Report of the National Heart, Lung, and Blood Institute Working Group on the Role of Microbiota in Blood Pressure Regulation

Current Status and Future Directions

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A recent American Heart Association report shows that 34% of US adults ≥20 years of age have hypertension representing ≈86 million adults.[1] A substantial increase in the prevalence of hypertension has occurred globally.[2] The projected number of individuals with systolic blood pressure of ≥140 mm Hg has doubled from 442 million in 1990 to 874 million in 2015. Hypertension is one of the most prevalent risk factors for cardiovascular disease (CVD).[1] Results of the recently concluded SPRINT (Systolic Blood Pressure Intervention Trial) funded by the National Heart, Lung, and Blood Institute showed that among older adults with hypertension but without diabetes mellitus, lowering systolic blood pressure to a target goal of 120 mm Hg, compared with the standard goal of 140 mm Hg, resulted in significantly lower rates of fatal and nonfatal cardiovascular events and death from any cause.[3]

Some groups such as blacks who display both disproportionately earlier onset and higher prevalence of hypertension have increased risk of blood pressure (BP)–related cardiovascular and renal disease complications compared with non-Hispanic whites. Despite intensive attempts to influence lifestyle changes, nutritional counseling, and intensive antihypertensive drug treatment strategies, ≈14% of all hypertension patients seem to be resistant to antihypertensive interventions.[1] Resistant hypertension is defined as BP above goal (>140/90 mm Hg) on ≥3 BP-lowering medications or needing ≥4 medications prescribed at optimal dose to control BP to goal. Thus, both increased prevalence and inability to achieve BP goals in a large patient population with hypertension are creating a tremendous healthcare burden. In addition, no novel antihypertensive drugs have been added to our formulary since 1995, when the first angiotensin receptor antagonist was approved in the United States. The recent attempts to use percutaneous renal artery sympathetic denervation, as a novel means to control hypertension, have been largely unsuccessful to date.[4,5] Therefore, there is a crucial need to discover novel and innovative ways to address the BP control issue.

Recent studies showing a role for microbiota in BP regulation might provide promising new therapeutic approaches. Increasing evidence indicates that dysregulation of commensal microbiota is linked to a variety of chronic disorders, some of which (including diabetes mellitus, obesity, chronic kidney disease [CKD], heart failure, etc) have an impact on BP regulation and are risk factors for hypertension.[6,7] Thus, it is reasonable to infer that microbiota could be a major participant in BP homeostasis. In fact, several recent studies have identified gut dysbiosis being associated with hypertension and new links between brain–gut, kidney–gut, and microbial metabolites–host interactions in BP homeostasis.[8–11] These results, coupled with a sense of urgency to discover novel mechanism-based therapeutic strategies for hypertension, led the National Heart, Lung, and Blood Institute to convene a Working Group (WG) on June 10, 2016, to discuss this emerging area of the role of microbiota in BP regulation. The WG brought together 16 experts from diverse backgrounds in hypertension; cancer; and cardiovascular, renal, nutritional, inflammatory, microbiome/microbial, and oral diseases.

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The WG reviewed existing and emerging scientific evidence connecting gut and oral microbiota to BP regulation. The WG was organized into 4 thematic sessions: (1) the link between microbiota and hypertension session included presentations on gut–brain axis and microbiota in animal models of hypertension and vascular dysfunction; (2) a session on the role of microbiota in human disease included CVD, kidney disease, and metabolic syndrome; (3) the oral microbiota session discussed the Human Oral Microbiome Database advances in metagenomic and metabolomic technologies and the role of nitrate in BP regulation; and (4) discussion on microbiota as a potential therapeutic target included dietary modifications, circadian regulation, and impact of chronic stress on the gut–brain axis. Because the WG members came from diverse backgrounds, and most have not collaborated before, the meeting provided an excellent opportunity for cross-disciplinary thinking and discussions and highlighted potential for future collaborations and research topics. In this report, we provide a summary of the WG presentations, discussion, and its recommendations for future research directions.

Gut Microbiota and Hypertension

Indirect involvement of gut microbiota in BP regulation and hypertension has been known for some time, primarily from studies involving fermented milk, probiotics, and meta-analysis of randomized trials.[12–14] Evidence for a more direct link was first presented in 2 simultaneous reports, using several different animal models of hypertension (ie, Dahl salt–sensitive and salt-resistant rats, spontaneously hypertensive rats, and angiotensin II-infusion rat models).[8,11] Significant changes in gut microbiota and alterations in Firmicutes/Bacteroidetes ratio were found to be linked with high BP.[11] Furthermore, the microbiota of hypertensive animals demonstrated significant decreases in butyrate- and acetate-producing bacterial populations. Interestingly, in the angiotensin II-infusion rat models, treatment with minocycline, an anti-inflammatory antibiotic that prevented dysbiosis, expanded both acetate- and butyrate-producing bacteria and lowered high BP.[11] Contributions of antibiotic and anti-inflammatory properties of minocycline on its antihypertensive actions remain to be investigated. These original observations have been validated with other hypertension animal models, strengthening the connection between gut dysbiosis and high BP.[15–17]

Changes in gut microbiota in hypertension were associated with changes in gut inflammatory status and pathology.[18] Reported structural changes in the gut of hypertensive animals include an increase in gut wall fibrosis and decreases in villi length and number of goblet cells, coupled with alterations in gastrointestinal cell tight junction proteins, in turn associated with increased gut leakiness. Increased gut permeability is an important factor in enhancing bidirectional flow of biological mediators, including microbes, microbial products, hormones, immune cells, and so on, thus affecting peripheral and central BP control mechanisms.

In summary, several recent studies have provided the initial bases for the fundamental concept that the gut microbiota is involved in BP control and hypertension. Of course, the story is just beginning, and further research is needed to fully unravel the translational and clinical potential of these observations. Some of the questions that need to be explored include the following: (1) Microbiome comparisons among control, hypertensive, and resistant hypertensive patients with respect to sex, race, and antihypertensive therapy. (2) Brain–gut causal relationship, ie, is the autonomic dysregulation involved in gut pathology and dysbiosis? (3) What is the primary site for perception of prohypertensive signals: the brain or the gut, or perhaps both contribute equally?

Microbial–Host Interactions in a Genetic Model of Hypertension

The gut is the first organ exposed to any dietary components; thus, the role of gut microbiota in chronic diseases, including CVD and hypertension, has been questioned. On the basis of previous evidence connecting dietary salt and BP regulation, microbial–host interactions were investigated in various models of hypertension, including in the salt-sensitive rat model of hypertension, that is, the Dahl salt–sensitive and salt-resistant rats fed with high-salt diet.[8] The hypothesis was that the gut microbiota composition in the Dahl salt–sensitive rat would be different from that in salt-resistant rats despite identical diets and housing conditions and that changes in microbiota composition would result in BP modifications. The genome of salt-resistant rats is resistant to alterations in BP, consistent with the finding that their BP was not altered by fecal microbiota transplant from hypertensive Dahl salt–sensitive rats.[8] On the contrary, fecal microbiota transplant from salt-resistant rats induced sustained and exacerbated high BP in Dahl salt–sensitive rats. These induced effects on BP were associated with post-transplantation changes in the gut microbial composition, providing the fundamental basis to explore further the host–microbiota interactions in BP regulation.

Genetics of the host and metagenomic (microbiota genome) factors from the microbiota may interact to confer susceptibility or resistance to the development of hypertension. System biology approach for dissecting the host genomic factors and for dissecting the metagenomic factors through a metagenomic, metatranscriptomic, metabolomics approaches will be required. Although it is useful to study the connection between the microbiome, genetic, and dietary conditions, analyses limited to the use of experimental animal models cannot explore all the sources for potential variations, specifically those related to inherited causal factors. Thus, population studies to track Mendelian inheritance of host–microbiota interactions in hypertension will be necessary in the future.

Gut Microbial Metabolites, Host Sensory Receptors, and BP Control

Short-chain fatty acids (SCFAs) are a relatively well-studied class of gut microbial metabolites, primarily comprised acetate, propionate, and butyrate. SCFAs and SCFA receptors have been implicated in 2 well-established areas of microbiota–host interactions: immune responses and metabolism.[19–21] It is important to note that virtually all the SCFAs in the circulating bloodstream are microbial in origin, as evidenced by the nearly nondetectable levels of SCFAs in the plasma of germ-free mice.[22] Therefore, changes in SCFAs would indicate an involvement of the gut microbiota.

A recent finding suggests that SCFAs play a role in BP regulation.[23] This work largely relies on the analysis of phenotypes of 2 different SCFA receptor-null mice: Olfr78 (olfactory receptor 78) and Gpr41 (G-protein–coupled receptor 41). Olfr78 is localized in the renal afferent arteriole and in vascular smooth muscle cells of large vessels, whereas Gpr41 is localized in the vascular endothelium. Consistent with the localization of Olfr78 to the afferent arteriole (the site of renin storage and secretion), Olfr78-null mice have lowered plasma renin levels and lowered baseline BP.[9] By contrast, Gpr41-null animals have isolated systolic hypertension, consistent with an altered vascular tone in vivo, which results in functionally stiffer vessels.[24] In support of this SCFA-driven vasoactive response, studies have been reported that SCFAs can induce dilatation of resistance vessels ex vivo.[23] Similarly, the use of acetate in hemodialysis buffers has been reported to cause hypotension,[25] consistent with a role of SCFAs in the dilatation of resistance vessels. Furthermore, population-based human studies found that interventions that alter SCFAs production correlate with changes in BP.[12] Recent studies investigating a link between the gut microbiota and BP regulation have indicated potential roles of SCFAs in influencing phenotypic changes.[8,11] Further investigations are needed to define the specific mechanisms and contribution of these new pathways among many other pathways that influence both the gut microbiota and BP.

Gut Microbiota and Age-Related Vascular Dysfunction

The risk of CVD of all causes increases progressively with advancing age. The primary event that drives aging-associated increases in CVD risk is related to arterial dysfunction: primarily large elastic artery (ie, aorta and carotid artery) stiffening and vascular endothelial dysfunction.[26,27] Substantial evidence indicates that the key underlying mechanisms mediating vascular aging include excessive superoxide-associated vascular oxidative stress and chronic low-grade vascular inflammation.[26,27] Despite this understanding, the upstream events that drive vascular aging are largely unknown at present.

Remodeling of the gut microbiota has recently been linked to aging, including evidence of alterations in both diversity and abundance of specific taxa.[28,29] Although the role of such changes in the gut microbiota with aging on vascular dysfunction and arterial BP has not been systematically investigated, both published and preliminary findings support this possibility. For example, increases in aortic stiffening and endothelial dysfunction with aging in C57Bl/6 mice are causally associated with increased vascular superoxide production. A recent preliminary study showed that short-term (3–4 weeks) administration of broad-spectrum antibiotics, a treatment that suppresses many gut bacterial species, normalized vascular superoxide production and ameliorated vascular dysfunction in old mice (R.A. Gioscia-Ryan and D.R. Seals, unpublished data, 2017). Also, concentrations of plasma trimethylamine N-oxide (TMAO), a gut microbial-dependent metabolite, were increased 3-fold in healthy late-middle-aged and older adults (55±2 years) compared with young controls (22±1 year). In this regard, plasma TMAO concentrations are positively correlated with CVD in humans.[30,31] Moreover, brachial artery flow–mediated dilation, a measure of vascular endothelial function, was inversely related to plasma TMAO (r=0.43), whereas systolic arterial BP was positively related to TMAO levels (r=0.47; R.A. Gioscia-Ryan and D.R. Seals, unpublished data, 2017). Together, these data support the concept that changes in the gut microbiota contribute to vascular dysfunction and increases in arterial BP with aging.

Several knowledge gaps need to be explored in this field. These include the following: (1) Does the gut microbiota influence arterial function and BP with primary aging (aging in the absence of clinical disease) and in age-related CVDs such as essential hypertension? Additional cause-and-effect studies (eg, gut microbiota transfer experiments) are needed. (2) What specific changes to the microbiome (microbe composition and metabolite production) associated with aging or age-related diseases cause arterial dysfunction and effects on BP? (3) What are the integrated physiological mechanisms connecting the gut microbiota to arterial dysfunction and elevated BP? For example, are inflammatory mediators released into the circulation via a leaky gut? What is the potential role of elevated circulating TMAO with aging? (4) Do environmental influences (exercise, diet, sleep, stress, smoking, antibiotic use, etc) modulate the gut microbiota to alter arterial function, BP, and health with aging? If so, how? (5) What is the potential efficacy of preventive and therapeutic strategies that target the gut microbiota to mitigate arterial dysfunction and elevated BP with aging?

Diet-Induced Gut Microbial Metabolites in CVD and Hypertension

The gut microbiota can directly influence downstream pathways leading to autonomic imbalance and immune responses. In turn, microbiota composition can also be influenced by the environment—especially nutrients from dietary sources. Indeed, one of the largest environmental exposures of the gut microbiota comes from food, and the gut microbiota produces various metabolites. Metabolites generated by certain microbes can exert both paracrine and endocrine effects on the host, leading to either favorable or unfavorable consequences. One such metabolite has recently been described as a microbial nitrogenous metabolite from dietary phosphatidylcholine or L-carnitine known as trimethylamine, which is absorbed and converted by hepatic enzymes (flavin-containing monooxygenase 3) in human to form TMAO and excreted by the kidneys. Studies have demonstrated that the gut microbiota play an obligatory role in producing trimethylamine/TMAO, and its generation is a combined result of dietary exposure and microbial metabolism.[32,33] Accumulation of circulating TMAO can lead to a wide range of adverse effects including macrophage/foam cell activation, steroid and bile acid metabolism, vascular and endothelial cell dysfunction, and platelet hyperresponsiveness, leading to atherosclerosis, cardiorenal impairment, and thrombosis.[34] Although TMAO does not affect BP in normotensive animals, it prolongs the hypertensive effect of angiotensin II, suggesting the possibility that TMAO increases the susceptibility to developing hypertension.[35] Thus, further investigations on the role of gut microbial-derived metabolites

in the pathophysiology of hypertension are warranted for the development of novel therapeutic approaches.

Microbiota-Derived Toxins and CKD

Hypertension is an important risk factor for CKD and is the second leading cause of end-stage renal disease in the United States.[36] Evidence has been accumulating in recent years as to alterations in the gut microbiota in patients with CKD and end-stage renal disease.[37,38] Patients with CKD have an increase in aerobic and anaerobic bacteria in the upper intestinal tract. There are significant differences in abundance of 190 microbial operational taxonomic units between end-stage renal disease and normal control individuals.[38] The most notable changes are reductions in both the Lactobacillaceae and Prevotellaceae families. The number of aerobic bacteria such as *Enterobacteria* and *Enterococci* was found to be ≈100 times higher in patients on maintenance hemodialysis than in controls.[39] Among anaerobic bacteria, hemodialysis patients had lower counts for *Bifidobacteria* and higher counts for *Clostridium perfringens*. Multiple factors contribute to the pathogenesis of dysbiosis in uremia, including secretion of urea into the gut, decreased consumption of dietary fiber, frequent use of antibiotics, slow colonic transit, metabolic acidosis, intestinal wall edema, and possibly oral iron intake. Impaired protein assimilation in uremia leads to a large influx of undigested proteins into the distal intestine, which favors the proliferation of proteolytic bacteria. Increased protein fermentation by proteolytic bacteria then results in generation of potentially toxic metabolites, such as ammonia, phenols, amines, indoles, and thiols.[40] Uremia also increases intestinal permeability,[41] allowing translocation of intact bacteria, bacterial fragments, endotoxin, and uremic toxins into the systemic circulation. Interestingly, TMAO concentrations are also found high in patients with CKD.[42] In animal models, TMAO directly contributed to progressive renal fibrosis and dysfunction.[42] Taken together, it is possible to hypothesize that the gut microbiota and the products generated by them, coupled with predicted leaky gut, could have major implications in the development and establishment of CKD and perhaps high BP as well.

To move this field forward, new studies will need to respond to several key questions: (1) Is alteration in the microbiome progressive in CKD? (2) If so, is there a unique bacterial signature in CKD? (3) Does the metabolic potential of the gut microbiota change in CKD? Novel therapeutic strategies (ie, prebiotics, probiotics, or symbiotics) will need to be explored for CKD and end-stage renal disease and to examine any associated effects on BP. The National Institute of Diabetes and Digestive and Kidney Diseases is conducting 2 studies, the Hemodialysis Novel Therapies Consortium (NCT02572882) and the CKD Pilot Studies, to define variability in microbiome profile and link these with metabolite profiles.

Oral Microbiome and Hypertension

Remarkable progress has been made to advance our understanding of the complex bacterial communities in human oral cavities, their classification, and implications in human health and diseases.[43–45] The oral microbiome comprised ≈700 species, and ≈67% of oral bacterial species have been cultivated (Human Oral Microbiome Database, www.homd.org). Recent advances in metagenomic and metabolomic technologies, coupled with successful cultivation of oral bacterial species, have all contributed to an explosion of oral microbiome research and understanding. For example, using sequencing data combined with spectral fluorescence imaging, Mark Welch et al[44] have recently demonstrated a direct visualization of the highly organized microbial consortium in human dental plaque at the micron scale. Because the oral cavity is the first to experience environmental, nutritional, and other external influences, a delicate balance in its microbial communities can have a major impact on human health and diseases. The significance of oral microbiome is continuously emerging, implicating oral dysbiosis in many periodontal diseases and other chronic diseases, such as CVD and hypertension.[46,47] For example, a direct relationship between the levels of subgingival periodontal bacteria and high BP has recently been reported.[47]

The WG discussed the role of oral microbiota in NO production and its implication in hypertension. It is known that NO can be modulated by microbial communities in the oral cavity to effectively reduce dietary nitrates to nitrite and NO, independent of its enzymatic synthesis from L-arginine.[48] Dietary nitrate from vegetable matter or from the oxidation of endogenous NO production is absorbed from the upper digestive tract into the bloodstream and concentrated in the salivary glands by active transport. About 20% of salivary nitrate is reduced to nitrite in the mouth by facultative anaerobic bacteria that are found on the surface of the tongue.[49] The circulation of nitrate back to the oral cavity for reduction to nitrite/NO is termed the enterosalivary nitrate–nitrite–NO pathway.[48] This pathway in humans seems to serve as an alternative mechanism that can provide an endothelium-independent source of bioactive NO compensating for insufficient host NO production (eg, with aging). This pathway is dependent on commensal oral bacteria to perform the first step (2-electron reduction) because mammals lack a functional nitrate reductase.[46] The presence of nitrate-reducing bacteria in the oral cavity, concentrated on the tongue, has been well documented.[50,51]

A recent meta-analysis has shown that increasing the dietary intake of nitrate-rich food products is effective in reducing BP.[52] Therapeutically, this information can offer an effective strategy to promote NO production and treat hypertension by modulating the specific oral nitrate- and nitrite-reducing bacterial communities. Understanding and harnessing this redundant compensatory pathway may prove to be a viable and cost-effective strategy. Furthermore, this pathway may explain the biochemical and physiological link between oral health and CVD through maintenance of NO production.

Diets, Probiotics, and Hypertension

Foods and dietary constituents contribute to the gut microbial community structure (ie, the types of microorganisms) and functional activity (ie, microbial gene expression). Given that components of host diet serve as energy and nutrient sources for microbial growth, substrates reaching the large intestine are a key contributor to the composition of the microbial community. Epidemiological studies indicate that high-fiber diet is associated with reduced BP and CVD.[13,53] Also, many studies have been reported regarding the beneficial effects of certain

probiotic bacterial strains that can decrease circulating levels of cholesterol and BP.[14] A recent meta-analysis of randomized controlled trials has also demonstrated beneficial effects of probiotics in reducing BP.[12] However, some studies have failed to find any beneficial effect of probiotics on BP, heart rate, or cardiovascular risk markers.[13,14,53] Thus, further studies are needed to resolve this conundrum.

Animal studies have demonstrated the role of diet and gut microbiota in BP control and hypertension. Treatment with probiotics *Lactobacillus* strains exerted antihypertensive effects in spontaneously hypertensive rats and improved endothelial function. This effect was associated with changes in the gut microbiota.[54] Another study showed that dietary intake of fiber and supplementation with acetate could modulate renal and cardiac pathways to produce cardiovascular beneficial effects and lower BP in mice.[16] Again, this treatment was associated with changes in the gut microbiota: that is, decreased ratio of Firmicutes and Bacteroidetes to induce eubiosis, induce acetate-producing bacteria, and attenuate adverse actions of mineralocorticoid excesses on BP. The emerging story is that the diets with beneficial cardiovascular effects likely influence gut microbial communities and influence microbial metabolites with profound effects on the cardiovascular system and BP. However, answers to many more questions will be needed to support this concept. Some include (1) Are there unique microbial communities enhanced by high-fiber diet? (2) If so, would they be beneficial as antihypertensive probiotics? (3) Are there ethnic- and sex-linked differences in hypertension prevalence associated with variability in diet-induced microbiome responsiveness? (4) Are there specific dietary probiotic formulations that could be used for BP regulation, either across the board or in a patient-tailored manner?

Metabolomic profiling of patients on different dietary conditions and associations with ethnic, sex, and microbiome composition needs to be performed. Similarly, interactions between salt and diet on gut pathophysiology and microbiome must be evaluated.

Conclusions, Recommendations, and Future Directions

The WG concluded that significant evidence exists to implicate the role of microbiota in BP regulation and agreed that this is a rapidly evolving field with tremendous potential for clinical implications and translation into therapeutic interventions for hypertension. The WG recognized several scientific questions, areas, challenges, and opportunities for further investigation.

1. Proof-of-concept investigations need to continue
   - i. Use of multiple animal models and development of novel animal models
   - ii. Metagenomics, metatranscriptomics, and metabolomics
   - iii. Identification, cultivation, and genomic and functional characterization of vascular-modifying microbial strains
   - iv. Host genome–microbiome cross-talk
   - v. Role of viruses, archaea, and fungi
2. Involvement of oral microbiome and its implication in the treatment of hypertension
3. Brain–gut axis in hypertension, mechanisms, gut pathophysiology, and implications in development of hypertension
4. Kidney–gut axis in hypertension, mechanisms, gut pathophysiology, and implications in development of hypertension
5. Nutritional factors and impact on microbiota-linked BP regulation
6. Preclinical investigations
   - i. Large-scale metagenomic studies: sex, race, and drug sensitivity
   - ii. Is there a unique microbial signature linked to sex, race, drug sensitivity, and so on?
   - iii. Metabolomics to identify hypertension and normal microbiota-derived metabolite profiles
   - iv. The therapeutic potential of fecal and oral transplant for control of hypertension
   - v. Investigation of pro- and prebiotics, alone or in combination with anti-inflammatory/antimicrobial drugs and antihypertensive drugs for resistant hypertension
7. Translational studies in humans
   - To confirm observations from preclinical investigations regarding the mechanistic role of microbiome in the cause of increases in BP/clinical hypertension and other changes in cardiovascular health with aging
   - To test and establish the efficacy of novel lifestyle and pharmacological interventions targeting microbiome for the prevention and treatment of clinical hypertension and other cardiovascular disorders

The WG also identified the following needs to move the research field forward:

1. Standardized technology to measure comprehensive metabolites in blood, saliva, and stool of animals and of patients with hypertension
2. Development of an integrated system to measure BP and hydrogen-specific, H_2S-specific, and methane-specific electrode systems to measure gut microbiota activity and diversity in vivo
3. National/international forum for microbiota in BP regulation
4. Standard protocols to measure gut blood flow

Perspectives

Dr Irvin Page proposed the mosaic theory of hypertension >60 years ago, suggesting that a complex interplay of multiple factors, including genetics, environmental, anatomic, adaptive, neural, endocrine, humoral, and hemodynamics, all of which were referred to as forces interdigitated to increase BP.[55] This theory has been iconic in providing a framework for elucidation of various cellular, molecular, and genetic dysregulated mechanisms that occur at the level of organs assessed for hypertension. These local events coordinate actions of multiple organs, including the brain, the vasculature, and the kidney, to affect BP. By sheer coincidence, the WG met on the day marking the anniversary of Dr Page's death (June 10, 1991), whereby the opportunity arose for the panel to reflect on this theory in the context of the recent discovery of links between microbiota and cause of hypertension. The WG noted that given the compelling evidence thus far linking microbiota to hypertension, it may be important to revisit the Page model

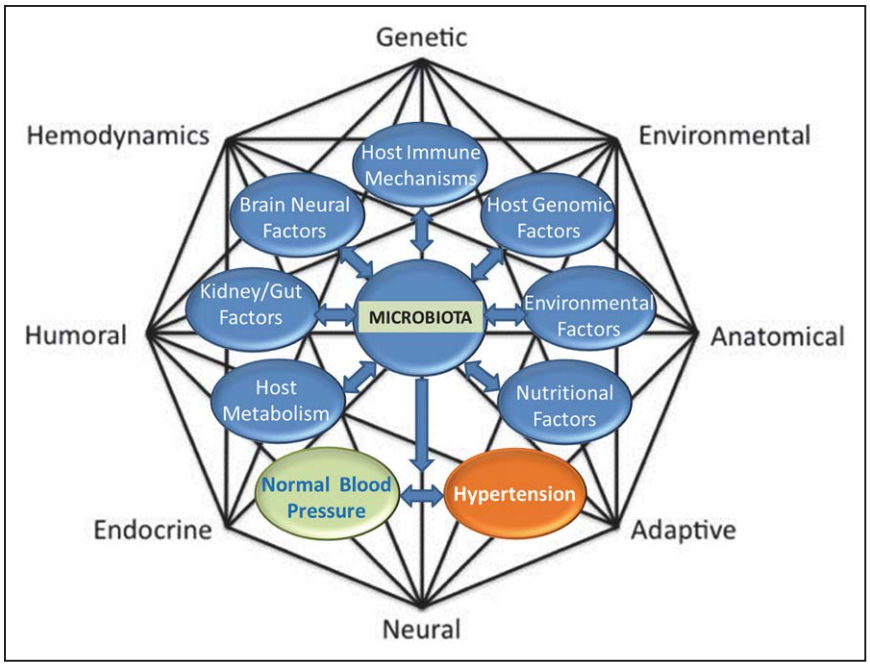

Figure. The revised mosaic theory of hypertension showing potential influences of microbiota in blood pressure control and hypertension.

to determine whether and how microbiota influences each of the forces that impact BP. Thus, we propose including microbiota along the previous guiding principles in the Page theory (Figure).

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Disclosures

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