and this was accompanied by an amelioration of clinical symptoms. High fiber intake and increased SCFA concentrations have also been shown to protect the CNS. Of note, patients with MS were shown to benefit from propionate treatment. Short-term propionate treatment resulted in a significant and sustained enrichment of functionally competent Tregs, while Th1 and Th17 cells were...
In addition, SCFA supplementation or high-fiber intake positively influences outcomes in rheumatoid arthritis, a chronic inflammatory disorder of the joints. Propionate can increase bone mass, and SCFAs were found to stimulate bone formation by increasing the number of Treg cells.

**Interplay between SCFA and the immune system**

Mechanistically, SCFAs can affect distinct immune cell populations. For instance, neutrophils were found to produce less inflammatory cytokines upon propionate and butyrate treatment. Butyrate can also reduce oxidative stress and phagocytic capacity. SCFAs modulate the inflammatory process by decreasing dendritic cell maturation and inhibiting CD4 and CD8 T cell proliferation. In contrast to acetate, butyrate or propionate affects dendritic cell maturation from bone marrow precursor cells by histone deacetylase (HDAC) inhibition. Moreover, butyrate was shown to provoke M1 macrophages to secrete fewer inflammatory cytokines, and increases secretion of the anti-inflammatory cytokine interleukin (IL)-10.

SCFAs also elicit the expression of anti-inflammatory signatures in human monocytes and T cells. For example, butyrate inhibits IL-12 production in *Staphylococcus aureus*-stimulated human monocytes and enhances the secretion of IL-10. Recently, we have demonstrated that propionate decreases rate of Th17 cell differentiation. Butyrate was also found to increase the secretion of IL-10 in Th1 differentiated cells via Gpr43. SCFA-driven induction of IL-10 activates STAT3 and mTOR, and consequently upregulates transcription factor B lymphocyte-induced maturation protein 1. Further, one of the most well-studied properties of SCFAs is their role in the induction of anti-inflammatory Tregs. We and others showed that butyrate and propionate increase the differentiation of murine and human Tregs and enhance their suppressive capacity. In addition to butyrate, *de novo* Treg cell formation in the periphery was also induced by propionate, but not acetate, via HDAC inhibition. Of note, *Clostridia*, a dominant class of commensal microbe, mediated the induction colonic Tregs, which is in line with findings that *Clostridium butyricum* induces Tregs and reduces Th17 cells, thereby ameliorating symptoms in experimental autoimmunity.

**Fasting as a novel treatment strategy for blood pressure control**

Accumulating evidence suggests that fasting is an effective tool to manage metabolic and inflammatory diseases. The rationale that caloric restriction impacts the microbiome is intriguing, nevertheless, robust data in humans is still scarce. Mesnage and colleagues investigated the effect of a 10-d periodic fast on the fecal microbiota of fifteen healthy men. Fasting caused a decrease in *Lachnospiraceae* and *Ruminococcaceae*. A small human pilot study showed that Ramadan fasting affected the microbiome of healthy subjects enriching several SCFA producers. We evaluated the impact of fasting in metabolic syndrome patients (Maifeld medRexiv 2020).
doi: https://doi.org/10.1101/2020.02.23.20027029; Nat. Commun. 2020 in press). In a clinical study, thirty-five metabolic syndrome patients underwent a 5 day fast followed by 3 weeks of DASH diet. A control group received DASH diet only. Fasting followed by the DASH diet reduced BP, need for antihypertensive medication and body weight 3 months post intervention, and altered the gut microbiome impacting SCFA producers. Stratification of the cohort to BP responsiveness showed that immune cell changes present in the fasting arm are more pronounced in BP responders than in non-responders. Furthermore, the immune shift in the fasting arm was fundamentally different from observed changes seen in the DASH arm. A BP responder-specific microbiome changes in the fasting arm post-intervention (enrichment of F. prausnitzii, Bacteroides and Firmicutes, depletion of Actinomyces) was observed. Of note, the enrichment of the butyrate producer F. prausnitzii remained even 3 months post fasting. Computational analyses revealed that BP responders and non-responders not only reacted differentially to fasting but differed at baseline with regards to their propionate synthesis capacity. Applying machine learning algorithms to either baseline immunome or 16S microbiome data, we were able to make effective predictions regarding which patients would respond to fasting with a reduction in BP. The prediction model was confirmed by reanalyzing the data from the only other existing cohort (the Mesnage dataset) investigating the effect of fasting and BP. A significant long-term BP decrease in the Mesnaga cohort was predicted with about 70% accuracy, further supporting the idea that these findings are likely generalizable. It is important to emphasize that the aforementioned studies established associations between the microbiome and BP in response to fasting. Fasting is a daunting challenge for many patients. Being able to manipulate the mechanisms responsible for the change in BP in response to fasting would be of high clinical utility. However, few studies have succeeded in establishing mechanisms in microbiome research which hold up in a clinical setting. The high-profile failure of a SER-109, a stool-derived bacterial spore mixture aimed at recurrent Clostridium difficile infections, during phase 2 trials is a prime example. The pharmacologic was designed for a different disease state, however, the translation challenges facing the microbiome field at large also apply in the context of hypertension.

From mice to men: translational challenges

As detailed throughout this review, the host-microbiome interaction is clearly influential in human health and disease. The interactions between the host and its various microbial inhabitants are reciprocal in nature, meaning the host genetics, microbial genetics, and the interplay of these two spaces all could be explanations for the resultant phenotype. In hypertension, model systems are often used in basic and preclinical research to study the pathogenesis and progression of disease. Mouse and rat models are extremely informative and can provide information which would not be available from a human cohort study. However, there are many barriers to the study of the human host-microbiome interaction in model systems.
There are many aspects of gastrointestinal physiology and morphology, which are distinct between human and rodent species\textsuperscript{14}. The cecum is perhaps the most obvious example of divergent speciation in humans and rodents, as well as the thinness of the mucus layer in the colon of mice compared to humans\textsuperscript{14}. Indeed, the genetics of model systems themselves is a double-edged sword; inbreeding and genetic homogeneity provides an easy platform for genetic manipulation, but one may question the utility of this oversimplification for translational research. The diversity of human genomes indelibly impacts individual susceptibility to disease, and the interactions between host and microbial genes is a growing area of research for the treatment of complex ailments such as Type 1 diabetes and Crohn’s disease\textsuperscript{14}. Additionally, the genomic responses to inflammatory stressors in mice and humans are starkly contrasting, which could be related to either host-specific or microbiome-specific features, or combination of the two\textsuperscript{14}. The microbes inhabiting the GI tract are often also distinct between mice and humans. Although they may be comparable on the genus or phylum level, species-specific changes are often shown to have clinical importance in hypertension\textsuperscript{143}. Additionally, it is known that within each microbial clade, the degree to which functional properties are shared between member species differ\textsuperscript{144}. For example, \textit{Firmicutes} species are particularly metabolically inconsistent as a clade\textsuperscript{144}, which again presents an issue when considering the ubiquitous use of the F/B ratio as a marker for dysbiosis. Furthermore, because of the isolation of lab-raised mouse communities for extended periods of time, which is frequently unavoidable, the microbiome and associated immune competency of sub-strains can also be divergent from one another\textsuperscript{145-147}. Interestingly, a recent study found that within two sub-strains of mice with subtle differences in the host genome, colonization with an IBD-associated \textit{Bacteroides} species induced disease in only one mouse strain, despite robust colonization of all mice\textsuperscript{148}. Littermate controls have therefore become an important standardization technique for microbiome studies\textsuperscript{140, 149}. Use of littermate controls can provide important context, for example, two recent studies suggested a role for Nlrp6- and ASC-mediated inflammasomes in shaping commensal gut microbiota composition\textsuperscript{150}, although none of these results were reproducible when the appropriate littermate controls were used\textsuperscript{152}. Additionally, the coprophagic nature of rodent models has been suggested to have a unique impact on the microbiome, which can be avoided using single housing strategies, although this induces a stress response in mice, adding an additional confounding factor.

Many researchers attempt to circumvent the issue of non-comparable species in mice compared to humans by using human microbes to colonize mice. This presents two important challenges, which should be considered. First, there is the issue of the reciprocal nature of interactions between the host and its microbes, of which the host is unavoidably different when using rodent models. Indeed, the importance of this interaction was exemplified in a recent study which showed that the colonization of germ-free mice with human or rat microbes did not induce immune maturation, and only mouse-specific microbes were able to induce full immune competence\textsuperscript{153}. 
Several differences have been noted between mouse and human immune composition which may be related to the immune-microbiome axis, for example, the proportion of neutrophils in the peripheral blood is about twice as high (50-70%) in humans compared to mice (10-25%).\textsuperscript{154, 155} Furthermore, the distribution of CD8+ T cells in nonlymphoid tissue in adult humans is much higher than what is found in specific pathogen free (SPF) mice, which could have implications for the progression of intracellular infections or cancer.\textsuperscript{156, 157} This is not surprising considering the coevolution of hosts and their respective microbiomes.\textsuperscript{140, 155} Human and mice only share about 15% of bacterial lineages.\textsuperscript{158} Due to the niche-specific specialization of microbes\textsuperscript{144}, even implantation of defined species may not induce the desired effect and can vary in effect depending on the rodent model used.\textsuperscript{24} Second, given that the human microbiome is so highly diverse, how should one determine which human microbiome to use for experimentation? Protocols in this sense vary between studies.\textsuperscript{159} Standardized procedures have been suggested throughout the literature\textsuperscript{159, 160}, such as household member or internal baseline controls, but have not been adopted universally. Despite these caveats, mice implanted with a microbiome grafted from diseased patients have been found to recapitulate clinical findings in several disease states, including in hypertension.\textsuperscript{56}

**From mice to men: future perspectives**

Despite the ubiquitousness of using SPF inbred rodent models for disease research, alternative approaches are gaining traction. The intention to avoid infections which may alter experimental results is logically sound. However, there is mounting evidence that suggests humans are relatively “dirty”, and that exposure to a full range of fungi, viruses, microbes and so on are needed for the robust development of a healthy immune system.\textsuperscript{161, 162} Indeed, a recent interventional study exposed children whose environment was otherwise highly hygienic to increased microbial biodiversity, which led to an increase in the amount of healthy commensal bacteria in the gut and skin, and a shift towards anti-inflammatory cytokine production in the skin\textsuperscript{163}. It has been suggested that preclinical modeling of immune response in rodents likely contributes to the high failure rate in clinical trials\textsuperscript{156, 164}. Humans are acutely and chronically infected with pathogens throughout their lifetime, which shapes their immune systems.\textsuperscript{165, 166} Furthermore, infections can also alter reactivity to vaccinations and subsequent unrelated infections, and it was recently shown that sequential infection in mice can recapitulate these effects.\textsuperscript{167}

Several studies have shown that using a mouse with a more natural environment or mice captured from the wild are quite different and may more accurately recapitulate human physiology than lab-raised SPF mice.\textsuperscript{164, 168, 169} It has been proposed that the scientific community may be able to improve preclinical pipelines with the use of mice with a “wild” mouse microbiome, rich in both commensals and opportunistic pathogens that are not normally present in lab-raised mice. Wild mice which were selected for their close similarity in genomic background were shown to have a distinct
immune profile compared to laboratory mice. The applicability of a strategy involving the capture of mice from the wild prior to experimentation is unlikely to be adopted widely. However, a recent study demonstrated that implantation of the full breadth of microbes from wild mice onto a C57BL/6NTac background (referred to as “wildling” mice) generates equally promising results (Figure 3). Wild and wildling mice, compared with standard lab-raised mice, had much higher concordance of mouse data with the results of clinical trials, and were more resistant to disease. The wildling mouse microbiome was also more resilient to a high-fat dietary challenge over the course of ten days than their lab-mouse counterparts, whose microbiome drastically shifted in response to the challenge, and was not able to fully recover. Lab mice in this regard are divergent from humans because of the relative stability of the human microbiome over time. Additionally, it has been shown that the introduction of a “wild” microbiome leads to the convergence of lab-raised mouse microbial communities. Co-housing experiments of lab mice with a “wild” counterpart also shifted the immune cell profile after about two weeks towards that of the wild mouse, which was more like the adult human phenotype. Interestingly, wild mice also have a thicker mucus layer in the colon than laboratory mice. These findings suggest the potential of this technique, particularly the wildling strategy, to be used to standardize the research into the host-microbiome interaction, thereby improving reproducibility.

Important considerations for ongoing research

Throughout this review, we have raised addressed many issues, and offered solutions to ongoing problems in the study of the microbiome. Nevertheless, microbiome science would also benefit from healthy doses of skepticism. Indeed, both animal and human studies present many challenges for ongoing research. In mouse studies, standardization of procedures throughout the field, such as the use of littermate controls and robust documentation of conditions which could impact the microbiome is essential. Factors like caging, bedding and diet are likely to substantially impact results. Furthermore, it has been recently shown that the microbiome has a diurnal circadian rhythm, in which case, time of sample collection should become a consideration. The site of sampling the microbiome is also important, as the microbial cell density, composition and the production of microbial metabolites varies throughout the GI tract. A recent study demonstrated that additional to the spatial dynamics of different GI regions, the microbiome sampled from the mucosa and the luminal space were unique in mice and in men. Because the GI tract is the site-of-action for polarization of immune cells and absorption of microbially produced metabolites, many have questioned whether fecal sampling is the right avenue for studying the host-microbiome interface. Feces represents the excretory products from this system. However, fecal sampling is most common and practically applicable way to examine the microbiome, particularly for longitudinal studies where non-invasive methods are necessitated. The collection of fecal matter no doubt
substantially contributes to our understanding of the host-microbiome interaction. Although the relevance of locally produced microbial biproducts is suspected to be of importance, particularly impacting the uptake of metabolites to the circulation and effecting the activity of GI immune cells, measurements of this compartment are underdeveloped. The ability to identify microbiologically-produced compounds at the site-of-action in the interstitial fluid would likely provide a different perspective on host-microbiome dynamics.

Microbiome analysis adds another layer of complexity to experimental design. Particularly in human studies, procedural standardization is necessary to increase reproducibility and accelerate progress. Recent studies have suggested that sample collection, storage, and extraction methods can all contribute to resultant microbiome quantification. Particularly the extraction method appears to play a major role in the overall microbiome signature, as it was found that the extraction method used resulted in taxonomic shifts mimicking important biologically relevant features like the enterotype or diet. Two sequencing methods, 16s rRNA sequencing and shotgun metagenomic sequencing, can be used to understand the composition of the microbiome. Recent findings suggest results from 16s and shotgun are largely concordant, regardless of which technology is selected, although importantly the resolution of shotgun is better than 16s. The main differences are that 16s sequencing only allows genus-level taxonomic resolution, where shotgun offers species-level information, and that shotgun data additionally can be used to assess the functional potential of the microbiome. Similar inferences can be made from 16s using projected annotations (e.g. using PICRUSt), however this is not as reliable or robust, and does not necessarily match up with comparable annotation from shotgun data. However, it should also be noted that 16s is more cost-effective than shotgun, and in the case that high-resolution data is not crucial, this remains a viable option for sequencing. Choice of software pipeline, settings and particularly reference databases also may have impact on results, though substantially less than biosample handling. However, when comparing multiple datasets, the analysis pipeline used for either 16s or shotgun data must be carefully considered so as not to introduce a source of bias. Beyond this, investigators must also ask themselves whether the quantification of absolute or relative abundance of microbes is best able to answer their research question. Because the variation in DNA yield from sequenced samples can influence relative abundance quantification, it is important to interpret these data with caution to avoid false positives (including through methods such as rarefaction or explicit modeling of proportions), and it may be useful to verify findings using absolute abundances, considering again possible sources of error.

Ultimately, results must always be interpreted in the context with which they were collected. Most microbiome research to date (particularly human studies) suggests associations between phenotypic data and microbes or metrics of microbial diversity. To move the field forward and begin to address whether the microbiome can be targeted or manipulated to influence the prevalence or
progression of hypertension, studies which can establish causation and uncover mechanisms are urgently needed. The two areas which promising in terms of the development of novel treatment strategies are the targeting of microbially-produced molecules (such as SCFAs) and modulation of the microbiome-immune axis.\textsuperscript{38} Considering the challenges in preclinical research using rodent models, we suggest the use of novel strategies to address these ongoing inquiries.

In conclusion, although prudence is needed in the interpretation of microbiome data\textsuperscript{37}, the study of the microbiome-host interface in hypertension is a promising and rapidly accelerating field of research. With a variety of opportunities for further advancement, we anticipate that both pharmacological and lifestyle-centered treatment options addressing the microbiome space are likely to emerge in the not-so-distant future.

Sources of Funding

D.N.M., H.B., N.W., S.F were supported by the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation; Projektnummer 394046635 - SFB 1365); D.N.M. supported by the DZHK (German Center for Cardiovascular Research, 81Z0100106). NW was supported by the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation program (NW: 852796). N.W. is supported by a grant from the Corona-Stiftung. N.W. is participant in the Clinician Scientist Program funded by the Berlin Institute of Health (BIH). Financial support from the Research Council of Norway (project number 262079), The Norwegian Health Association and from The Western Norway Regional Health Authority (project number 912168) to H. Wiig is gratefully acknowledged.
References


85. Union E. Implementation of the EU Salt Reduction Framework Results of Member States survey. 2012.


Microbiota-derived short-chain fatty acids promote Th1 cell IL-10 production to maintain intestinal homeostasis. *Nature communications*. 2018;9:3555.


Figure 1. The relationship between blood pressure and the gut microbiome. Ingested food is transformed by the gut microbiome into small metabolites. Food antigens, microbially-produced metabolites, and the microbes themselves all contribute to immune homeostasis. Perturbations to the symbiotic relationship between host and the microbiome can lead directly or indirectly, via the immune system, to changes in blood pressure and associated heart, vascular, or kidney damage.

Figure 2. Intestinal spatial variability can be found on both the host and microbiome side (adapted from Mowat & Agace, 2014). Relative levels of luminal and tissue-associated content are illustrated here, suggestive of the regional specialization of both features. The luminal contents of the intestines are known to vary significantly, in terms of microbial load, the microbial inhabitants, and the resultant microbially-produced metabolites. Shown here is a proposed scheme, although the inhabitants and regional specifications throughout the GI tract is subject to individual differences in both mice and men. In accordance with the variations in luminal content, the host immune system is likewise regionally specific. Shown here are immune cells where spatial dynamics have been demonstrated during immune homeostasis.

Figure 3. Modeling the human immune phenotype for basic and preclinical research. The mammalian phenotype is driven by the combination of the host genome and the microbial genome (microbiome), together referred to as the metagenome. However, the repertoire of microbes encountered in the wild is not replicated in a laboratory setting. This can substantially distort how the immune system develops and functions, leading to false assumptions of how our own “wild” immune system works. Thus, laboratory mice are too far removed from natural environmental conditions to faithfully mirror the physiology of free-living mammals such as humans. To address this shortcoming, embryos of laboratory mice can be transferred into wild mice to generate wildlings that more closely resemble the natural mammalian metaorganism with coevolved microbes and pathogens, while preserving the research benefits of tractable genetics of laboratory mice (interventional approaches, mechanistic studies etc.). Natural microbiota has been shown to be multi-generationally stable and resilient against environmental challenges, thereby providing a model system for long-term work and reproducible experimentation. Moreover, in two preclinical trials, where conventional lab mice as well as rat and non-human primate models had failed to predict the human response to harmful drug treatments, wildlings accurately mirrored the human phenotype. Such models may enhance the validity and reproducibility of biomedical studies among research institutes, facilitate the discovery of disease mechanisms and treatments that cannot be studied in conventional laboratory mice, and increase the safety as well as the success of translating results from animal models to humans.
Figure 2
Free-living mammals: natural metaorganism
Mature immune system possessing immune experience
Natural host genome
Natural microbial genome

Wildlings: seminatural metaorganism
Mature immune system possessing immune experience
Embryo transfer into wild mice to restore natural microbial genome
Laboratory host genome
Natural microbial genome

Specific pathogen-free mice: laboratory metaorganism
Immature immune system lacking immune experience
Laboratory host genome
Laboratory microbial genome

Creation of tratable laboratory host genome
Creation of laboratory microbial genome through germ-free rederivation

NATURAL WORLD
Diverse environment rich in inflammatory and microbial stimuli

LABORATORY WORLD
Restricted environment lacking inflammatory and microbial stimuli

REDUCED TRANSLATIONAL RESEARCH VALUE
INCREASED TRANSLATIONAL RESEARCH VALUE