Gut Dysbiosis Is Linked to Hypertension

Tao Yang,* Monica M. Santisteban,* Vermali Rodriguez,* Eric Li, Niousha Ahmari, Jessica Marulanda Carvajal, Mojgan Zadeh, Minghao Gong, Yanfei Qi, Jasenka Zubcevic, Bikash Sahay, Carl J. Pepine, Mohan K. Raizada, Mansour Mohamadzadeh

Abstract—Emerging evidence suggests that gut microbiota is critical in the maintenance of physiological homeostasis. This study was designed to test the hypothesis that dysbiosis in gut microbiota is associated with hypertension because genetic, environmental, and dietary factors profoundly influence both gut microbiota and blood pressure. Bacterial DNA from fecal samples of 2 rat models of hypertension and a small cohort of patients was used for bacterial genomic analysis. We observed a significant decrease in microbial richness, diversity, and evenness in the spontaneously hypertensive rat, in addition to an increased Firmicutes/Bacteroidetes ratio. These changes were accompanied by decreases in acetate- and butyrate-producing bacteria. In addition, the microbiota of a small cohort of human hypertensive patients was found to follow a similar dysbiotic pattern, as it was less rich and diverse than that of control subjects. Similar changes in gut microbiota were observed in the chronic angiotensin II infusion rat model, most notably decreased microbial richness and an increased Firmicutes/Bacteroidetes ratio. In this model, we evaluated the efficacy of oral minocycline in restoring gut microbiota. In addition to attenuating high blood pressure, minocycline was able to rebalance the dysbiotic hypertension gut microbiota by reducing the Firmicutes/Bacteroidetes ratios. These observations demonstrate that high blood pressure is associated with gut microbiota dysbiosis, both in animal and human hypertension. They suggest that dietary intervention to correct gut microbiota could be an innovative nutritional therapeutic strategy for hypertension. (Hypertension. 2015;65:00-00. DOI: 10.1161/HYPERTENSIONAHA.115.05315.) • Online Data Supplement

Key Words: butyrate ■ dysbiosis ■ hypertension ■ microbiota ■ minocycline

Hypertension is not only the most modifiable risk factor for cardiovascular disease and stroke but also a hallmark of obesity, diabetes mellitus, and metabolic syndrome.1 Despite recent advances in pharmacotherapy and widespread adoption of lifestyle changes, prevalence of hypertension remains high. By some estimates, the prevalence of resistant hypertension is ≈10% to 15% among the hypertensive patient population. Treatment-resistant hypertension is characterized by high norepinephrine spillover, enhanced sympathetic outflow, and dampened parasympathetic activity, thus suggesting a neurogenic component that is essential in maintaining elevated blood pressure (BP) levels.2-3 Increasing evidence suggests that aurotin dysfunctions, treatment-resistant hypertension is also accompanied by a chronic low-grade inflammatory profile that facilitates end-organ damage and perpetuates the hypertensive state.4-5 However, the intrinsic mechanisms that contribute to sustaining the systemic inflammatory profile found in these patients are yet to be fully understood.

In the periphery, gut microbiota plays an important role in shaping a robust systemic and intestinal immune system.6,7 It is commonly referred to as an essential acquired organ because its composition and richness are constantly adapting to the challenges presented by the environment or by the host, such as age, diet, lifestyle modifications, and disease states.8,9 Typically, changes in gut microbiota have been related to chronic inflammatory diseases, such as asthma, allergies, inflammatory bowel disease, and infectious diseases.10 However, recent data suggest that it may also play a role in the development and maintenance of cardiovascular disease and metabolic disorders, such as obesity, diabetes mellitus, and metabolic syndrome.11-14 Adult gut microbiota is diverse; it is made up of trillions of microorganisms but mainly dominated by 4 phyla: (1) Firmicutes, (2) Bacteroidetes, (3) Actinobacteria, and (4) Proteobacteria. A delicate balance in the gut microbiota composition is key in maintaining intestinal immunity and whole-body homeostasis; any disruption of this balance could lead to

Received February 9, 2015; first decision February 20, 2015; revision accepted March 17, 2015.
From the Department of Infectious Diseases and Pathology, College of Veterinary Medicine (T.Y., M.Z., M.G., B.S., M.M.), Division of Gastroenterology, Hematology and Nutrition, Department of Medicine (T.Y., M.Z., M.G., B.S., M.M.), Department of Physiology and Functional Genomics (M.M.S., V.R., J.M.C., M.K.R.), College of Medicine, Division of Cardiovascular Medicine, Department of Medicine (V.R., Y.Q., C.J.P.), Division of Infectious Diseases and Global Medicine, Department of Medicine (E.L.), and Department of Physiological Sciences, College of Veterinary Medicine (N.A., J.Z.), University of Florida, Gainesville.
*These authors contributed equally to this article.
The online-only Data Supplement is available with this article at http://hyper.ahajournals.org/lookup/suppl/doi:10.1161/HYPERTENSIONAHA.115.05315/-/DC1.
Correspondence to Mohan K. Raizada or Mansour Mohamadzadeh, University of Florida, Box 100274, Gainesville, FL 32610. E-mail mraizada@ufl.edu or m.zadeh@ufl.edu
© 2015 American Heart Association, Inc.
Hypertension is available at http://hyper.ahajournals.org DOI: 10.1161/HYPERTENSIONAHA.115.05315
devastating pathophysiological consequences. An imbalance in gut microbiota is commonly known as dysbiosis. Although characterization of a healthy microbiota is at its initial stages, increasing evidence suggests that changes in the ratio of the microbe communities Firmicutes (F) and Bacteroidetes (B), known as the F/B ratio, can be potentially used as a biomarker for pathological conditions.

Several clinical trials have been conducted evaluating the use of probiotics and its effects on BP regulation. A meta-analysis of 9 randomized trials showed a significant decrease in both systolic BP (SBP) and diastolic BP in patients who consumed a daily dose of ≥10⁹ CFU of probiotics. This evidence indirectly suggests that gut microbiota may play a key role in the control of BP homeostasis and that any change in microbiota composition or imbalance may potentially result in hypertension. Hence, we hypothesize that hypertensive risk factors, such as genetic predisposition and diet, induce changes in the gut microbiota that results in dysbiosis, and controlling this effect may prove as an alternative treatment for this disease. This study was designed to provide evidence for this hypothesis.

Methods

The detailed description of Materials and Methods is available in online-only Data Supplement. All animal procedures were approved by the University of Florida Institutional Animal Care and Use Committee. Human studies were approved by the University of Florida Institutional Review Board.

Results

Hypertension Is Associated With Alteration in Fecal Microbiota Composition in the Spontaneously Hypertensive Rat

Fecal DNA was isolated from both Wistar Kyoto rats (WKY; mean arterial pressure: 108±2 mmHg) and spontaneously hypertensive rats (SHRs; mean arterial pressure: 148±10 mmHg), and the bacterial loads were represented as fecal biomass DNA per milligram of feces. A significant reduction of bacterial load was found in SHRs when compared with WKY rats (Figure 1A). Subsequently, 16S ribosomal DNA sequencing and the bioinformatics alignment comparison against the SILVA nonredundant database were performed. Significant fecal microbial alterations were found in the SHRs when compared with the WKY rats. The compositions of bacterial communities were evaluated by calculating 3 major ecological parameters, including Chao richness (an estimate of a total number of operational taxonomic units present in the given community), Pielou evenness (to show how evenly the individuals in the community are distributed over different operational taxonomic units), and Shannon diversity (the combined parameter of richness and evenness). Microbial richness, evenness, and diversity were all found to be drastically decreased in the SHR group when compared with the WKY control (Figure 1A). Furthermore, weighted UniFrac analyses were used to calculate distances between the fecal samples from the WKY rats and SHRs, and 3-dimensional (3D) scatterplots were generated by principal coordinate analysis (PCoA) to visualize whether the experimental groups in the input phylogenetic tree have significantly different microbial communities. This method allows us to present dissimilarities of the data in terms of distance. Each principal coordinate axis percentage describes how much 1D can account for. The composition of the fecal microbial communities of the WKY rats and SHRs was found to be distinct, as presented in Figure 1B. A clear separation was observed between the 2 clusters, representing WKY rats and SHRs, respectively. Results were compared by Student t test; *P<0.05 and **P<0.01. PC1–3 indicates principal coordinates 1–3.

![Gut microbial communities in Wistar Kyoto (WKY) rats and spontaneously hypertensive rats (SHRs).](image)
microbiota pattern, we analyzed the proportions of 16S rDNA reads assigned to each phylum. The F/B ratio in the SHR was >5-fold higher compared with WKY rats (Figure 2A). Fecal samples from both WKY rats and SHRs were dominated by Firmicutes and Bacteroidetes and with smaller proportions of Actinobacteria and Proteobacteria (Figure 2B). However, significant differences were observed between the groups in terms of the relative abundances of Actinobacteria, Bacteroidetes, and Firmicutes. The diminished proportion of Actinobacteria indicates a less diverse microbiota in the SHR, which is consistent with the decrease in Chao richness and Shannon diversity.

**Acetate and Butyrate Fermenters Decreased in the SHR**

Given an imbalanced F/B ratio in the SHR, we asked what genera of bacteria contributed to the alteration of microbiota composition toward dysbiosis. LEfSe performed at the genus level demonstrated that butyrate-producing bacteria were found to be highly accumulated in WKY rats, including *Coprococcus* and *Pseudobutyryviridio* (Figure 3A). In contrast, lactate-producing bacteria, *Streptococcus* and *Turicibacter*, were in higher quantities in the SHR. Two uncultured genera operational taxonomic units (109 and 177) that belonged to Bacteroidetes, and Firmicutes. The diminished proportion of Actinobacteria indicates a less diverse microbiota in the SHR, which is consistent with the decrease in Chao richness and Shannon diversity.

<table>
<thead>
<tr>
<th>Phylum</th>
<th>WKY Mean abundance (%)</th>
<th>SHR Mean abundance (%)</th>
<th>P value</th>
<th>F/B Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinobacteria</td>
<td>0.0026**</td>
<td>0.26</td>
<td>&lt;0.0001</td>
<td>2.50</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>&lt;0.0001**</td>
<td>4.21</td>
<td>&gt;0.05</td>
<td>18.30</td>
</tr>
<tr>
<td>Firmicutes</td>
<td>&lt;0.0001**</td>
<td>94.78</td>
<td>&gt;0.05</td>
<td>78.56</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>0.9996</td>
<td>0.094</td>
<td>&gt;0.05</td>
<td>0.094</td>
</tr>
</tbody>
</table>

Figure 2. Comparison of microbiota composition between Wistar Kyoto (WKY) rats and spontaneously hypertensive rats (SHRs). A, The Firmicutes/Bacteroidetes ratio (F/B ratio) was calculated as a biomarker of gut dysbiosis. B, Phylum breakdown of the 4 most abundant bacterial communities in the WKY rat (n=5) and SHR (n=6) fecal samples. A decrease of Bacteroidetes along with an increase of Firmicutes resulted in a dysbiosis signature of gut microbiota in SHRs. A significant reduction of the Actinobacteria phylum correlated with a lower diversity value. Results were compared by Student t test; **P<0.01 and ***P<0.001.

**Lower Richness and Diversity of Gut Microbiota in Hypertensive Patients**

On the basis of these data, we speculated that the composition of the microbiota would also differ between patients with normal (119±2 mm Hg; n=10) and high (144±9 mm Hg; n=7) SBP (Figure 4A). Fecal samples were processed and analyzed via Illumina sequencing as described for rodent experiments. We observed a reduction in bacterial Chao richness and Shannon diversity in the patients with high SBP when compared with normal SBP (Figure 4A). There was a trend toward a decrease in Pielou evenness as well, but it did not reach statistical significance with this limited number of patients.

Next, weighted UniFrac analyses were used to calculate distances, and 3D scatterplots were generated using PCoA. As shown in Figure 4B, the 2 patient populations formed separate clusters. The analyzed human data clearly confirmed the observation made in our animal model where gut dysbiosis is observed between the high and normal SBP cohorts. Nonetheless, further studies to strengthen this observation need to be conducted using a larger cohort of hypertensive patients.

**Gut Microbial Diversity Is Increased by Treatment of Minocycline**

Because gut microbiota can be modified through the use of broad-spectrum antibiotics, we evaluated the efficacy of using minocycline in restoring gut microbiota. Minocycline is an anti-inflammatory antibiotic that freely crosses the blood–brain barrier and has been shown to produce beneficial effects in combating hypertension. In addition, minocycline

*Yang et al*   *Dysbiosis in Hypertension* 3
is the drug approved in our patient studies (NCT numbers, 02133872 and 02133885), which has shown an impressive ability to decrease BP, improve hemoglobin A1c levels, and weight loss in our pilot study.28 Therefore, understanding the effects of minocycline on microbiota will be greatly beneficial. For this purpose, we used the chronic angiotensin II (Ang II) infusion model, which is a well validated and established model of hypertension. As expected, oral minocycline for 4 weeks was able to significantly lower mean arterial pressure (24-h mean arterial pressure: 124±2 versus 168±2 mm Hg; Figure 5A). Consistent with the SHRs, rats infused with Ang II showed a trend toward lower bacterial load, although this finding did not reach significance (Figure 5B). Minocycline treatment did not result in a further reduction of bacterial load. This is not surprising because previous studies have indicated that the depletion of microbiota required a combination of at least 3 antibiotics for 3 to 4 weeks,29,30 and single antibiotics may not be sufficient to reduce the total bacterial load.31

To test the effect of minocycline on microbial composition, 16S rDNA sequencing was performed and data were statistically analyzed as before. Rats infused with Ang II alone demonstrated a lower richness value when compared with control (Figure 5B). Despite this lower richness value,
we did not observe a clear separation between the clusters of these 2 groups in PCoA analysis (Figure 5C). However, Ang II+minocycline rats displayed a significant separation from the other 2 groups, indicating a shift in the gut bacterial composition by the administration of minocycline.

Most importantly, the F/B ratio was considerably increased in the Ang II–infused rats compared with controls (Figure 6A). This was significantly reduced by minocycline treatment. In addition, the pie charts that represent the composition of microbiota at the phylum level show a trend toward relative higher abundance of Firmicutes and a lower abundance of Bacteroidetes in Ang II–infused rats when compared with controls (Figure 6B), although the differences between those 2 groups did not reach individual significance. This indicates that...
the dysbiosis in this model is characterized by both an increase in Firmicutes and a decrease in Bacteroidetes. This signature of gut microbiota dysbiosis is consistent with that previously shown in the SHR model. Interestingly, oral administration of minocycline was capable of increasing Bacteroidetes in the gut, which has been reported as a positive sign after successful fecal microbiota transplantation for the treatment of recurrent *C. difficile* infection–induced dysbiosis. In addition, we observed a striking bloom of other bacteria phyla (including Verrucomicrobia) beyond Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, and Tenericutes, indicating a significant shift of intestinal microbial composition toward diversity. In summary, we demonstrated that minocycline administration was able to restore the Bacteroidetes population and reshape the microbiota composition, by drastically increasing microbial diversity and restoring the F/B ratio.

**Acetate- and Butyrate-Producing Bacteria Are Expanded by Minocycline Treatment**

Because minocycline treatment caused a remarkable shift in the gut microbial environment, we investigated the specific bacteria with notable alteration of abundance that could be verified at the genus level by LEfSe analysis and identified the 4 most expanded genera in the Ang II+minocycline–treated rats. Figure 7A shows the enrichment of *Akkermansia*, *Bacteroides*, *Enterorhabdus*, and *Marvinbryantia* in the Ang II+minocycline–treated group. Functionally, both *Bacteroides* and *Marvinbryantia* produce acetate as their main fermentation product, and *Akkermansia* and *Enterorhabdus* have been found to be inversely associated with obesity and diabetes mellitus.

Similar to the SHR, the acetate- and butyrate-producing bacteria genera were also depleted in chronically infused Ang II rats, although this finding failed to reach significance (Figure 7B). However, oral administration of minocycline resulted in a remarkable increase of both the acetate- and butyrate-producing bacteria. This was associated with a decrease in the abundance of lactate-producing bacteria.

**Discussion**

This study provides the first evidence, to our knowledge, of an association of hypertension with altered gut microbiota with the use of 2 different rat models of hypertension and a small cohort of hypertensive patients. The major findings of this study are (1) decreases in the microbial richness and marked increases in the F/B ratio in the animal models of hypertension, implicating the existence of gut dysbiosis in hypertension; (2) this dysbiosis was associated with decreases in acetate- and butyrate-producing bacteria and an increase in the lactate-producing bacterial population; (3) gut microbiota dysbiosis was confirmed in a small cohort of human hypertensive patients, pointing to the potential clinical significance of this work; (4) oral minocycline could rebalance the gut microbiota in a rat model of hypertension. These findings clearly implicate the role of gut microbiota in the pathophysiology of both animal and human hypertension.

Gut microbial dysbiosis has been connected to many chronic diseases, including pathophysiologies that are associated with the cardiovascular system, such as diabetes mellitus, obesity, and cardiac dysfunctions. In this study, we used 3 commonly used parameters to study the gut microbiota: Chao richness, Pielou evenness, and Shannon diversity. The discrepancy...
Finally, we found a reduction in the fecal samples; however, its significance in pig models requires note that the F/B ratio is well validated in rodent and human diabetes mellitus, and cardiovascular disease. It is important to gut dysbiosis and has been associated with obesity, diabetes of Bacteroidetes, has been widely considered a signature of both the SHR and Ang II infusion models. An increased F/B ratio, caused by an expansion of Firmicutes or a contraction of Bacteroidoites, has been widely considered a signature of gut dysbiosis is characterized by an imbalance in specific microbial populations and their corresponding metabolites, specifically decreased acetate and butyrate-producing bacteria were found in both models of hypertension. Some butyrate-producing bacteria can use acetate as an energy source to generate butyrate. Butyrate, one of the most beneficial SCFAs, harbors multiple effects for the host, such as reduction of inflammation in the intestine and adipose tissue, improved insulin sensitivity in type 2 diabetes mel- litus, protection against diet induced obesity, and cardiovascular disease. This SCFA plays a regulatory role in the epithelial cell differentiation, barrier function, stimulation of regulatory T cells, amelioration of mucosal inflammation, and suppression of colorectal cancer to name a few effects in the intestine. Systematically, butyrate has been shown to prevent immune cell infiltration and improve renal function following ischemic damage. Another SCFA, propionate, has been shown to induce vasodilation and acute hypertensive response in wild-type mice, mediated by Gpr41 receptor. Therefore, we can conclude that the hypertension-associated gut dysbiosis is characterized by an imbalance in specific microbial populations and their corresponding metabolites, specifically decreased acetate and butyrate-producing bacteria and increased lactate-producing bacteria. However, further experiments demonstrating the direct and indirect effects of SCFAs on BP are necessary.

Our previous studies have indicated that minocycline influences microglial activation and neuroinflammation in auto-nomic brain regions. Minocycline is an anti-inflammatory antibiotic that penetrates through the blood–brain barrier and causes a reduction in high BP. Doxycycline belongs to the same antibiotic class but has limited ability to pass through the blood–brain barrier, and its effects on BP are inconclusive because there are multiple conflicting reports. In this study, we chose to focus on minocycline as it is the focus of can be attributed to the differences in pathogenesis in both of these animal models and may represent an important area of future research focus. However, the most important and recognized biomarker, the F/B ratio, was dramatically increased in both the SHR and Ang II infusion models. An increased F/B ratio, caused by an expansion of Firmicutes or a contraction of Bacteroidoites, has been widely considered a signature of gut dysbiosis and has been associated with obesity, diabetes mellitus, and cardiovascular disease. It is important to note that the F/B ratio is well validated in rodent and human samples; however, its significance in pig models requires further studies. Finally, we found a reduction in the fecal biomass in the SHR, as well as a trend toward reduction in the Ang II infusion model. This is consistent with the previous reports that dysbiosis is associated with a decrease of bacterial loads. Previous studies failed to identify differences in the gut microbiota between WKY rats and SHRs, and L-NAME hypertension. However, the discrepancies may be accounted for by (1) significant pathophysiological differences between WKY rat and SHR colonies depending on origin, breeding, and maintenance protocols; (2) lack of details about animal housing or pathogen-free facilities; (3) using a large enough number of rats necessary to detect a differences; and (4) choosing the best models available for human hypertension.

In contrast, the changes in the microbiota were associated with changes in SCFAs. Increased acetate and butyrate and reduced microbial diversity are linked to diabetes mellitus, obesity, and cardiac dysfunctions. Our observations are consistent with these reports and support the important role of microbial diversity in hypertension. Of particular interest is the finding that lactate-producing bacteria were increased in both animal models of hypertension because plasma lactate levels have been associated with increased BP. However, we did not measure plasma lactate in any of our experiments, and this remains a future direction. In addition, a greater microbial diversity of the gut is conducive to enrichment of bacterial colonies that are known to produce metabolites, such as short-chain fatty acids (SCFAs), that are beneficial in maintaining normal physiological homeostasis. Interestingly, decreases in the butyrate- and acetate-producing bacteria were found in both models of hypertension. Some butyrate-producing bacteria can use acetate as an energy source to generate butyrate. Butyrate, one of the most beneficial SCFAs, harbors multiple effects for the host, such as reduction of inflammation in the intestine and adipose tissue, improved insulin sensitivity in type 2 diabetes mellitus, protection against diet induced obesity, and cardiovascular disease. This SCFA plays a regulatory role in the epithelial cell differentiation, barrier function, stimulation of regulatory T cells, amelioration of mucosal inflammation, and suppression of colorectal cancer to name a few effects in the intestine. Systematically, butyrate has been shown to prevent immune cell infiltration and improve renal function following ischemic damage. Another SCFA, propionate, has been shown to induce vasodilation and acute hypertensive response in wild-type mice, mediated by Gpr41 receptor. Therefore, we can conclude that the hypertension-associated gut dysbiosis is characterized by an imbalance in specific microbial populations and their corresponding metabolites, specifically decreased acetate and butyrate-producing bacteria and increased lactate-producing bacteria. However, further experiments demonstrating the direct and indirect effects of SCFAs on BP are necessary.
our approved clinical studies, and therefore, understanding the effects of this broad-spectrum antibiotic on the gut microbiota homeostasis is crucial. Oral minocycline treatment results in the attenuation of high BP induced by chronic Ang II infusion. The first important finding was that minocycline did not reduce fecal biomass, providing evidence that this antibiotic does not have negative effects on the gut microbiota. In addition, our data show that this treatment resulted in a drastic shift in the microbial environment characterized by a bloom of diverse bacteria in the gut. A dramatic increase in Verrucomicrobia phylum bacteria was observed, indicating that minocycline produced an intestinal environment that was extremely conducive to nurturing the growth of bacteria of this phylum. Further analysis of Verrucomicrobia phylum revealed a significant increase in the mucin-degrading bacterium, Akkermansia. It is pertinent to point out that a decrease in Akkermansia has been associated with obesity and diabetes mellitus, and introduction of Akkermansia could reverse the metabolic disorder and improve inflammation in diabetic and obese patients. In addition, preliminary data indicate that minocycline treatment was able to reverse the Ang II–induced decrease in cingulin in the colon. Cingulin is a tight junction protein whose decreased levels have been associated with gut barrier dysfunction leading to increased inflammation. Thus, these gut-specific effects of minocycline seem to be linked with minocycline’s beneficial effects on inflammation and hypertension.

This raises an important question, is minocycline’s antihypertensive effect primarily on the gut, or on the brain or simultaneously on both, resulting in the regulation of gut–brain axis? There is no evidence to rule out either possibility at the present time because minocycline can freely cross the blood–brain barrier26 and can directly influence both the gut and the brain. Minocycline has profound effects on the gut: (1) it inhibits expression of proinflammatory cytokines and intestinal damage in mouse model of colitis,52,53 (2) it attenuates gut damage and intestinal mucositis because of cancer chemotherapy,54 and (3) our data show that it improves gut microbial homeostasis during hypertension dysbiosis and is able to rebalance the gut acetate-, butyrate-, and lactate-producing bacterial populations. Furthermore, evidence of direct anti-inflammatory effects of minocycline in the brain in many neurodegenerative and neurobehavioral diseases also exists.49,55

We propose the following hypothesis: prohypertensive signals (eg, diet, genetic predisposition, and obesity) affect gut microbial composition, inducing dysbiosis, increase inflammatory cells in the bone marrow, and blood, as well as hematopoietic stem cells. This increase in hematopoietic stem cells has been implicated in their extravasation into the brain and establishment of neuroinflammation in many neural diseases.57,58 The combination of gut dysbiosis, peripheral inflammation, and neuroinflammation leads to the development, establishment, and maintenance of high BP. Further studies are needed to determine whether the gut dysbiosis precedes the high BP and is crucial for the development of hypertension or is rather important in the late stages of the disease. In addition, we postulate the following sequence of events in minoinduced antihypertensive effects: (1) in addition to crossing the blood–brain barrier and inhibiting microglial activation, minocycline treatment induces an anti-inflammatory environment in the gut, which is conducive to enrichment of bacterial colonies that are known to produce metabolites, such as SCFAs, that are beneficial in maintaining normal physiological homeostasis; (2) these metabolites directly or indirectly affect the generation of hematopoietic stem cells from the bone marrow, decreasing the levels of myeloid progenitors; (3) this would result in attenuation of peripheral inflammation and decrease in mobilization of myeloid cells to the brain. The latter would be associated with attenuation of neuroinflammation. Future experiments will be aimed to provide evidence for this hypothesis.

In summary, our study demonstrates that hypertension is associated with gut microbiota dysbiosis, characterized by an increased F/B ratio, as well as a drastic decrease in acetate-, butyrate-, and an accumulation of lactate-producing microbial populations in 2 rat models of hypertension. Treatment with oral minocycline dose that attenuates hypertension also produces beneficial effects on dysbiosis. Most importantly, this study does indeed demonstrate a dysbiotic gut profile in patients with high BP. It is important to mention that the 2 control subjects observed within the hypertension PCoA plot distribution pertain to subjects who were currently under anti-hypertension treatment although optimum medication combination and dosage had not been met. Therefore, this suggests that reversing the gut dysbiosis might be affected by the stability of BP control and the time undergoing such treatment. This clinical trial continues to recruit patients to be able to further establish the profile of the hypertension gut dysbiosis, in addition to provide enough patients to perform subgroup analyses. Nonetheless, these observations support our overall proposal of a microbial dysbiosis linked to hypertension.

### Perspectives

The involvement of gut dysbiosis in the pathogenesis of many diseases, including diabetes mellitus, obesity, cancer, and mental disorders, is rapidly emerging. However, little is known about the role of microbiota in hypertension. In this study, we present evidence of gut dysbiosis in hypertension for the first time. This includes (1) significant depletion of bacterial richness and increased F/B ratio in 2 rat models of hypertension, (2) decreases in SCFA-producing bacteria and increase in lactate-producing bacterial populations, (3) treatment with minocycline that attenuates high BP and is able to rebalance gut microbiota, and (4) validation of this concept in a small cohort of hypertensive patients. These observations suggest that innovative dietary strategies that affect gut microbiota could be developed for control and treatment of hypertension.

### Sources of Funding

This work was supported by National Institutes of Health grants HL33610, AI093370, and UL1 TR000064 Clinical and Translational Science Award, to the University of Florida, and a grant from Gatorade Trust Funds distributed by the University of Florida, Department of Medicine. M.M. Santisteban is a predoctoral fellow of the Greater Southeast Affiliate, American Heart Association (14PRE18590018).

### Disclosures

None.

References


Hypertension  June 2015


Gut Dysbiosis Is Linked to Hypertension
Tao Yang, Monica M. Santisteban, Vermali Rodriguez, Eric Li, Niousha Ahmari, Jessica Marulanda Carvajal, Mojgan Zadeh, Minghao Gong, Yanfei Qi, Jasenka Zubcevic, Bikash Sahay, Carl J. Pepine, Mohan K. Raizada and Mansour Mohamadzadeh

Hypertension. published online April 13, 2015;
Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2015 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/early/2015/04/13/HYPERTENSIONAHA.115.05315

Data Supplement (unedited) at:
http://hyper.ahajournals.org/content/suppl/2015/04/13/HYPERTENSIONAHA.115.05315.DC1
http://hyper.ahajournals.org/content/suppl/2016/04/11/HYPERTENSIONAHA.115.05315.DC2

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/
SUPPLEMENTAL MATERIAL

GUT MICROBIOTA DYSBIOSIS IS LINKED TO HYPERTENSION

Tao Yang\textsuperscript{1,2\*}, Monica M. Santisteban\textsuperscript{3\*}, Vermali Rodriguez\textsuperscript{3,4\*}, Eric Li\textsuperscript{5}, Niousha Ahmari\textsuperscript{6}, Jessica Marulanda Carvajal\textsuperscript{3}, Mojgan Zadeh\textsuperscript{1,2}, Minghao Gong\textsuperscript{1,2}, Yanfei Qi\textsuperscript{4}, Jasenka Zubcevic\textsuperscript{6}, Bikash Sahay\textsuperscript{1,2}, Carl J. Pepine\textsuperscript{4}, Mohan K. Raizada\textsuperscript{3\*}, Mansour Mohamadzadeh\textsuperscript{1,2\*}

\textsuperscript{1}Department of Infectious Diseases and Pathology, College of Veterinary Medicine, \textsuperscript{2}Division of Gastroenterology, Hematology and Nutrition, Department of Medicine, \textsuperscript{3}Department of Physiology and Functional Genomics, College of Medicine, \textsuperscript{4}Division of Cardiovascular Medicine, Department of Medicine, \textsuperscript{5}Division of Infectious Diseases and Global Medicine, Department of Medicine, and \textsuperscript{6}Department of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL, 32610, USA

* Equal Contribution
+ Co-corresponding Authors

Co-Corresponding authors:

Mohan K. Raizada, Ph.D.
Department of Physiology and Functional Genomics
Email: mraizada@ufl.edu
Phone: 352-392-9299
Fax: 352-294-0191

and

Mansour Mohamadzadeh, Ph.D.
Department of Infectious Diseases and Pathology, Department of Medicine
Email: m.zadeh@ufl.edu
Phone: 352-394-4117
Methods

Animals
All experimental procedures were approved by the University of Florida (UF) Institutional Animal Care and Use Committee and were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All animals were purchased from Charles River Laboratories and housed individually in a temperature controlled room with a 12:12 hour light-dark cycle. Specific pathogen free (SPF) rats were housed individually in positive pressure ventilated racks, supplied with autoclaved bedding and cages, and ad libitum irradiated diets and sterile reverse osmosis water systems. Conventional housing followed proper barrier procedures to provide elevated levels of protection from spread of pathogens and diseases. Rats were housed for a minimum of one month in the facility prior to being used in the outlined experiments. Experiments were conducted at least twice for consistent observations.

Hypertension models
Two rat models of HTN were utilized in this study: (1) eight to ten week old male spontaneously hypertensive rats (SHR) and control Wistar Kyoto rats (WKY), and (2) eight week old male Sprague Dawley (SD) rats chronically infused with 0.9% saline or 200ng/kg/min angiotensin II (Ang II) by subcutaneously implanted mini osmotic pumps (No. 2004 ALZET) for 4 weeks. BP recordings were carried out by radiotelemetry as described previously\(^1\). BP data is presented as mean arterial pressure (MAP). Additionally, SD rats were used to study the effects of minocycline (mino) on Ang II-induced HTN. For this experiment, vehicle (sterile H\(_2\)O) or mino (50 mg/kg/day; Sigma-Aldrich)\(^2,3\) was administered by oral gavage (1ml per animal) two days prior to implantation of Ang II osmotic pumps and continued for 4 weeks. Fecal samples were collected in a sterile environment directly from the rectum into a sterile Eppendorf tube. Samples were flash frozen and stored at \(-80^\circ\)C until microbiota analysis.

Patient Study
This study is approved by the UF Institutional Review Board and registered at ClinicalTrials.gov under NCT#02188381. Briefly, Control (not taking anti-hypertensive medication) and Hypertensive (currently under hypertensive treatment) subjects were identified during their routine clinic care at UF Health Shands and UF Health Cardiology at Springhill, Gainesville FL. Major exclusion criteria for this study were as follows: (1) currently or have been pregnant in the last six months, (2) currently or previous antibiotic treatment within the last 2 months of study enrollment, (3) currently taking anti-inflammatory agents, glucocorticoids or other immune regulating medications, (4) taking probiotics, or (5) history of intestinal surgery, inflammatory bowel disease, celiac disease, lactose intolerance, chronic pancreatitis or other malabsorption disorder. Each subject was older than 18 years of age and provided written informed consent prior to enrollment. Upon enrollment, an office ambient BP measurement was recorded for each individual and stool sample collection kits containing sample collection and return instructions were provided. Stool samples were collected using disposable “nuns” hat by the patients, immediately placed in a stool collection tube (Stratec molecular, Germany) and sent to the UF Center for Inflammation and Mucosal Immunity for analysis. Data
analysis was based on office BP measurements. Despite having a history of hypertension, subjects with SBP < 125 mmHg were classified as controls while subjects with SBP ≥ 125 mmHg were classified as hypertensive.

16s Ribosomal DNA Sequencing
Rat fecal DNA was extracted by using ZR fecal DNA MiniPrep (Zymo Research, Irvine, CA). Primers with adaptor sequences for Illumina Miseq (Illumina, Inc., San Diego, CA) were used to amplify the bacterial 16S ribosomal DNA V4-V5 region. PCR amplicons were purified (Qiagen, Madison, WI) and subsequently quantified by Qubit 2.0 Fluorometer (Invitrogen, Grand Island, NY) and Kapa SYBR fast qPCR kit (Kapa Biosystems, Inc., Woburn, MA). DNA library was finalized with equal amount of amplicons for all data analysis.

Data analysis
Standard bioinformatics alignment comparison was utilized for data analysis. Paired end reads were demultiplexed according to a combination of forward and reverse indices. Additional quality filtering included exact match to sequencing primers and an average quality score of 30 or higher on each read. Prior to further analysis, each paired end read was stitched into one contiguous read using the FLASh (Fast Length Adjustment of Short reads) software tool. Reads that could not be joined were excluded from downstream analysis. All sequences passing filters were aligned against a Silva non-redundant 16S reference database (v108) and assigned taxonomic classifications using USEARCH at a 97% identity threshold, and dereplication to unique reference sequence-based Operational Taxonomic Units (refOTU) was performed using UCLUST at a 97% clustering threshold and summarized in a refOTU table. Additional alpha diversity measures and normalized per level taxonomic abundances were created using custom scripts written in R. Differentially significant features at each level were identified using linear discriminant analysis (LDA) along with effect size measurements (LEfSe). Three-dimensional principal coordinates analyses (PCoA) plots using the tree-based UniFrac distance metric were generated through custom scripts in R and scripts from the QIIME package. Classification of bacterial taxonomy based on the end product was performed as previously described. Briefly, genera were classified into more than one group correspondingly if they were defined as producers of multiple metabolites. Genera that were defined as producing equol, histamine, hydrogen and propionate constituted only a minor portion of the population and were therefore excluded from this analysis.
References


微生物与高血压（摘要）

肠道微生态失调与高血压有关

Gut Dysbiosis Is Linked to Hypertension

Tao Yang, Monica M. Santisteban, Vernali Rodriguez, Eric Li, Niousha Ahmari, Jessica Marulanda Carvajal, Mojgan Zadeh, Minhaeong Gong, Yanfei Qi, Jasenka Zubcevic, Bikash Sahay, Carl J. Pepine, Mohan K. Raizada, Mansour Mohamadzadeh

田野 审校

最新的证据表明，肠道菌群对维持生理动态平衡至关重要。由于遗传、环境和饮食因素对肠道菌群和高血压有明显影响，这项研究目的旨在检验肠道菌群生态失调与高血压是否存在相关关联。研究对2个高血压大鼠模型和一个小的队列患者的粪便样本进行细菌基因组分析。研究观察到自发性高血压大鼠的微生物丰富性、多样性和均匀度都显著减少，除厚壁菌门/拟杆菌的比率增高之外。这些变化伴随着酪酸和产丁酸菌的减少。另外还发现，小型高血压患者群的微生物菌群具有类似的现象。当肠道菌群症状和高血压存在相关性。在慢性血管紧张素II灌注大鼠模型中观察到类似的肠道菌群变化；微生物丰富性显著减少和厚壁菌门/拟杆菌比例升高。这一模型中，我们评估了口服二甲酸双环素恢复肠道菌群的功效。除了减轻高血压之外，二甲酸双环素能够降低厚壁菌门/拟杆菌的比率重新平衡高血压肠道菌群失调。这些观察结果表明，在动物实验中人群研究中高血压和肠道菌群失调存在相关性。研究认为，通过饮食干预来改善肠道菌群可能是一种创新性的高血压治疗策略。

（Hypertension. 2015;65:1331-1340）

原发性醛固酮增多症（摘要）

外周血18-氧皮质醇的测定可区分原发性醛固酮增多症患者的单侧腺瘤及双侧病变

Measurement of Peripheral Plasma 18-Oxocortisol Can Discriminate Unilateral Adenoma From Bilateral Diseases in Patients With Primary Aldosteronism

Fumitoshi Satoh, Ryo Morimoto, Yoshikiyo Ono, Yoshitsugu Iwakura, Kei Omata, Masataka Kudo, Kei Takase, Kazumasa Seiji, Hitotoku Kasamoto, Seijiro Honma, Mitsunobu Okuyama, Kouwa Yamashita, Celso E. Gomez-Sanchez, William E. Rainey, Yoichi Araki, Hironobu Sasano, Yasuhiro Nakamura, Sadayoshi Ito

王硕 译 高传玉 审校

肾上腺静脉血取样是当前区分原发性醛固酮增多症（primary aldosteronism）患者为单侧还是双侧病变的唯一可靠方法。在本研究中，我们试图对已经确诊的234例原发性醛固酮增多症患者一一包括613例计算机断层扫描（computed tomography, CT）可检测到腺瘤的患者及121例双侧病变型醛固酮增多症（bilateral hyperaldosteronism）的患者，评估18-氧皮质醇（18-oxocortisol）及18-氧皮质醇（18-hydroxocortisol）的水平是否有助于鉴别单侧醛固酮腺瘤及双侧醛固酮增多病，所有患者均进行了CT检查及肾上腺静脉血取样。所有的醛固酮腺瘤均进行了手术切除，并以临床特征及组织病理学检查证实诊断的准确性。18-氧皮质醇及18-氧皮质醇通过液相色谱串联质谱法（liquid chromatography tandem mass spectrometry）测定。18-氧皮质醇鉴别腺瘤及双侧病变的临界值为4.7 ng/dL。其敏感度/特异度为0.83/0.99，与之相比，18-氧皮质醇鉴别腺瘤及双侧病变的敏感度/特异度为0.62/0.96，在113例醛固酮腺瘤中，95例（84%）的18-氧皮质醇水平高于6.1 ng/dL，醛固酮水平高于32.7 ng/dL；而在121例双侧病变型醛固酮增多症患者中，9例（8%）的18-氧皮质醇水平高于6.1 ng/dL，醛固酮水平高于32.7 ng/dL。进一步分析发现，27例CT未检测到微腺醛固酮的患者中，8例患者CT可检测到双侧肾上腺结节，其中8-氧皮质醇及醛固酮的最高水平分别为4.8 ng/dL及24.5 ng/dL，分别低于上述的临界值。结论：外周血18-氧皮质醇浓度不仅有助于区分醛固酮瘤和双侧醛固酮增多症，还有助于预测对同时性有增生或微腺瘤的无功能性肾上腺皮质结节行不必要的手术治疗。

（Hypertension. 2015;65:1096-1102.）