# 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.



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

# The gut microbiome in hypertension: recent advances and future perspectives

**Running head:** Microbiome and blood pressure

Ellen Avery[1,3,4,5,6], Hendrik Bartolomaeus[1,2,3,4,5], Andras Maifeld[1,2,4,5], Lajos Marko[1,2,4,5], Helge Wiig[7], Nicola Wilck[1,2,4,5], Stephan P. Rosshart[8], Sofia K. Forslund[1,2,3,4,5], and Dominik N. Muller[1,2,3,4,5]

**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**

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

| | |
|---|---|
| GPR43 | G protein-coupled receptor 43 |
| GPR109a | G protein-coupled receptor 109 A |
| Olfr558 | Olfactory receptor 558 |
| Olfr78 | Olfactory receptor 78 |
| FFAR | Free fatty acid receptor |
| F/B ratio | Firmicutes-to-Bacteroidetes ratio |
| AT1 | Angiotensin II type 1 |
| MS | Multiple sclerosis |
| CNS | Central nervous system |
| HDAC | Histone deacetylase |
| STAT3 | Signal transducer and activator of transcription 3 |
| mTOR | Mechanistic target of rapamycin |
| IBD | Inflammatory bowel disease |
| SPF | Specific pathogen free |

## 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 ($3.0*10^{13}$). During pregnancy, the developing fetus is virtually sterile, though upon birth newborns immediately collect up to $3.8*10^{13}$ bacteria from 500 to 1000 different microbial species[1] 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.[2] 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.[2,3] 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).[4]

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.[5] 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.[5, 6] 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.[7-11] 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.[12] 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[13]). 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.[14]). 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.[14] 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.[13] 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.[15] In humans, the volume of microbes is far smaller than in mice, but this compartment still plays an important role in facultative anaerobic fermentation.[16]

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.[13] 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.[17] Mucus lining the gut lumen represents a physiological barrier against bacterial infections and can bind toxins.[18] 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.[19] *Akkermansia muciniphila* and *Citrobacter rodentium* are capable of degrading mucin, and the latter blooms during fiber deprivation.[20] 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.[21] Altogether, the evidence pinpoints to the pivotal role of the gut microbiota in gut epithelial health.[22]

The intestine is continually exposed 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.[23, 24] 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.[25] GF mice are also severely compromised in immune cell function and lymphoid organ development.[26] 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.[27] They have also provide detailed insight as to how human-resident bacterial species may modulate the host immune system.[28] 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.[29] Furthermore, microbial colonization in early life is critical for the maturation of the human immune system[30], and perturbations during this phase have been shown to influence susceptibility to allergies[31] and infectious diseases.[32]

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.[33-35] Pro-inflammatory effector memory T cells ($T_{EM}$), T helper cell subtypes $T_H17$ (producing IL-17) and $T_H1$ (producing IFN-γ) promote hypertension and cardiovascular target organ damage while regulatory T cells (Treg) typically produce high amounts of anti-inflammatory IL-10 and can ameliorate vascular, cardiac and renal damage.[36-41] In addition, gamma delta (γδ) T cells[42] and myeloid derived suppressor cells[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.[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 *Bifidobacterium adolescentis* induced $T_H17$ cells [27, 45-47] while *Lactobacillus murinus* and their tryptophan metabolite indole-3 lactic acid inhibits $T_H17$ cells.[48] Further, *Clostridium* spp. and the short-chain fatty acid (SCFA) butyrate are outstanding inducers of Tregs in the colon.[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[51]. Additionally, population data from the UK Biobank suggests that lifestyle factors can account for up to 4–5mmHg.[52] Of note, even a 2 mm Hg BP reduction would decrease overall cardiovascular disease (CVD) mortality by 7%.[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.[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[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 *Klebsiella*, *Parabacteroides*, *Desulfovibrio*, and *Prevotella* and higher BP, although not all studies were able to identify this pattern.[54, 56, 59, 61] Data from the cross-sectional HELIUS cohort study[58] demonstrated that *Klebsiella* spp. and *Streptococcaceae* spp. were positively correlated with BP, as found previously.[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.[56] In addition, there are several valuable rodent hypertension models which have analyzed the role of the gut microbiome and BP.[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.[64] Furthermore, FMT from stroke-prone SHR into WKY controls increased the systolic BP of these otherwise normotensive rats.[64] Dysbiosis was also described for hypertension models such as angiotensin II-infused mice[66], Dahl salt-sensitive rats[65] as well as high salt-treated mice[48], and deoxycorticosterone acetate-salt hypertensive mice.[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.[68] Furthermore, intestinal permeability and dysbiosis in SHR could be remedied by treating rats with the anti-hypertensive drug losartan.[69]

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

**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.[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.[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.[76, 77] Accordingly, production of metabolites by the bacterial community is shifted[78], which promotes inflammation[79] and ultimately can lead to the development of diseases such as obesity[80] and atherosclerosis.[81] While historically meals were often cooked freshly, nowadays humans more frequently consume processed foods, which generally have a higher salt content.[82] This lifestyle often leads to a higher salt intake than the recommendations of medical guidelines or experts.[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[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.[87] Lifestyle and diet-induced perturbations of the microbiota and its metabolites can directly affect epithelial cell and immune cell homeostasis.[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.[4, 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 Bacteriodetes, 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.[92] SCFAs are rapidly absorbed in the colon[93], and butyrate is utilized to a large extent as fuel to supply energy to colonic epithelial cells.[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.[92] Propionate uptake by the liver serves as precursor for gluconeogenesis, liponeogenesis, and protein synthesis[95], while acetate enters the circulation and is metabolized by several tissues and

is a substrate for cholesterol synthesis.[96] SCFA can bind to the G-protein coupled receptors Gpr41, Gpr43, Gpr109a, Olfr558 and Olfr78 in mice, which are also known as free fatty acid receptors (FFAR).[97] 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.[98] SCFA-induced G-protein coupled receptor signal transduction has been elegantly reviewed by Poll et al.[97] Of note, Gpr41 and Olfr78 are both involved in the regulation of BP, although they seem to promote opposing actions.[99] Renin secretion is induced upon Olfr78 activation.[99] In line with this, Gpr41 knock out mice are hypertensive, while Olfr78 null animals are hypotensive.[100] Interestingly, acetate was formerly used in hemodialysis buffers, but was largely abandoned, because of its hypotensive action[101], 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.[102-104] 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.[105, 106] 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.[107] Since that study, a high F/B ratio is often used 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.[64]

**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.[99] 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.[66] 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.[66] 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.[66] Similarly, our group recently tested the properties of oral propionate treatment in hypertensive mice, with and without atherosclerosis.[108] Propionate treatment decreased

systemic and local inflammatory responses, BP, and cardiac damage in both models.[108] The therapeutic effects of propionate are mediated by Treg cells.[108] 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. [108] Furthermore, others have shown that Th17 cells and the balance of Th17 to Tregs mediate the role of SCFAs in BP regulation.[109, 110]

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.[57-59, 61, 62] 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.[111] In the HELIUS cohort[58], 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.[58] Conversely, fecal SCFA levels were higher in patients with higher BP.[58] This positive correlation is in line with previous studies[55, 59], 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.[112] 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.[113] In the human MetaCardis cohort[114, 115], 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.[46, 60, 99, 100, 108, 116-122] For instance, postbiotic treatment with acetate, propionate or butyrate ameliorates acute kidney injury.[121] Renoprotection was associated with a reduced local and systemic inflammatory response, oxidative cellular stress, and apoptosis.[121] 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.[46] High fiber intake and increased SCFA concentrations have also been shown to protect the CNS.[117] Of note, patients with MS were shown to benefit from propionate treatment.[123] Short-term propionate treatment resulted a significant and sustained enrichment of functionally competent Tregs, while Th1 and Th17 cells were

depleted simultaneously.[123] In addition, SCFA supplementation or high-fiber intake positively influences outcomes in rheumatoid arthritis, a chronic inflammatory disorder of the joints.[116, 124, 125] Propionate can increase bone mass, and SCFAs were found to stimulate bone formation by increasing the number of Treg cells.[116]

**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.[126] Butyrate can also reduce oxidative stress and phagocytic capacity.[127] SCFAs modulate the inflammatory process by decreasing dendritic cell maturation and inhibiting $CD_4$ and CD8 T cell proliferation.[121] In contrast to acetate, butyrate or propionate affects dendritic cell maturation from bone marrow precursor cells by histone deacetylase (HDAC) inhibition.[128] Moreover, butyrate was shown to provoke M1 macrophages to secrete fewer inflammatory cytokines, and increases secretion of the anti-inflammatory cytokine interleukin (IL)-10.[129]

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.[130] Recently, we have demonstrated that propionate decreases rate of Th17 cell differentiation[46, 123]. Butyrate was also found to increase the secretion of IL-10 in Th1 differentiated cells via $Gpr43$[131]. SCFA-driven induction of IL-10 activates $STAT_3$ and mTOR, and consequently upregulates transcription factor B lymphocyte- induced maturation protein 1.[131] 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.[46, 50, 123, 132] In addition to butyrate, *de novo* Treg cell formation in the periphery was also induced by propionate, but not acetate, via HDAC inhibition.[132] Of note, *Clostridia*, a dominant class of commensal microbe, mediated the induction colonic Tregs[50], which is in line with findings that *Clostridium butyricum* induces Tregs and reduces Th17 cells, thereby ameliorating symptoms in experimental autoimmunity.[133]

**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.[87] The rationale that caloric restriction impacts the microbiome is intriguing, nevertheless, robust data in humans is still scarce.[90, 134-137] Mesnage and colleagues investigated the effect of a 10-d periodic fast on the fecal microbiota of fifteen healthy men.[90] Fasting caused a decrease in *Lachnospiraceae* and *Ruminococcaceae*.[90] A small human pilot study showed that Ramadan fasting affected the microbiome of healthy subjects enriching several SCFA producers.[135] 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.[90] A significant long-term BP decrease in the Mesnage 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.[138] 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.[138, 139] 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[140]. 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[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[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.[141] 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[142]. 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.[143] Additionally, it is known that within each microbial clade, the degree to which functional properties are shared between member species differ.[144] For example, *Firmicutes* species are particularly metabolically inconsistent as a clade[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.[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 Bacteroides species induced disease in only one mouse strain, despite robust colonization of all mice.[148] Littermate controls have therefore become an important standardization technique for microbiome studies.[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[150, 151], although none of these results were reproducible when the appropriate littermate controls were used.[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.[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%).[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.[156, 157] This is not surprising considering the coevolution of hosts and their respective microbiomes.[140, 155] Human and mice only share about 15% of bacterial lineages.[158] Due to the niche-specific specialization of microbes[144], even implantation of defined species may not induce the desired effect and can vary in effect depending on the rodent model used.[14] 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.[159] Standardized procedures have been suggested throughout the literature[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.[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.[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[163]. It has been suggested that preclinical modeling of immune response in rodents likely contributes to the high failure rate in clinical trials[156, 164]. Humans are acutely and chronically infected with pathogens throughout their lifetime, which shapes their immune systems.[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.[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.[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.[170] 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).[164] Wild and wildling mice, compared with standard lab-raised mice, had much higher concordance of mouse data with the results of clinical trials[164], and were more resistant to disease.[168] 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.[164] Lab mice in this regard are divergent from humans because of the relative stability of the human microbiome over time.[5] Additionally, it has been shown that the introduction of a "wild" microbiome leads to the convergence of lab-raised mouse microbial communities.[156, 164] 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.[156] Interestingly, wild mice also have a thicker mucus layer in the colon than laboratory mice.[171] 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.[172] 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.[173, 174] 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.[175] 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.[13] 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.[176] Because the GI tract is the site-of-action for polarization of immune cells and absorption of microbially produced metabolites[177], 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 microbially-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[178], storage[179], and extraction methods[180, 181] 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.[181] 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[182], 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.[183] Similar inferences can be made from 16s using projected annotations (e.g. using PICRUSt[184]), however this is not as reliable or robust, and does not necessarily match up with comparable annotation from shotgun data.[182] 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.[183] 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.[160, 185]

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.[138] 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[172], 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**


1. Sender R, Fuchs S and Milo R. Revised Estimates for the Number of Human and Bacteria Cells in the Body. *PLoS Biol*. 2016;14:e1002533.
2. Tierney BT, Yang Z, Luber JM, Beaudin M, Wibowo MC, Baek C, Mehlenbacher E, Patel CJ and Kostic AD. The Landscape of Genetic Content in the Gut and Oral Human Microbiome. *Cell host & microbe*. 2019;26:283-295 e8.
3. Human Microbiome Project C. Structure, function and diversity of the healthy human microbiome. *Nature*. 2012;486:207-14.
4. Zhang LS and Davies SS. Microbial metabolism of dietary components to bioactive metabolites: opportunities for new therapeutic interventions. *Genome medicine*. 2016;8:46.
5. David LA, Materna AC, Friedman J, Campos-Baptista MI, Blackburn MC, Perrotta A, Erdman SE and Alm EJ. Host lifestyle affects human microbiota on daily timescales. *Genome biology*. 2014;15:R89.
6. Thompson AL, Monteagudo-Mera A, Cadenas MB, Lampl ML and Azcarate-Peril MA. Milk- and solid-feeding practices and daycare attendance are associated with differences in bacterial diversity, predominant communities, and metabolic and immune function of the infant gut microbiome. *Front Cell Infect Microbiol*. 2015;5:3.
7. Forslund K, Hildebrand F, Nielsen T, Falony G, Le Chatelier E, Sunagawa S, Prifti E, Vieira-Silva S, Gudmundsdottir V, Krogh Pedersen H, et al. Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature*. 2015;528:262-6.
8. Qin J, Li Y, Cai Z, Li S, Zhu J, Zhang F, Liang S, Zhang W, Guan Y, Shen D, et al. A metagenome-wide association study of gut microbiota in type 2 diabetes. *Nature*. 2012;490:55-60.
9. Koeth RA, Wang Z, Levison BS, Buffa JA, Org E, Sheehy BT, Britt EB, Fu X, Wu Y, Li L, et al. Intestinal microbiota metabolism of l-carnitine, a nutrient in red meat, promotes atherosclerosis. *Nature medicine*. 2013;19:576-85.
10. Tang WH, Wang Z, Fan Y, Levison B, Hazen JE, Donahue LM, Wu Y and Hazen SL. Prognostic value of elevated levels of intestinal microbe-generated metabolite trimethylamine-N-oxide in patients with heart failure: refining the gut hypothesis. *Journal of the American College of Cardiology*. 2014;64:1908-14.
11. Tang WH, Wang Z, Levison BS, Koeth RA, Britt EB, Fu X, Wu Y and Hazen SL. Intestinal microbial metabolism of phosphatidylcholine and cardiovascular risk. *The New England journal of medicine*. 2013;368:1575-84.
12. Donia MS and Fischbach MA. HUMAN MICROBIOTA. Small molecules from the human microbiota. *Science*. 2015;349:1254766.
13. Mowat AM and Agace WW. Regional specialization within the intestinal immune system. *Nature reviews Immunology*. 2014;14:667-85.
14. Nguyen TL, Vieira-Silva S, Liston A and Raes J. How informative is the mouse for human gut microbiota research? *Dis Model Mech*. 2015;8:1-16.
15. Brown K, Abbott DW, Uwiera RRE and Inglis GD. Removal of the cecum affects intestinal fermentation, enteric bacterial community structure, and acute colitis in mice. *Gut microbes*. 2018;9:218-235.
16. Marteau P, Pochart P, Dore J, Bera-Maillet C, Bernalier A and Corthier G. Comparative study of bacterial groups within the human cecal and fecal microbiota. *Appl Environ Microbiol*. 2001;67:4939-42.
17. Stevens CE and Hume ID. Contributions of microbes in vertebrate gastrointestinal tract to production and conservation of nutrients. *Physiological reviews*. 1998;78:393-427.

18. Lievin-Le Moal V and Servin AL. The front line of enteric host defense against unwelcome intrusion of harmful microorganisms: mucins, antimicrobial peptides, and microbiota. *Clin Microbiol Rev*. 2006;19:315-37.
19. Derrien M, Vaughan EE, Plugge CM and de Vos WM. Akkermansia muciniphila gen. nov., sp. nov., a human intestinal mucin-degrading bacterium. *Int J Syst Evol Microbiol*. 2004;54:1469-1476.
20. Collado MC, Derrien M, Isolauri E, de Vos WM and Salminen S. Intestinal integrity and Akkermansia muciniphila, a mucin-degrading member of the intestinal microbiota present in infants, adults, and the elderly. *Applied and environmental microbiology*. 2007;73:7767-70.
21. Camilleri M. Leaky gut: mechanisms, measurement and clinical implications in humans. *Gut*. 2019;68:1516-1526.
22. Desai MS, Seekatz AM, Koropatkin NM, Kamada N, Hickey CA, Wolter M, Pudlo NA, Kitamoto S, Terrapon N, Muller A, et al. A Dietary Fiber-Deprived Gut Microbiota Degrades the Colonic Mucus Barrier and Enhances Pathogen Susceptibility. *Cell*. 2016;167:1339-1353 e21.
23. Krebs CF, Paust HJ, Krohn S, Koyro T, Brix SR, Riedel JH, Bartsch P, Wiech T, Meyer-Schwesinger C, Huang J, et al. Autoimmune Renal Disease Is Exacerbated by S1P-Receptor-1-Dependent Intestinal Th17 Cell Migration to the Kidney. *Immunity*. 2016;45:1078-1092.
24. Hart AL, Ng SC, Mann E, Al-Hassi HO, Bernardo D and Knight SC. Homing of immune cells: role in homeostasis and intestinal inflammation. *Inflammatory bowel diseases*. 2010;16:1969-77.
25. Hayes CL, Dong J, Galipeau HJ, Jury J, McCarville J, Huang X, Wang XY, Naidoo A, Anbazhagan AN, Libertucci J, et al. Commensal microbiota induces colonic barrier structure and functions that contribute to homeostasis. *Scientific reports*. 2018;8:14184.
26. MacDonald TT and Carter PB. Requirement for a bacterial flora before mice generate cells capable of mediating the delayed hypersensitivity reaction to sheep red blood cells. *Journal of immunology (Baltimore, Md : 1950)*. 1979;122:2624-9.
27. Ivanov, II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch SV, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell*. 2009;139:485-98.
28. Geva-Zatorsky N, Sefik E, Kua L, Pasman L, Tan TG, Ortiz-Lopez A, Yanortsang TB, Yang L, Jupp R, Mathis D, et al. Mining the Human Gut Microbiota for Immunomodulatory Organisms. *Cell*. 2017;168:928-943 e11.
29. Schluter J, Peled JU, Taylor BP, Markey KA, Smith M, Taur Y, Niehus R, Staffas A, Dai A, Fontana E, et al. The gut microbiota is associated with immune cell dynamics in humans. *Nature*. 2020.
30. Gensollen T, Iyer SS, Kasper DL and Blumberg RS. How colonization by microbiota in early life shapes the immune system. *Science*. 2016;352:539-44.
31. Russell SL, Gold MJ, Hartmann M, Willing BP, Thorson L, Wlodarska M, Gill N, Blanchet MR, Mohn WW, McNagny KM, et al. Early life antibiotic-driven changes in microbiota enhance susceptibility to allergic asthma. *EMBO reports*. 2012;13:440-7.
32. Zhang X, Zhivaki D and Lo-Man R. Unique aspects of the perinatal immune system. *Nature reviews Immunology*. 2017;17:495-507.
33. Wilck N, Balogh A, Marko L, Bartolomaeus H and Muller DN. The role of sodium in modulating immune cell function. *Nat Rev Nephrol*. 2019;15:546-558.
34. Libby P, Pasterkamp G, Crea F and Jang IK. Reassessing the Mechanisms of Acute Coronary Syndromes. *Circulation research*. 2019;124:150-160.
35. Xiao L and Harrison DG. Inflammation in Hypertension. *The Canadian journal of cardiology*. 2020;36:635-647.

36. Itani HA and Harrison DG. Memories that last in hypertension. *American journal of physiology Renal physiology*. 2015;308:F1197-9.
37. Itani HA, Xiao L, Saleh MA, Wu J, Pilkinton MA, Dale BL, Barbaro NR, Foss JD, Kirabo A, Montaniel KR, et al. CD70 Exacerbates Blood Pressure Elevation and Renal Damage in Response to Repeated Hypertensive Stimuli. *Circulation research*. 2016;118:1233-43.
38. Madhur MS, Lob HE, McCann LA, Iwakura Y, Blinder Y, Guzik TJ and Harrison DG. Interleukin 17 promotes angiotensin II-induced hypertension and vascular dysfunction. *Hypertension*. 2010;55:500-7.
39. Marko L, Kvakan H, Park JK, Qadri F, Spallek B, Binger KJ, Bowman EP, Kleinewietfeld M, Fokuhl V, Dechend R, et al. Interferon-gamma signaling inhibition ameliorates angiotensin II-induced cardiac damage. *Hypertension*. 2012;60:1430-6.
40. Didion SP, Kinzenbaw DA, Schrader LI, Chu Y and Faraci FM. Endogenous interleukin-10 inhibits angiotensin II-induced vascular dysfunction. *Hypertension*. 2009;54:619-24.
41. Kvakan H, Kleinewietfeld M, Qadri F, Park JK, Fischer R, Schwarz I, Rahn HP, Plehm R, Wellner M, Elitok S, et al. Regulatory T cells ameliorate angiotensin II-induced cardiac damage. *Circulation*. 2009;119:2904-12.
42. Caillon A, Mian MOR, Fraulob-Aquino JC, Huo KG, Barhoumi T, Ouerd S, Sinnaeve PR, Paradis P and Schiffrin EL. gammadelta T Cells Mediate Angiotensin II-Induced Hypertension and Vascular Injury. *Circulation*. 2017;135:2155-2162.
43. Shah KH, Shi P, Giani JF, Janjulia T, Bernstein EA, Li Y, Zhao T, Harrison DG, Bernstein KE and Shen XZ. Myeloid Suppressor Cells Accumulate and Regulate Blood Pressure in Hypertension. *Circulation research*. 2015;117:858-69.
44. Ferguson JF, Aden LA, Barbaro NR, Van Beusecum JP, Xiao L, Simmons AJ, Warden C, Pasic L, Himmel LE, Washington MK, et al. High dietary salt-induced dendritic cell activation underlies microbial dysbiosis-associated hypertension. *JCI Insight*. 2019;5.
45. Honda K and Littman DR. The microbiota in adaptive immune homeostasis and disease. *Nature*. 2016;535:75-84.
46. Haghikia A, Jorg S, Duscha A, Berg J, Manzel A, Waschbisch A, Hammer A, Lee DH, May C, Wilck N, et al. Dietary Fatty Acids Directly Impact Central Nervous System Autoimmunity via the Small Intestine. *Immunity*. 2015;43:817-29.
47. Tan TG, Sefik E, Geva-Zatorsky N, Kua L, Naskar D, Teng F, Pasman L, Ortiz-Lopez A, Jupp R, Wu HJ, et al. Identifying species of symbiont bacteria from the human gut that, alone, can induce intestinal Th17 cells in mice. *Proceedings of the National Academy of Sciences of the United States of America*. 2016;113:E8141-E8150.
48. Wilck N, Matus MG, Kearney SM, Olesen SW, Forslund K, Bartolomaeus H, Haase S, Mahler A, Balogh A, Marko L, et al. Salt-responsive gut commensal modulates TH17 axis and disease. *Nature*. 2017;551:585-589.
49. Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, Cheng G, Yamasaki S, Saito T, Ohba Y, et al. Induction of colonic regulatory T cells by indigenous Clostridium species. *Science*. 2011;331:337-41.
50. Furusawa Y, Obata Y, Fukuda S, Endo TA, Nakato G, Takahashi D, Nakanishi Y, Uetake C, Kato K, Kato T, et al. Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature*. 2013.
51. Evangelou E, Warren HR, Mosen-Ansorena D, Mifsud B, Pazoki R, Gao H, Ntritsos G, Dimou N, Cabrera CP, Karaman I, et al. Genetic analysis of over 1 million people identifies 535 new loci associated with blood pressure traits. *Nature genetics*. 2018;50:1412-1425.

52. Pazoki R, Dehghan A, Evangelou E, Warren H, Gao H, Caulfield M, Elliott P and Tzoulaki I. Genetic Predisposition to High Blood Pressure and Lifestyle Factors: Associations With Midlife Blood Pressure Levels and Cardiovascular Events. *Circulation*. 2018;137:653-661.
53. Lewington S, Clarke R, Qizilbash N, Peto R, Collins R and Prospective Studies C. Age-specific relevance of usual blood pressure to vascular mortality: a meta-analysis of individual data for one million adults in 61 prospective studies. *Lancet*. 2002;360:1903-13.
54. Dan X, Mushi Z, Baili W, Han L, Enqi W, Huanhu Z and Shuchun L. Differential Analysis of Hypertension-Associated Intestinal Microbiota. *Int J Med Sci*. 2019;16:872-881.
55. de la Cuesta-Zuluaga J, Mueller NT, Alvarez-Quintero R, Velasquez-Mejia EP, Sierra JA, Corrales-Agudelo V, Carmona JA, Abad JM and Escobar JS. Higher Fecal Short-Chain Fatty Acid Levels Are Associated with Gut Microbiome Dysbiosis, Obesity, Hypertension and Cardiometabolic Disease Risk Factors. *Nutrients*. 2018;11.
56. Li J, Zhao F, Wang Y, Chen J, Tao J, Tian G, Wu S, Liu W, Cui Q, Geng B, et al. Gut microbiota dysbiosis contributes to the development of hypertension. *Microbiome*. 2017;5:14.
57. Sun S, Lulla A, Sioda M, Winglee K, Wu MC, Jacobs DR, Jr., Shikany JM, Lloyd-Jones DM, Launer LJ, Fodor AA, et al. Gut Microbiota Composition and Blood Pressure. *Hypertension*. 2019;73:998-1006.
58. Verhaar BJH, Collard D, Prodan A, Levels JHM, Zwinderman AH, Backhed F, Vogt L, Peters MJL, Muller M, Nieuwdorp M, et al. Associations between gut microbiota, faecal short-chain fatty acids, and blood pressure across ethnic groups: the HELIUS study. *European heart journal*. 2020;41:4259-4267.
59. Yan Q, Gu Y, Li X, Yang W, Jia L, Chen C, Han X, Huang Y, Zhao L, Li P, et al. Alterations of the Gut Microbiome in Hypertension. *Front Cell Infect Microbiol*. 2017;7:381.
60. Yang T, Santisteban MM, Rodriguez V, Li E, Ahmari N, Carvajal JM, Zadeh M, Gong M, Qi Y, Zubcevic J, et al. Gut dysbiosis is linked to hypertension. *Hypertension*. 2015;65:1331-40.
61. Kim S, Goel R, Kumar A, Qi Y, Lobaton G, Hosaka K, Mohammed M, Handberg EM, Richards EM, Pepine CJ, et al. Imbalance of gut microbiome and intestinal epithelial barrier dysfunction in patients with high blood pressure. *Clinical science (London, England : 1979)*. 2018;132:701-718.
62. Huart J, Leenders J, Taminiau B, Descy J, Saint-Remy A, Daube G, Krzesinski JM, Melin P, de Tullio P and Jouret F. Gut Microbiota and Fecal Levels of Short-Chain Fatty Acids Differ Upon 24-Hour Blood Pressure Levels in Men. *Hypertension*. 2019;74:1005-1013.
63. Jackson MA, Verdi S, Maxan ME, Shin CM, Zierer J, Bowyer RCE, Martin T, Williams FMK, Menni C, Bell JT, et al. Gut microbiota associations with common diseases and prescription medications in a population-based cohort. *Nature communications*. 2018;9:2655.
64. Adnan S, Nelson JW, Ajami NJ, Venna VR, Petrosino JF, Bryan RM, Jr. and Durgan DJ. Alterations in the gut microbiota can elicit hypertension in rats. *Physiological genomics*. 2017;49:96-104.
65. Mell B, Jala VR, Mathew AV, Byun J, Waghulde H, Zhang Y, Haribabu B, Vijay-Kumar M, Pennathur S and Joe B. Evidence for a link between gut microbiota and hypertension in the Dahl rat. *Physiological genomics*. 2015;47:187-97.
66. Kaye DM, Shihata WA, Jama HA, Tsyganov K, Ziemann M, Kiriazis H, Horlock D, Vijay A, Giam B, Vinh A, et al. Deficiency of Prebiotic Fiber and Insufficient Signaling Through Gut Metabolite-Sensing Receptors Leads to Cardiovascular Disease. *Circulation*. 2020;141:1393-1403.
67. Marques FZ, Nelson E, Chu PY, Horlock D, Fiedler A, Ziemann M, Tan JK, Kuruppu S, Rajapakse NW, El-Osta A, et al. High-Fiber Diet and Acetate Supplementation Change the Gut

765 Microbiota and Prevent the Development of Hypertension and Heart Failure in Hypertensive
766 Mice. *Circulation*. 2017;135:964-977.
767 68. Santisteban MM, Qi Y, Zubcevic J, Kim S, Yang T, Shenoy V, Cole-Jeffrey CT, Lobaton
768 GO, Stewart DC, Rubiano A, et al. Hypertension-Linked Pathophysiological Alterations in the
769 Gut. *Circulation research*. 2017;120:312-323.
770 69. Robles-Vera I, Toral M, de la Visitacion N, Sanchez M, Gomez-Guzman M, Munoz R,
771 Algieri F, Vezza T, Jimenez R, Galvez J, et al. Changes to the gut microbiota induced by losartan
772 contributes to its antihypertensive effects. *British journal of pharmacology*. 2020;177:2006-
773 2023.
774 70. Chen L, He FJ, Dong Y, Huang Y, Wang C, Harshfield GA and Zhu H. Modest Sodium
775 Reduction Increases Circulating Short-Chain Fatty Acids in Untreated Hypertensives: A
776 Randomized, Double-Blind, Placebo-Controlled Trial. *Hypertension*. 2020;76:73-79.
777 71. Toral M, Gomez-Guzman M, Jimenez R, Romero M, Sanchez M, Utrilla MP, Garrido-
778 Mesa N, Rodriguez-Cabezas ME, Olivares M, Galvez J, et al. The probiotic Lactobacillus
779 coryniformis CECT5711 reduces the vascular pro-oxidant and pro-inflammatory status in
780 obese mice. *Clinical science (London, England : 1979)*. 2014;127:33-45.
781 72. Muller DN, Wilck N, Haase S, Kleinewietfeld M and Linker RA. Sodium in the
782 microenvironment regulates immune responses and tissue homeostasis. *Nature reviews*
783 *Immunology*. 2019;19:243-254.
784 73. Khalesi S, Sun J, Buys N and Jayasinghe R. Effect of probiotics on blood pressure: a
785 systematic review and meta-analysis of randomized, controlled trials. *Hypertension*.
786 2014;64:897-903.
787 74. Maier L, Pruteanu M, Kuhn M, Zeller G, Telzerow A, Anderson EE, Brochado AR,
788 Fernandez KC, Dose H, Mori H, et al. Extensive impact of non-antibiotic drugs on human gut
789 bacteria. *Nature*. 2018;555:623-628.
790 75. Zeevi D, Korem T, Zmora N, Israeli D, Rothschild D, Weinberger A, Ben-Yacov O, Lador
791 D, Avnit-Sagi T, Lotan-Pompan M, et al. Personalized Nutrition by Prediction of Glycemic
792 Responses. *Cell*. 2015;163:1079-1094.
793 76. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML,
794 Jones WJ, Roe BA, Affourtit JP, et al. A core gut microbiome in obese and lean twins. *Nature*.
795 2009;457:480-4.
796 77. Turnbaugh PJ, Ridaura VK, Faith JJ, Rey FE, Knight R and Gordon JI. The effect of diet
797 on the human gut microbiome: a metagenomic analysis in humanized gnotobiotic mice.
798 *Science translational medicine*. 2009;1:6ra14.
799 78. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, Griffin NW, Lombard V,
800 Henrissat B, Bain JR, et al. Gut microbiota from twins discordant for obesity modulate
801 metabolism in mice. *Science*. 2013;341:1241214.
802 79. Christ A, Gunther P, Lauterbach MAR, Duewell P, Biswas D, Pelka K, Scholz CJ, Oosting
803 M, Haendler K, Bassler K, et al. Western Diet Triggers NLRP3-Dependent Innate Immune
804 Reprogramming. *Cell*. 2018;172:162-175 e14.
805 80. Cani PD, Bibiloni R, Knauf C, Waget A, Neyrinck AM, Delzenne NM and Burcelin R.
806 Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat
807 diet-induced obesity and diabetes in mice. *Diabetes*. 2008;57:1470-81.
808 81. Wang Z, Klipfell E, Bennett BJ, Koeth R, Levison BS, Dugar B, Feldstein AE, Britt EB, Fu
809 X, Chung YM, et al. Gut flora metabolism of phosphatidylcholine promotes cardiovascular
810 disease. *Nature*. 2011;472:57-63.
811 82. He FJ and MacGregor GA. Reducing population salt intake worldwide: from evidence
812 to implementation. *Progress in cardiovascular diseases*. 2010;52:363-82.

83. Taylor J. 2013 ESH/ESC guidelines for the management of arterial hypertension. *European heart journal*. 2013;34:2108-9.
84. Organization WH. Sodium intake for adults and children. 2012;ISBN 978-92-4-150483-6, 56.
85. Union E. Implementation of the EU Salt Reduction Framework Results of Member States survey. 2012.
86. Whelton PK, Carey RM, Aronow WS, Casey DE, Jr., Collins KJ, Dennison Himmelfarb C, DePalma SM, Gidding S, Jamerson KA, Jones DW, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults: A Report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *Circulation*. 2018;138:e484-e594.
87. de Cabo R and Mattson MP. Effects of Intermittent Fasting on Health, Aging, and Disease. *The New England journal of medicine*. 2019;381:2541-2551.
88. Backhed F, Manchester JK, Semenkovich CF and Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proceedings of the National Academy of Sciences of the United States of America*. 2007;104:979-84.
89. Tremaroli V and Backhed F. Functional interactions between the gut microbiota and host metabolism. *Nature*. 2012;489:242-9.
90. Mesnage R, Grundler F, Schwiertz A, Le Maho Y and Wilhelmi de Toledo F. Changes in human gut microbiota composition are linked to the energy metabolic switch during 10 d of Buchinger fasting. *J Nutr Sci*. 2019;8:e36.
91. Zmora N, Suez J and Elinav E. You are what you eat: diet, health and the gut microbiota. *Nature reviews Gastroenterology & hepatology*. 2019;16:35-56.
92. Cummings JH, Pomare EW, Branch WJ, Naylor CP and Macfarlane GT. Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut*. 1987;28:1221-7.
93. Moore WE, Cato EP and Holdeman LV. Some current concepts in intestinal bacteriology. *The American journal of clinical nutrition*. 1978;31:S33-42.
94. Roediger WE. Utilization of nutrients by isolated epithelial cells of the rat colon. *Gastroenterology*. 1982;83:424-9.
95. Wolever TM, Spadafora P and Eshuis H. Interaction between colonic acetate and propionate in humans. *The American journal of clinical nutrition*. 1991;53:681-7.
96. Wolever TM, Brighenti F, Royall D, Jenkins AL and Jenkins DJ. Effect of rectal infusion of short chain fatty acids in human subjects. *The American journal of gastroenterology*. 1989;84:1027-33.
97. Poll BG, Cheema MU and Pluznick JL. Gut Microbial Metabolites and Blood Pressure Regulation: Focus on SCFAs and TMAO. *Physiology (Bethesda)*. 2020;35:275-284.
98. Muralitharan RR, Jama HA, Xie L, Peh A, Snelson M and Marques FZ. Microbial Peer Pressure: The Role of the Gut Microbiota in Hypertension and Its Complications. *Hypertension*. 2020:HYPERTENSIONAHA12014473.
99. Pluznick JL, Protzko RJ, Gevorgyan H, Peterlin Z, Sipos A, Han J, Brunet I, Wan LX, Rey F, Wang T, et al. Olfactory receptor responding to gut microbiota-derived signals plays a role in renin secretion and blood pressure regulation. *Proceedings of the National Academy of Sciences of the United States of America*. 2013;110:4410-5.
100. Natarajan N, Hori D, Flavahan S, Steppan J, Flavahan NA, Berkowitz DE and Pluznick JL. Microbial short chain fatty acid metabolites lower blood pressure via endothelial G protein-coupled receptor 41. *Physiological genomics*. 2016;48:826-834.

101. Pagel MD, Ahmad S, Vizzo JE and Scribner BH. Acetate and bicarbonate fluctuations and acetate intolerance during dialysis. *Kidney international*. 1982;21:513-8.
102. Stephen AM, Champ MM, Cloran SJ, Fleith M, van Lieshout L, Mejborn H and Burley VJ. Dietary fibre in Europe: current state of knowledge on definitions, sources, recommendations, intakes and relationships to health. *Nutr Res Rev*. 2017;30:149-190.
103. Reynolds A, Mann J, Cummings J, Winter N, Mete E and Te Morenga L. Carbohydrate quality and human health: a series of systematic reviews and meta-analyses. *Lancet*. 2019;393:434-445.
104. Hartley L, May MD, Loveman E, Colquitt JL and Rees K. Dietary fibre for the primary prevention of cardiovascular disease. *Cochrane Database Syst Rev*. 2016:CD011472.
105. Dewulf EM, Cani PD, Claus SP, Fuentes S, Puylaert PG, Neyrinck AM, Bindels LB, de Vos WM, Gibson GR, Thissen JP, et al. Insight into the prebiotic concept: lessons from an exploratory, double blind intervention study with inulin-type fructans in obese women. *Gut*. 2013;62:1112-21.
106. Maier TV, Lucio M, Lee LH, VerBerkmoes NC, Brislawn CJ, Bernhardt J, Lamendella R, McDermott JE, Bergeron N, Heinzmann SS, et al. Impact of Dietary Resistant Starch on the Human Gut Microbiome, Metaproteome, and Metabolome. *mBio*. 2017;8.
107. De Filippo C, Cavalieri D, Di Paola M, Ramazzotti M, Poullet JB, Massart S, Collini S, Pieraccini G and Lionetti P. Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proceedings of the National Academy of Sciences of the United States of America*. 2010;107:14691-6.
108. Bartolomaeus H, Balogh A, Yakoub M, Homann S, Marko L, Hoges S, Tsvetkov D, Krannich A, Wundersitz S, Avery EG, et al. Short-Chain Fatty Acid Propionate Protects From Hypertensive Cardiovascular Damage. *Circulation*. 2019;139:1407-1421.
109. Robles-Vera I, Toral M, de la Visitacion N, Sanchez M, Gomez-Guzman M, Romero M, Yang T, Izquierdo-Garcia JL, Jimenez R, Ruiz-Cabello J, et al. Probiotics Prevent Dysbiosis and the Rise in Blood Pressure in Genetic Hypertension: Role of Short-Chain Fatty Acids. *Mol Nutr Food Res*. 2020;64:e1900616.
110. Robles-Vera I, de la Visitacion N, Toral M, Sanchez M, Romero M, Gomez-Guzman M, Yang T, Izquierdo-Garcia JL, Guerra-Hernandez E, Ruiz-Cabello J, et al. Probiotic Bifidobacterium breve prevents DOCA-salt hypertension. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2020;34:13626-13640.
111. Roshanravan N, Mahdavi R, Alizadeh E, Jafarabadi MA, Hedayati M, Ghavami A, Alipour S, Alamdari NM, Barati M and Ostadrahimi A. Effect of Butyrate and Inulin Supplementation on Glycemic Status, Lipid Profile and Glucagon-Like Peptide 1 Level in Patients with Type 2 Diabetes: A Randomized Double-Blind, Placebo-Controlled Trial. *Horm Metab Res*. 2017;49:886-891.
112. Yang T, Magee KL, Colon-Perez LM, Larkin R, Liao YS, Balazic E, Cowart JR, Arocha R, Redler T, Febo M, et al. Impaired butyrate absorption in the proximal colon, low serum butyrate and diminished central effects of butyrate on blood pressure in spontaneously hypertensive rats. *Acta physiologica*. 2019;226:e13256.
113. Wu D, Tang X, Ding L, Cui J, Wang P, Du X, Yin J, Wang W, Chen Y and Zhang T. Candesartan attenuates hypertension-associated pathophysiological alterations in the gut. *Biomed Pharmacother*. 2019;116:109040.
114. Verger EO, Armstrong P, Nielsen T, Chakaroun R, Aron-Wisnewsky J, Gobel RJ, Schutz T, Delaere F, Gausseres N, Clement K, et al. Dietary Assessment in the MetaCardis Study: Development and Relative Validity of an Online Food Frequency Questionnaire. *J Acad Nutr Diet*. 2017;117:878-888.

115. Vieira-Silva S, Falony G, Belda E, Nielsen T, Aron-Wisnewsky J, Chakaroun R, Forslund SK, Assmann K, Valles-Colomer M, Nguyen TTD, et al. Statin therapy is associated with lower prevalence of gut microbiota dysbiosis. *Nature*. 2020;581:310-315.
116. Lucas S, Omata Y, Hofmann J, Bottcher M, Iljazovic A, Sarter K, Albrecht O, Schulz O, Krishnacoumar B, Kronke G, et al. Short-chain fatty acids regulate systemic bone mass and protect from pathological bone loss. *Nature communications*. 2018;9:55.
117. Mizuno M, Noto D, Kaga N, Chiba A and Miyake S. The dual role of short fatty acid chains in the pathogenesis of autoimmune disease models. *PloS one*. 2017;12:e0173032.
118. Pluznick JL. Microbial Short-Chain Fatty Acids and Blood Pressure Regulation. *Current hypertension reports*. 2017;19:25.
119. Trompette A, Gollwitzer ES, Yadava K, Sichelstiel AK, Sprenger N, Ngom-Bru C, Blanchard C, Junt T, Nicod LP, Harris NL, et al. Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nature medicine*. 2014;20:159-66.
120. Zaiss MM, Rapin A, Lebon L, Dubey LK, Mosconi I, Sarter K, Piersigilli A, Menin L, Walker AW, Rougemont J, et al. The Intestinal Microbiota Contributes to the Ability of Helminths to Modulate Allergic Inflammation. *Immunity*. 2015;43:998-1010.
121. Andrade-Oliveira V, Amano MT, Correa-Costa M, Castoldi A, Felizardo RJ, de Almeida DC, Bassi EJ, Moraes-Vieira PM, Hiyane MI, Rodas AC, et al. Gut Bacteria Products Prevent AKI Induced by Ischemia-Reperfusion. *Journal of the American Society of Nephrology : JASN*. 2015;26:1877-88.
122. Bartolomaeus H, Avery EG, Bartolomaeus TUP, Kozhakhmetov S, Zhumadilov Z, Muller DN, Wilck N, Kushugulova A and Forslund SK. Blood Pressure Changes Correlate with Short-Chain Fatty Acids Production Shifts Under a Synbiotic Intervention. *Cardiovascular research*. 2020.
123. Duscha A, Gisevius B, Hirschberg S, Yissachar N, Stangl GI, Eilers E, Bader V, Haase S, Kaisler J, David C, et al. Propionic Acid Shapes the Multiple Sclerosis Disease Course by an Immunomodulatory Mechanism. *Cell*. 2020;180:1067-1080 e16.
124. Hager J, Bang H, Hagen M, Frech M, Trager P, Sokolova MV, Steffen U, Tascilar K, Sarter K, Schett G, et al. The Role of Dietary Fiber in Rheumatoid Arthritis Patients: A Feasibility Study. *Nutrients*. 2019;11.
125. Hui W, Yu D, Cao Z and Zhao X. Butyrate inhibit collagen-induced arthritis via Treg/IL-10/Th17 axis. *International immunopharmacology*. 2019;68:226-233.
126. Vinolo MA, Rodrigues HG, Hatanaka E, Sato FT, Sampaio SC and Curi R. Suppressive effect of short-chain fatty acids on production of proinflammatory mediators by neutrophils. *The Journal of nutritional biochemistry*. 2011;22:849-55.
127. Vinolo MA, Hatanaka E, Lambertucci RH, Newsholme P and Curi R. Effects of short chain fatty acids on effector mechanisms of neutrophils. *Cell Biochem Funct*. 2009;27:48-55.
128. Singh N, Thangaraju M, Prasad PD, Martin PM, Lambert NA, Boettger T, Offermanns S and Ganapathy V. Blockade of dendritic cell development by bacterial fermentation products butyrate and propionate through a transporter (Slc5a8)-dependent inhibition of histone deacetylases. *The Journal of biological chemistry*. 2010;285:27601-8.
129. Wang F, Liu J, Weng T, Shen K, Chen Z, Yu Y, Huang Q, Wang G, Liu Z and Jin S. The Inflammation Induced by Lipopolysaccharide can be Mitigated by Short-chain Fatty Acid, Butyrate, through Upregulation of IL-10 in Septic Shock. *Scand J Immunol*. 2017;85:258-263.
130. Saemann MD, Bohmig GA, Osterreicher CH, Burtscher H, Parolini O, Diakos C, Stockl J, Horl WH and Zlabinger GJ. Anti-inflammatory effects of sodium butyrate on human monocytes: potent inhibition of IL-12 and up-regulation of IL-10 production. *FASEB journal :*

*official publication of the Federation of American Societies for Experimental Biology*. 2000;14:2380-2.
131. Sun M, Wu W, Chen L, Yang W, Huang X, Ma C, Chen F, Xiao Y, Zhao Y, Ma C, et al. Microbiota-derived short-chain fatty acids promote Th1 cell IL-10 production to maintain intestinal homeostasis. *Nature communications*. 2018;9:3555.
132. Arpaia N, Campbell C, Fan X, Dikiy S, van der Veeken J, deRoos P, Liu H, Cross JR, Pfeffer K, Coffer PJ, et al. Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature*. 2013;504:451-5.
133. Chen H, Ma X, Liu Y, Ma L, Chen Z, Lin X, Si L, Ma X and Chen X. Gut Microbiota Interventions With Clostridium butyricum and Norfloxacin Modulate Immune Response in Experimental Autoimmune Encephalomyelitis Mice. *Frontiers in immunology*. 2019;10:1662.
134. Frost F, Storck LJ, Kacprowski T, Gartner S, Ruhlemann M, Bang C, Franke A, Volker U, Aghdassi AA, Steveling A, et al. A structured weight loss program increases gut microbiota phylogenetic diversity and reduces levels of Collinsella in obese type 2 diabetics: A pilot study. *PLoS One*. 2019;14:e0219489.
135. Ozkul C, Yalinay M and Karakan T. Structural changes in gut microbiome after Ramadan fasting: a pilot study. *Beneficial microbes*. 2020;11:227-233.
136. Louis S, Tappu RM, Damms-Machado A, Huson DH and Bischoff SC. Characterization of the Gut Microbial Community of Obese Patients Following a Weight-Loss Intervention Using Whole Metagenome Shotgun Sequencing. *PLoS One*. 2016;11:e0149564.
137. Guevara-Cruz M, Flores-Lopez AG, Aguilar-Lopez M, Sanchez-Tapia M, Medina-Vera I, Diaz D, Tovar AR and Torres N. Improvement of Lipoprotein Profile and Metabolic Endotoxemia by a Lifestyle Intervention That Modifies the Gut Microbiota in Subjects With Metabolic Syndrome. *J Am Heart Assoc*. 2019;8:e012401.
138. Fischbach MA. Microbiome: Focus on Causation and Mechanism. *Cell*. 2018;174:785-790.
139. Ratner M. Seres's pioneering microbiome drug fails mid-stage trial. *Nat Biotechnol*. 2016;34:1004-1005.
140. Stappenbeck TS and Virgin HW. Accounting for reciprocal host-microbiome interactions in experimental science. *Nature*. 2016;534:191-9.
141. Virgin HW and Todd JA. Metagenomics and personalized medicine. *Cell*. 2011;147:44-56.
142. Seok J, Warren HS, Cuenca AG, Mindrinos MN, Baker HV, Xu W, Richards DR, McDonald-Smith GP, Gao H, Hennessy L, et al. Genomic responses in mouse models poorly mimic human inflammatory diseases. *Proc Natl Acad Sci U S A*. 2013;110:3507-12.
143. Bartolomaeus H, Marko L, Wilck N, Luft FC, Forslund SK and Muller DN. Precarious Symbiosis Between Host and Microbiome in Cardiovascular Health. *Hypertension*. 2019;73:926-935.
144. Vieira-Silva S, Falony G, Darzi Y, Lima-Mendez G, Garcia Yunta R, Okuda S, Vandeputte D, Valles-Colomer M, Hildebrand F, Chaffron S, et al. Species-function relationships shape ecological properties of the human gut microbiome. *Nat Microbiol*. 2016;1:16088.
145. Randall DW, Kieswich J, Swann J, McCafferty K, Thiemermann C, Curtis M, Hoyles L and Yaqoob MM. Batch effect exerts a bigger influence on the rat urinary metabolome and gut microbiota than uraemia: a cautionary tale. *Microbiome*. 2019;7:127.
146. Enríquez JA. Mind your mouse strain. *Nature Metabolism*. 2019;1:5-7.
147. Omary MB, Cohen DE, El-Omar EM, Jalan R, Low MJ, Nathanson MH, Peek RM, Jr. and Turner JR. Not all mice are the same: Standardization of animal research data presentation. *Hepatology*. 2016;63:1752-4.

148. Bloom SM, Bijanki VN, Nava GM, Sun L, Malvin NP, Donermeyer DL, Dunne WM, Jr., Allen PM and Stappenbeck TS. Commensal Bacteroides species induce colitis in host-genotype-specific fashion in a mouse model of inflammatory bowel disease. *Cell host & microbe*. 2011;9:390-403.
149. Rogers GB, Kozlowska J, Keeble J, Metcalfe K, Fao M, Dowd SE, Mason AJ, McGuckin MA and Bruce KD. Functional divergence in gastrointestinal microbiota in physically-separated genetically identical mice. *Sci Rep*. 2014;4:5437.
150. Elinav E, Strowig T, Kau AL, Henao-Mejia J, Thaiss CA, Booth CJ, Peaper DR, Bertin J, Eisenbarth SC, Gordon JI, et al. NLRP6 inflammasome regulates colonic microbial ecology and risk for colitis. *Cell*. 2011;145:745-57.
151. Levy M, Thaiss CA, Zeevi D, Dohnalova L, Zilberman-Schapira G, Mahdi JA, David E, Savidor A, Korem T, Herzig Y, et al. Microbiota-Modulated Metabolites Shape the Intestinal Microenvironment by Regulating NLRP6 Inflammasome Signaling. *Cell*. 2015;163:1428-43.
152. Mamantopoulos M, Ronchi F, Van Hauwermeiren F, Vieira-Silva S, Yilmaz B, Martens L, Saeys Y, Drexler SK, Yazdi AS, Raes J, et al. Nlrp6- and ASC-Dependent Inflammasomes Do Not Shape the Commensal Gut Microbiota Composition. *Immunity*. 2017;47:339-348 e4.
153. Chung H, Pamp SJ, Hill JA, Surana NK, Edelman SM, Troy EB, Reading NC, Villablanca EJ, Wang S, Mora JR, et al. Gut immune maturation depends on colonization with a host-specific microbiota. *Cell*. 2012;149:1578-93.
154. Doeing DC, Borowicz JL and Crockett ET. Gender dimorphism in differential peripheral blood leukocyte counts in mice using cardiac, tail, foot, and saphenous vein puncture methods. *BMC Clin Pathol*. 2003;3:3.
155. Mestas J and Hughes CC. Of mice and not men: differences between mouse and human immunology. *J Immunol*. 2004;172:2731-8.
156. Beura LK, Hamilton SE, Bi K, Schenkel JM, Odumade OA, Casey KA, Thompson EA, Fraser KA, Rosato PC, Filali-Mouhim A, et al. Normalizing the environment recapitulates adult human immune traits in laboratory mice. *Nature*. 2016;532:512-6.
157. Selin LK, Brehm MA, Naumov YN, Cornberg M, Kim SK, Clute SC and Welsh RM. Memory of mice and men: CD8+ T-cell cross-reactivity and heterologous immunity. *Immunol Rev*. 2006;211:164-81.
158. Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD and Gordon JI. Obesity alters gut microbial ecology. *Proceedings of the National Academy of Sciences of the United States of America*. 2005;102:11070-5.
159. Arrieta MC, Walter J and Finlay BB. Human Microbiota-Associated Mice: A Model with Challenges. *Cell Host Microbe*. 2016;19:575-8.
160. Marques FZ, Jama HA, Tsyganov K, Gill PA, Rhys-Jones D, Muralitharan RR, Muir J, Holmes A and Mackay CR. Guidelines for Transparency on Gut Microbiome Studies in Essential and Experimental Hypertension. *Hypertension*. 2019;74:1279-1293.
161. Bach JF. The hygiene hypothesis in autoimmunity: the role of pathogens and commensals. *Nat Rev Immunol*. 2018;18:105-120.
162. Okada H, Kuhn C, Feillet H and Bach JF. The 'hygiene hypothesis' for autoimmune and allergic diseases: an update. *Clin Exp Immunol*. 2010;160:1-9.
163. Roslund MI, Puhakka R, Gronroos M, Nurminen N, Oikarinen S, Gazali AM, Cinek O, Kramna L, Siter N, Vari HK, et al. Biodiversity intervention enhances immune regulation and health-associated commensal microbiota among daycare children. *Sci Adv*. 2020;6.
164. Rosshart SP, Herz J, Vassallo BG, Hunter A, Wall MK, Badger JH, McCulloch JA, Anastasakis DG, Sarshad AA, Leonardi I, et al. Laboratory mice born to wild mice have natural microbiota and model human immune responses. *Science*. 2019;365.

165. Oldstone MB. Molecular mimicry, microbial infection, and autoimmune disease: evolution of the concept. *Curr Top Microbiol Immunol*. 2005;296:1-17.
166. Foxman EF and Iwasaki A. Genome-virome interactions: examining the role of common viral infections in complex disease. *Nat Rev Microbiol*. 2011;9:254-64.
167. Reese TA, Bi K, Kambal A, Filali-Mouhim A, Beura LK, Burger MC, Pulendran B, Sekaly RP, Jameson SC, Masopust D, et al. Sequential Infection with Common Pathogens Promotes Human-like Immune Gene Expression and Altered Vaccine Response. *Cell Host Microbe*. 2016;19:713-9.
168. Rosshart SP, Vassallo BG, Angeletti D, Hutchinson DS, Morgan AP, Takeda K, Hickman HD, McCulloch JA, Badger JH, Ajami NJ, et al. Wild Mouse Gut Microbiota Promotes Host Fitness and Improves Disease Resistance. *Cell*. 2017;171:1015-1028 e13.
169. Sbierski-Kind J, Kath J, Brachs S, Streitz M, von Herrath MG, Kuhl AA, Schmidt-Bleek K, Mai K, Spranger J and Volk HD. Distinct Housing Conditions Reveal a Major Impact of Adaptive Immunity on the Course of Obesity-Induced Type 2 Diabetes. *Frontiers in immunology*. 2018;9:1069.
170. Abolins S, King EC, Lazarou L, Weldon L, Hughes L, Drescher P, Raynes JG, Hafalla JCR, Viney ME and Riley EM. The comparative immunology of wild and laboratory mice, Mus musculus domesticus. *Nat Commun*. 2017;8:14811.
171. Jakobsson HE, Rodriguez-Pineiro AM, Schutte A, Ermund A, Boysen P, Bemark M, Sommer F, Backhed F, Hansson GC and Johansson ME. The composition of the gut microbiota shapes the colon mucus barrier. *EMBO Rep*. 2015;16:164-77.
172. Hanage WP. Microbiology: Microbiome science needs a healthy dose of scepticism. *Nature*. 2014;512:247-8.
173. Zhang C, Zhang M, Pang X, Zhao Y, Wang L and Zhao L. Structural resilience of the gut microbiota in adult mice under high-fat dietary perturbations. *ISME J*. 2012;6:1848-57.
174. Ericsson AC, Gagliardi J, Bouhan D, Spollen WG, Givan SA and Franklin CL. The influence of caging, bedding, and diet on the composition of the microbiota in different regions of the mouse gut. *Sci Rep*. 2018;8:4065.
175. Thaiss CA, Levy M, Korem T, Dohnalova L, Shapiro H, Jaitin DA, David E, Winter DR, Gury-BenAri M, Tatirovsky E, et al. Microbiota Diurnal Rhythmicity Programs Host Transcriptome Oscillations. *Cell*. 2016;167:1495-1510 e12.
176. Zmora N, Zilberman-Schapira G, Suez J, Mor U, Dori-Bachash M, Bashiardes S, Kotler E, Zur M, Regev-Lehavi D, Brik RB, et al. Personalized Gut Mucosal Colonization Resistance to Empiric Probiotics Is Associated with Unique Host and Microbiome Features. *Cell*. 2018;174:1388-1405 e21.
177. Belkaid Y and Hand TW. Role of the microbiota in immunity and inflammation. *Cell*. 2014;157:121-41.
178. Wang Z, Zolnik CP, Qiu Y, Usyk M, Wang T, Strickler HD, Isasi CR, Kaplan RC, Kurland IJ, Qi Q, et al. Comparison of Fecal Collection Methods for Microbiome and Metabolomics Studies. *Front Cell Infect Microbiol*. 2018;8:301.
179. Choo JM, Leong LE and Rogers GB. Sample storage conditions significantly influence faecal microbiome profiles. *Sci Rep*. 2015;5:16350.
180. Costea PI, Zeller G, Sunagawa S, Pelletier E, Alberti A, Levenez F, Tramontano M, Driessen M, Hercog R, Jung FE, et al. Towards standards for human fecal sample processing in metagenomic studies. *Nat Biotechnol*. 2017;35:1069-1076.
181. Bartolomaeus TUP, Birkner T, Bartolomaeus H, Lober U, Avery EG, Mahler A, Weber D, Kochlik B, Balogh A, Wilck N, et al. Quantifying Technical Confounders in Microbiome Studies. *Cardiovascular research*. 2020.

182. Rausch P, Ruhlemann M, Hermes BM, Doms S, Dagan T, Dierking K, Domin H, Fraune S, von Frieling J, Hentschel U, et al. Comparative analysis of amplicon and metagenomic sequencing methods reveals key features in the evolution of animal metaorganisms. *Microbiome*. 2019;7:133.
183. Jovel J, Patterson J, Wang W, Hotte N, O'Keefe S, Mitchel T, Perry T, Kao D, Mason AL, Madsen KL, et al. Characterization of the Gut Microbiome Using 16S or Shotgun Metagenomics. *Front Microbiol*. 2016;7:459.
184. Langille MG, Zaneveld J, Caporaso JG, McDonald D, Knights D, Reyes JA, Clemente JC, Burkepile DE, Vega Thurber RL, Knight R, et al. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nat Biotechnol*. 2013;31:814-21.
185. Michalska-Smith MJ and Allesina S. Telling ecological networks apart by their structure: A computational challenge. *PLoS Comput Biol*. 2019;15:e1007076.
186. Donaldson GP, Lee SM and Mazmanian SK. Gut biogeography of the bacterial microbiota. *Nat Rev Microbiol*. 2016;14:20-32.
187. Suntharalingam G, Perry MR, Ward S, Brett SJ, Castello-Cortes A, Brunner MD and Panoskaltsis N. Cytokine storm in a phase 1 trial of the anti-CD28 monoclonal antibody TGN1412. *The New England journal of medicine*. 2006;355:1018-28.
188. Fisher CJ, Jr., Agosti JM, Opal SM, Lowry SF, Balk RA, Sadoff JC, Abraham E, Schein RM and Benjamin E. Treatment of septic shock with the tumor necrosis factor receptor:Fc fusion protein. The Soluble TNF Receptor Sepsis Study Group. *N Engl J Med*. 1996;334:1697-702.

**Figures Legends**

**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[13]). 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.[13, 186] 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.[13, 177] 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 (interventionalist 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[187, 188], 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.[164] 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.

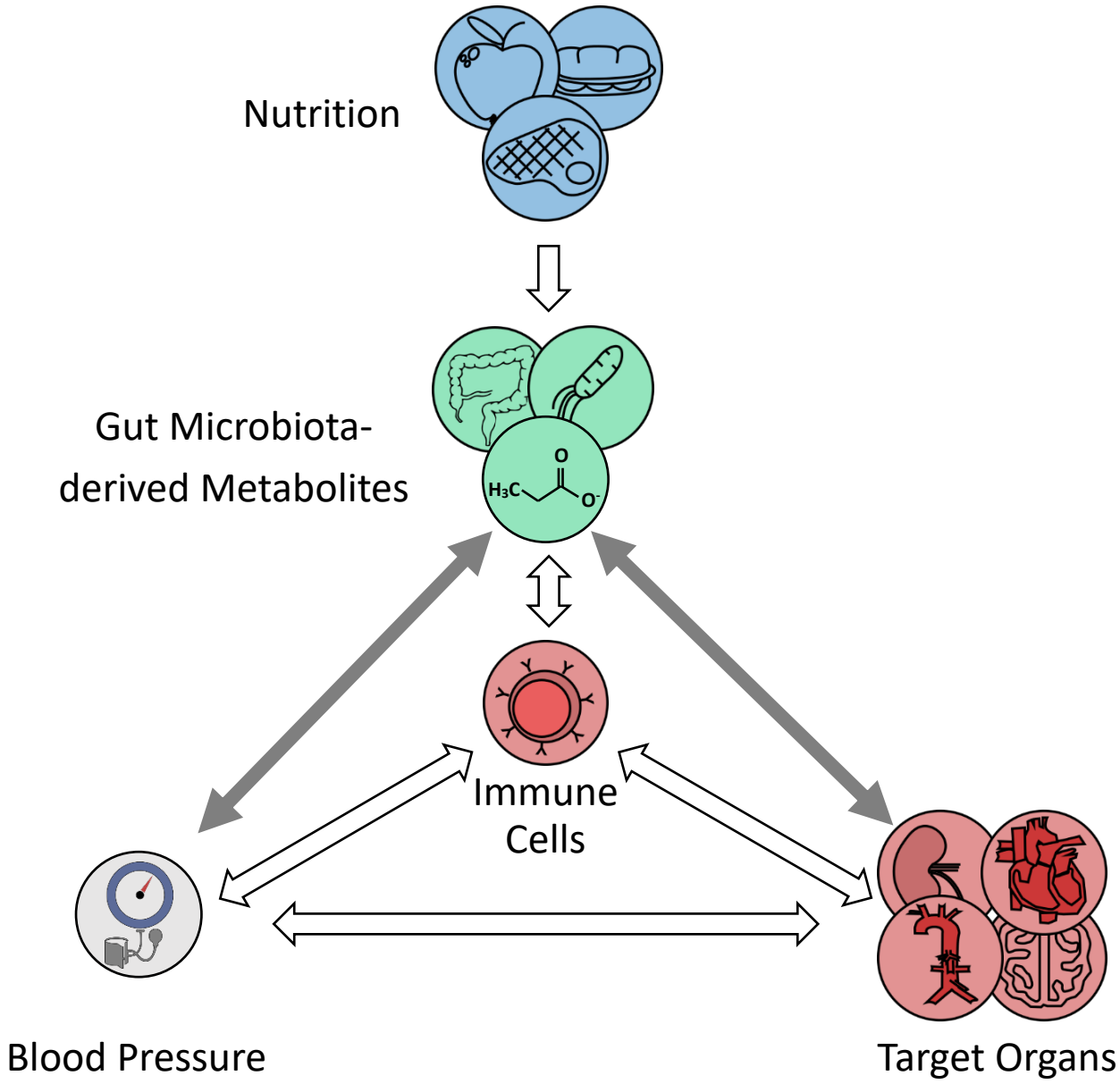

Nutrition
Gut Microbiota-
derived Metabolites
Immune
Cells
Blood Pressure
Target Organs

Figure 1

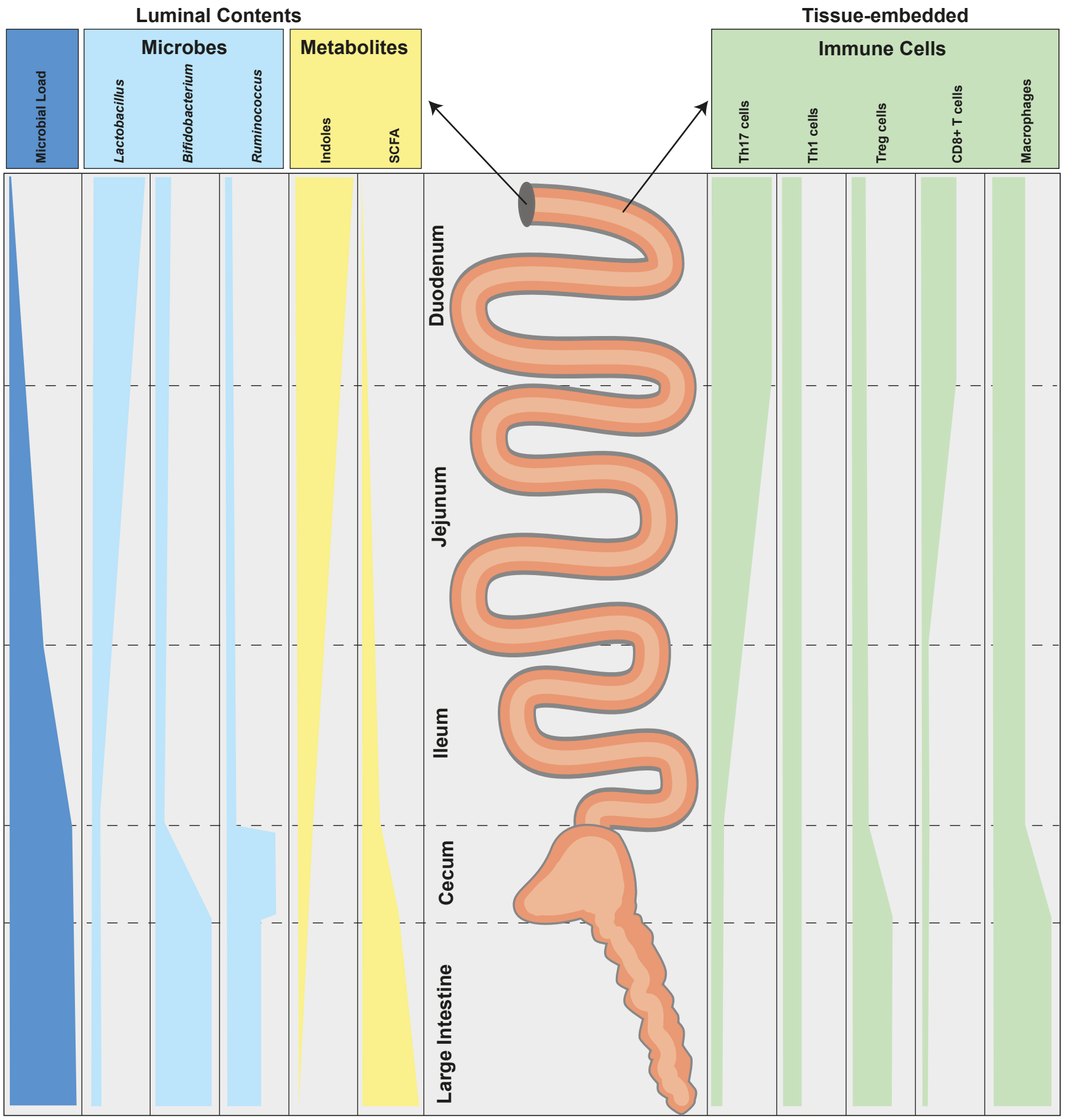
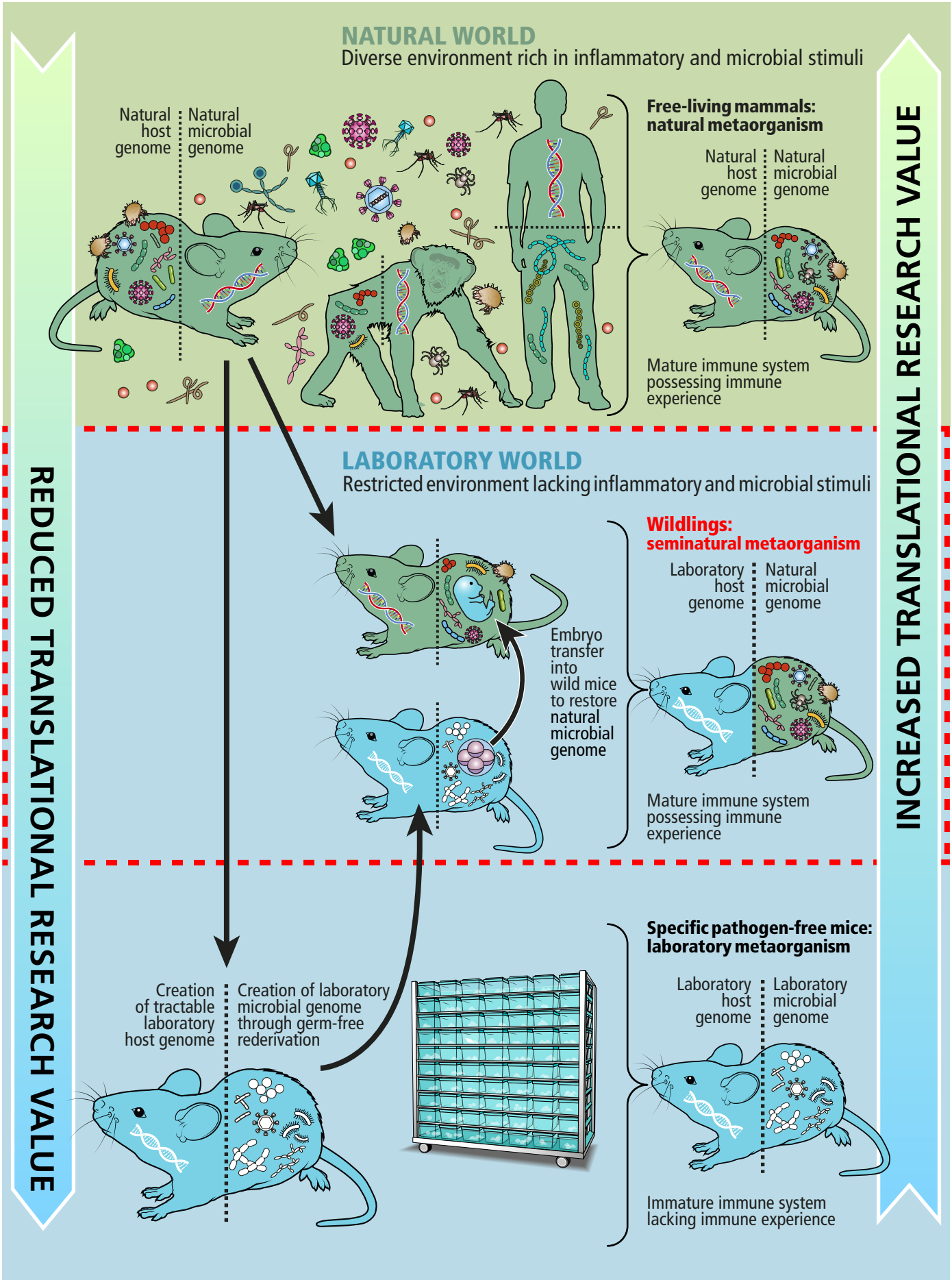

Luminal Contents
Tissue-embedded
Microbial Load
Microbes
Lactobacillus
Bifidobacterium
Ruminococcus
Metabolites
Indoles
SCFA
Immune Cells
Th17 cells
Th1 cells
Treg cells
CD8+ T cells
Macrophages
Duodenum
Jejunum
Ileum
Cecum
Large Intestine

Figure 2


NATURAL WORLD
Diverse environment rich in inflammatory and microbial stimuli
Natural host genome
Natural microbial genome
Free-living mammals: natural metaorganism
Natural host genome
Natural microbial genome
Mature immune system possessing immune experience
LABORATORY WORLD
Restricted environment lacking inflammatory and microbial stimuli
Wildlings: seminatural metaorganism
Laboratory host genome
Natural microbial genome
Embryo transfer into wild mice to restore natural microbial genome
Mature immune system possessing immune experience
Specific pathogen-free mice: laboratory metaorganism
Creation of tractable laboratory host genome
Creation of laboratory microbial genome through germ-free rederivation
Laboratory host genome
Laboratory microbial genome
Immature immune system lacking immune experience
REDUCED TRANSLATIONAL RESEARCH VALUE
INCREASED TRANSLATIONAL RESEARCH VALUE