The Microbiome and Hypertension

Elaine M. Richards for Mohan K. Raizada
Department of Physiology and Functional Genomics
College of Medicine
Why Do We Worry About Hypertension?

• 46% of Americans have hypertension (SBP>130 mmHg and DBP>80, 130/80, 2017 AHA/ACC guidelines)
• Most of us will develop it as we age
• Leading cause of death and disability-adjusted life years worldwide in 2010
• It is the major, modifiable risk factor for cardiovascular disease, stroke, heart failure, chronic kidney disease, etc. and second only to cigarette smoking as a cause of preventable deaths in the US
• Earlier onset, worse disease Black > Hispanic > White Non-Hispanic Americans

• BUT ABOUT 30% OF PEOPLE WITH HYPERTENSION DO NOT ACHIEVE BLOOD PRESSURE CONTROL WITH CURRENT TREATMENTS (neurogenic)

Brain-Gut-Bone Marrow Interactions: Triangular Hypothesis for Hypertension

HTN RISK FACTORS
- Diet
- Alcohol
- Toxins, pollutants
- Exercise

Hypertension. 2015; 65:1331-40

Reviews:
Gut Microbial Dysbiosis and Gut Pathology in Animal Models of Hypertension: Summary

Spontaneously Hypertensive Rat (SHR), Chronic Angiotensin II infusion (both rat and mouse)

- Decreased richness, diversity and evenness.
- Increased Firmicutes/Bacterioidetes, \([F/B]\) ratio.
- Decreased acetate- and butyrate-producing bacterial populations.
- Butyrate supplementation is antihypertensive.

- Gut pathophysiology: \(\downarrow\) villi length, \(\downarrow\) goblet cells, \(\uparrow\) fibrosis, \(\uparrow\) tunica muscularis thickness

### Evidence from Others

_Yang et.al. (Raizada), 2015, Hypertension, PMID: 25870193_

<table>
<thead>
<tr>
<th>Authors</th>
<th>Year</th>
<th>Journal</th>
<th>PMID</th>
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<tbody>
<tr>
<td>Mell et.al, (Joe)</td>
<td>2015</td>
<td>Physiol. Genomics</td>
<td>258293993</td>
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<td>Karbach et.al. (Wenzel)</td>
<td>2016</td>
<td>JAHA</td>
<td>27577581</td>
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<td>Marques et.al. (Kay)</td>
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<td>27927713</td>
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<td>Adan et.al. (Durgan)</td>
<td>2017</td>
<td>Physiol. Genomics</td>
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<td>Li et.al. (Cai)</td>
<td>2017</td>
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<td>28143587</td>
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<td>Yan et.al. (Ma)</td>
<td>2017</td>
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<td>28884091</td>
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<td>Wilck et.al. (Muller)</td>
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<td>Ramos-Romero et.al. (Torres)</td>
<td>2018</td>
<td>AJP: Endo.</td>
<td>29351480</td>
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Gut Dysbiosis: Cause or Consequence of Hypertension?

- Fecal Microbial Transfer (FMT) confers hypertensive phenotype
  - Rat to rat: Sleep apnea + high fat diet cecal contents to normal diet rats (Durgan et al, PMID: 28011881).
  - SHR to WKY rat (personal communication, Yang and Zubcevic).
  - Human hypertensive patient to germ-free mice (Li et al, 2017, PMID: 28143587).

Correct Gut Microbiome, Fix Hypertension?
Minocycline Treatment Influences Gut Dysbiosis and Attenuates Hypertension in Angiotensin II Infusion Model

Tao Yang et al. Hypertension. 2015;65:1331-1340
Captopril (an ACEi) lowered blood pressure in SHR and it remained lower 4 weeks after drug was discontinued.

Fix Hypertension, Correct Gut Microbiome?

Captopril (an ACEi) lowered blood pressure in SHR and it remained lower 4 weeks after drug was discontinued.
Captopril Treatment Alters Gut Microbiota in both WKY and SHR: PCoA Plot

Unpublished
Captopril Increases Anaerobic Bacterial Genera in WKY and SHR

Unpublished
Gut Microbiota Folate Biosynthesis is Decreased in SHR and Corrected by Captopril

Phylogenetic Investigation of Communities by Reconstitution of Unobserved States (PICRUSt) of 16S rRNA gene sequencing
16 Weeks After Captopril Withdrawal
Gut Pathophysiology was Still Reduced
Summary of Animal Studies

• Gut dysbiosis in 2 models of hypertension, with gut pathology.
• Correcting the microbiota alleviates hypertension.
• Correcting hypertension improves dysbiosis.

• But what about people?
Microbiome Trial Team:

Departments of Physiology and Medicine, University of Florida, Gainesville

Clinicaltrials.gov: NCT02188381
# Patients’ Characteristics

<table>
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<tr>
<th>Characteristics</th>
<th>Reference (Control)</th>
<th>High Blood Pressure</th>
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<tr>
<td>Number</td>
<td>18</td>
<td>22</td>
</tr>
<tr>
<td>Gender (% Female)</td>
<td>61%</td>
<td>63%</td>
</tr>
<tr>
<td>Age, years (SEM)</td>
<td>57.1 (3.5)</td>
<td>63.6 (3.3)</td>
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<tr>
<td>SBP, mmHg (SEM)</td>
<td>121.1 (1.5)</td>
<td>155.8 (3.4)</td>
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<tr>
<td>DBP, mmHg (SEM)</td>
<td>72.6 (2.1)</td>
<td>83.9 (1.5)</td>
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<td>BMI, kg/m² (SEM)</td>
<td>31.5 (2.0)</td>
<td>35.7 (2.1)</td>
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<tr>
<td>Triglycerides, mg/dl (SEM)</td>
<td>122.8 (16.2)</td>
<td>143.7 (28.2)</td>
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<td>Serum glucose, mg/dl (SEM)</td>
<td>128.0 (18.9)</td>
<td>132.0 (12.5)</td>
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<tr>
<td>LDL, mg/dl (SEM)</td>
<td>80.2 (7.6)</td>
<td>99.1 (8.2)</td>
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<td>Individuals not on Anti-HTN</td>
<td>33.3%</td>
<td>18.1%</td>
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Blood Pressures Of Reference Subjects and Those with High BP

[Graph showing blood pressures of reference subjects and those with high blood pressure (HBP). The graph indicates that the median blood pressure in the reference group is 22 mmHg higher compared to the HTN group, with a significant difference denoted by ****.]

- Red squares = 22 HBP
- Green circles = 18 NBP

[Graph showing diastolic blood pressure (DBP) comparison between reference and HTN subjects, with a significant difference denoted by ****.]
Microbiome is Functionally Rather than Taxonomically Changed with HBP (WGS)

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Metabolome is Different in Hypertensive Patients

Scores Plot

Component 1 (8.5%)

Component 2 (5.7%)

Scores Plot

Component 2 (7.6%)

Component 1 (7.4%)

Global untargeted mass spectrometry of serum

Unpublished
Butyrate Bacterial Communities and Plasma Butyrate Negatively Correlate with SBP

\[ R^2 = 0.1002 \quad P < 0.045 \]

\[ R^2 = 0.1011 \quad P < 0.045 \]

Gut Permeability Biomarkers Increased In HTN Patients

IFABP = Intestinal Fatty Acid Binding Protein: LPS = Lipopolysaccharides

Can We Predict Blood Pressure Using Gut Microbiome and Factors?

Stepwise linear regression analysis to determine top predictors of systolic blood pressure.

**Dependent variable:** Systolic BP

**Independent variables:**

- Butyrate kinase
- Acetate CoA transferase (α subunit)
- Acetate CoA transferase (β subunit)
- Aminotransferase
- Butyrate producing bacteria (%)
- Acetate producing bacteria (%)
- SCFA Transporter
- I-FABP
- LPS
- Zonulin

Best Predictors of Systolic Blood Pressure: Zonulin and Butyrate-Producing Bacteria

**Model 1: Zonulin:**
Estimated SBP = 90.1 + 1.27 (Zonulin)  
[R=0.72, R² 0.50, SD= 15.0]

**Model 2: Zonulin + Butyrate:**
Estimated SBP = 98.3 + 1.27 (Zonulin) - 0.35 (% Butyrate producer)  
R=0.76, R² 0.55, SD= 14.0]

Gut Microbiome is Functionally Different in Black Compared to White Non-Hispanic Americans


◇ = de novo synthesis of amino acids
* = interconversion of amino acids
✝ = synthesis of inflammatory antigens
Metabolome Differs in Black vs White Hypertensive Americans

Differential metabolites, some gut microbiota derived, suggest increased inflammation and oxidative stress in Black vs White hypertensive Americans

Problems

- Correlating human vs bacterial metabolites: need searchable database
- Microbiome discovery, how to scale up to tens of thousands of subjects (Dr. Carl Pepine, MD).
- Collaborate between countries doing large amounts of sequencing to predict regional, racial, etc. differences and commonalities in disease (Dr. Seungbum (Sam) Kim, Ph.D).
Can We Fix Hypertension and Microbiome in People?

• Resistant hypertension, generally considered to have a neurogenic component, patients taking 3 or more antihypertensive drugs including a diuretic.
• Minocycline, an anti-inflammatory antibiotic.
• Safe and effective.
• Well-tolerated, minimal side effects.
• Crosses blood brain barrier.

• Theoretically could act on both the gut microbiota and brain.
Minocycline Trial

• 50mg/day, 100mg/day, 200 mg/day for 14 days
• Goal: ambulatory blood pressure decrease of 5 mmHg in daytime
• Minocycline responders: drop blood pressure by 5 mmHg
• Minocycline Non-Responders: No reduction in blood pressure even at 200 mg/day
Minocycline Lowered Blood Pressure in Resistant Hypertensives with High Blood Pressure

Before Minocycline  
After Minocycline

SBP (mmHg)

100  120  140  160

p = 0.02

nsd

Unpublished
Gut microbiomes were not different before, but were after, minocycline treatment.
Gut Bacterial Functions Enriched in Minocycline Responders

Responder
Non-Responder

P<0.05, LDA>2.0

Unpublished
Gut Bacterial Functions Enriched in Minocycline Non-Responders

Unpublished

![Graph showing RPKM Normalization count (X1000) for Fatty acid degradation, Biofilm formation, and Xenobiotics degradation for Responders and Non-Responders.](image-url)
Summary

• Gut microbiota, gut structure and metabolites are altered in hypertension in animals and humans.
• Functional changes in the gut microbiota appear more relevant than bacterial taxonomic changes.
• Correcting hypertension ameliorates gut dysbiosis and *vice versa* in animals and perhaps in people.
• Changes in gut microbial functional capacity occur in animals and humans when blood pressure is corrected, e.g. folate biosynthesis, could these be exploited for therapy?
Acknowledgements

Co-PI’s: Mohan K Raizada, Carl J Pepine, Colin Sumners

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<tr>
<th>Team: Present</th>
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<tr>
<td>Elaine Richards</td>
<td>Yanfei Qi</td>
</tr>
<tr>
<td>Sam Kim</td>
<td>Vermali Rodriguez</td>
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<tr>
<td>Tao Yang</td>
<td>Monica Santisteban</td>
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<tr>
<td>Jasenka Zubcevic</td>
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<tr>
<td>Aline Oliveira</td>
<td>Ruby Goel</td>
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<tr>
<td>Eric Krause</td>
<td>Victor Aquino</td>
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<tr>
<td>Cardiology Clinical Trial Team</td>
<td>Gilberto Lobaton</td>
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<td>Jackie Walejko</td>
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Thanks to our study cohort for their contributions!
NHLBI Working group recommendations

- Use of multiple animal models and development of novel animal models
- Metagenomics, metatranscriptomics, and metabolomics
- Identification, cultivation, genomic and functional characterization of vascular-modifying microbial strains
- Host genome–microbiome cross-talk
- Role of viruses, archaea, and fungi
- Oral microbiome and HTN3.
- Brain–gut axis in hypertension, mechanisms, gut pathophysiology, and implications in development of hypertension
- Kidney–gut axis in hypertension, mechanisms, gut pathophysiology, and implications in development of hypertension
- Nutritional factors and impact on microbiota-linked BP regulation
- Preclinical investigations
- Large-scale metagenomic studies: sex, race, and drug sensitivity
- Is there a unique microbial signature linked to sex, race, drug sensitivity, and so on?
- Metabolomics to identify hypertension and normal microbiota-derived metabolite profiles
- The therapeutic potential of fecal and oral transplant for control of hypertension
- Investigation of pro- and prebiotics, alone or in combination with anti-inflammatory/antimicrobial drugs and antihypertensive drugs for resistant hypertension
- 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
- Test and establish the efficacy of novel lifestyle and pharmacological interventions targeting microbiome for the prevention and treatment of clinical hypertension and CVDs

Raizada, MK et al: Hypertension 2017 doi: 10.1161/HYPERTENSIONAHA.117.09699
NHLBI Working group recommendations, cont.

- Standardized technology to measure comprehensive metabolites in blood, saliva, and stool of animals and of patients with hypertension
- Development of an integrated system to measure BP and hydrogen-specific, H2S-specific, and methane-specific electrode systems to measure gut microbiota activity and diversity in vivo
- National/international forum for microbiota in BP regulation
- Standard protocols to measure gut blood flow

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