The Microbiome and Hypertension

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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)**

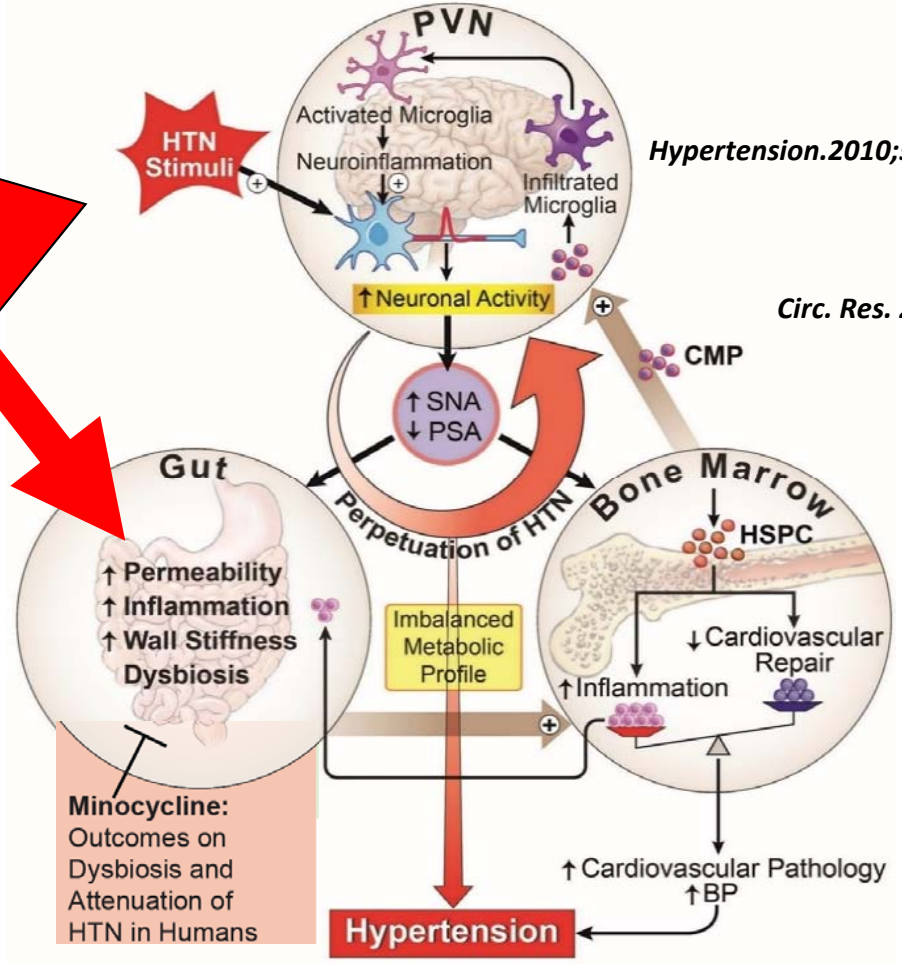
Whelton, PK et al. J. Am. Coll. Cardiol. DOI: 10.1016/j.jacc.2017.11.006

Brain-Gut-Bone Marrow Interactions:

Triangular Hypothesis for Hypertension

HTN RISK FACTORS
Diet
Alcohol
Toxins, pollutants

Exercise



Hypertension.2010;56:297-303.

Circ. Res. 2015;117:178-91.

Hypertension. 2014;63:542-50
Front Physiol. 2017, Apr 12;8:220

Hypertension. 2015; 65:1331-40
Circ. Res. 2016; 118: 1327-36.
Circ. Res. 2017;120(2):312-323.

Int J Cardiol. 2015;201:157-158.

Reviews:
Circ. Res.2016; 118:1327-36
Curr Hypertens Rep. 2017;19(4):36.

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: ↓ villi length, ↓ goblet cells, ↑fibrosis, ↑tunica muscularis thickness

Yang et.al. Hypertension. 2015 Jun;65(6):1331-40; Kim et.al. J Hypertens. 2015;33 Suppl 1:e77-8; Stewart et.al. Acta Biomater. 2016;45:296-302; Kim et.al. J Hypertens. 2016;34 Suppl e60-e61; Santisteban et.al. Circ Res. 2017;120:312-323.

Evidence from Others

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

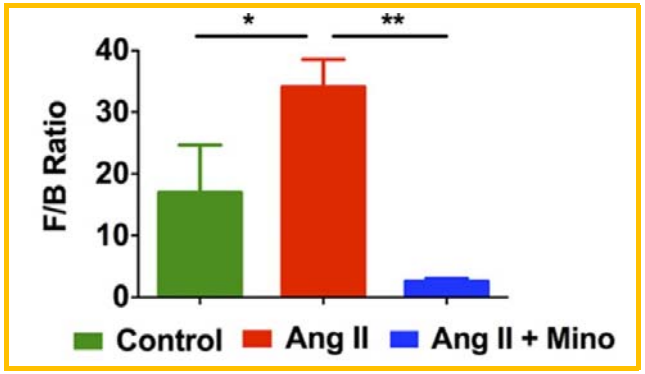
Authors	Year	Journal	PMID
Mell et.al, (Joe)	2015	Physiol. Genomics	258293993
Karbach et.al. (Wenzel)	2016	JAHA	27577581
Marques et.al. (Kay)	2017	Circulation	27927713
Adan et.al. (Durgan)	2017	Physiol. Genomics	28011881
Li et.al. (Cai)	2017	Microbiome	28143587
Yan et.al. (Ma)	2017	Front. Cell. Inf. Immu.	28884091
Wilck et.al. (Muller)	2017	Nature	29143823
Ramos-Romero et.al. (Torres)	2018	AJP: Endo.	29351480

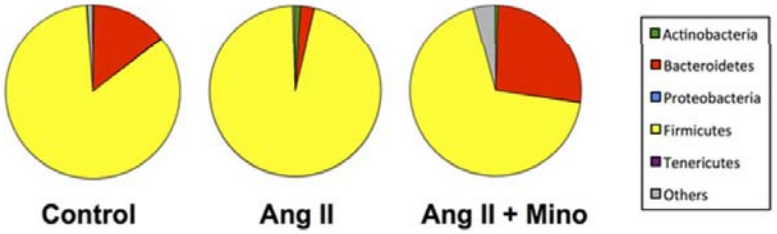
Gut Dysbiosis: Cause or Consequence of Hypertension?

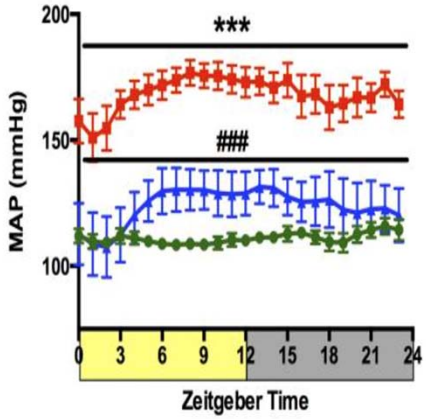
- Fecal Microbial Transfer (FMT) confers hypertensive phenotype
  - Rat to rat : Sleep apnea+high fat diet cecal contents to normal diet rats (Durgan et al, PMID: 28011881).
  - SHR to WKY rat (personal communication, Yang and Zubcevic).
  - Human hypertensive patient to germ-free mice (Li et al, 2017,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

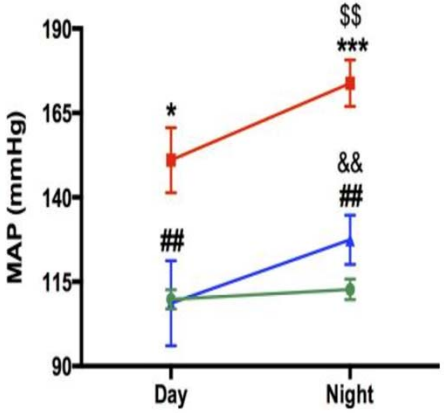
Correct Gut Microbiome, Fix Hypertension?

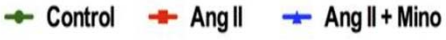
Minocycline Treatment Influences Gut Dysbiosis and Attenuates Hypertension in Angiotensin II Infusion Model









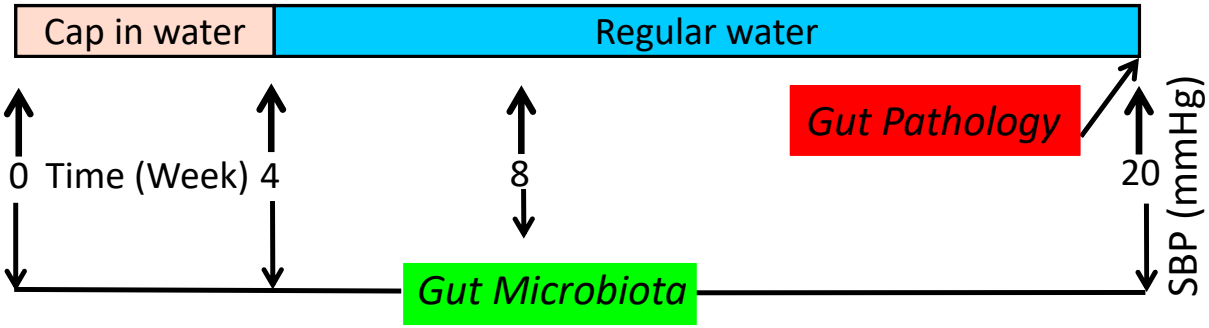


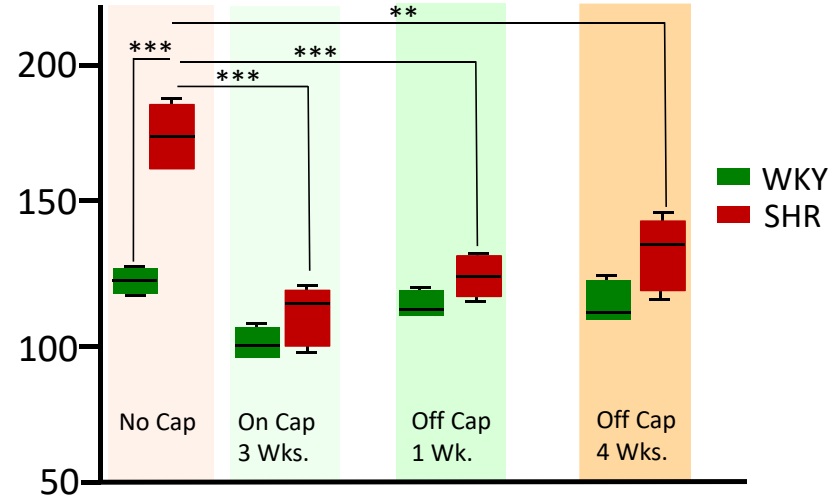
Phylum	P value			Mean value (%)		
	Con *vs.* AngII	Con *vs.* AngII + Mino	AngII *vs.* AngII + Mino	Control	AngII	AngII + Mino
Actinobacteria	0.1121	0.5609	0.4803	0.2167	1.096	0.6293
Bacteroidetes	0.4398	0.1425	**0.0055****	13.03	2.581	27.20
Firmicutes	0.5251	0.0968	**0.0045****	85.98	96.06	69.93
Proteobacteria	0.3006	0.9999	0.5631	0.1496	0.0508	0.1259

Tao Yang et al. Hypertension. 2015;65:1331-1340

American Heart Association.

Fix Hypertension, Correct Gut Microbiome?



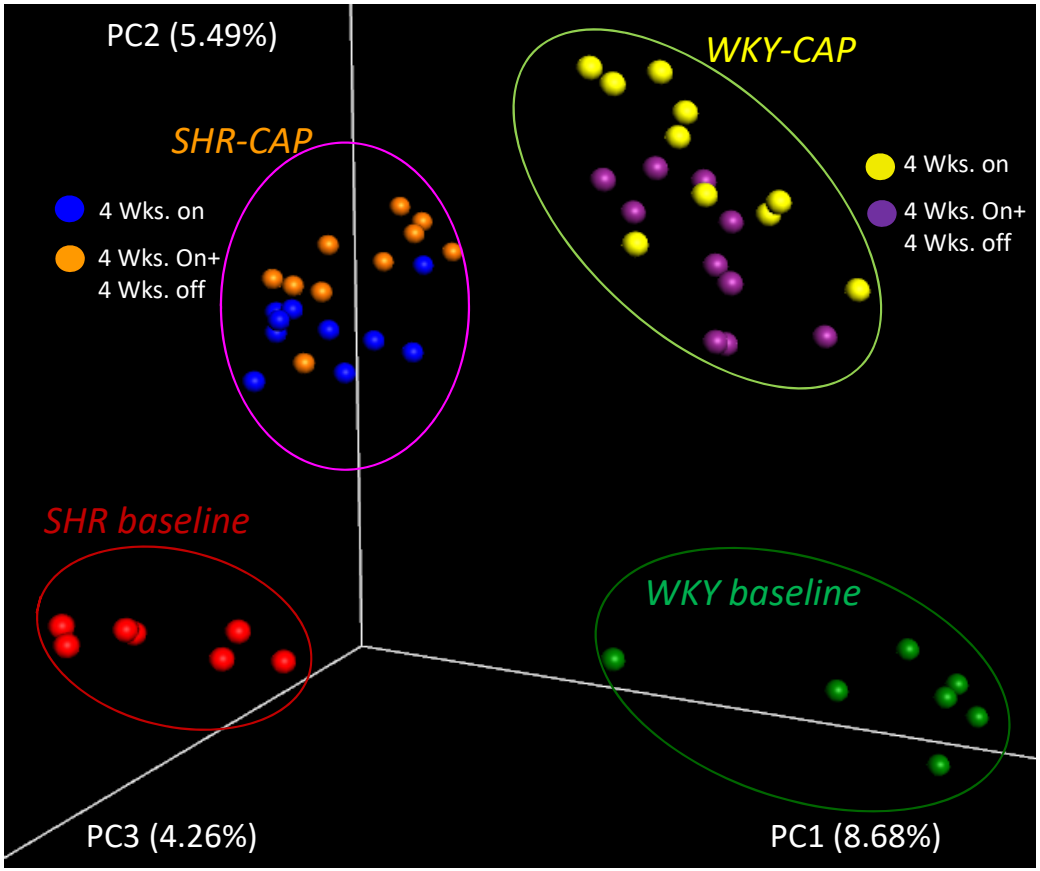


Captopril (an ACEi) lowered blood pressure in SHR and it remained lower 4 weeks after drug was discontinued

Unpublished

Captopril Treatment Alters Gut Microbiota in both WKY and SHR: PCoA Plot

PCoA Plot ()



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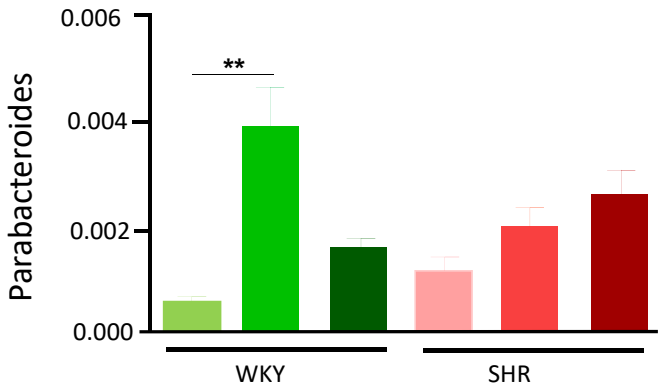
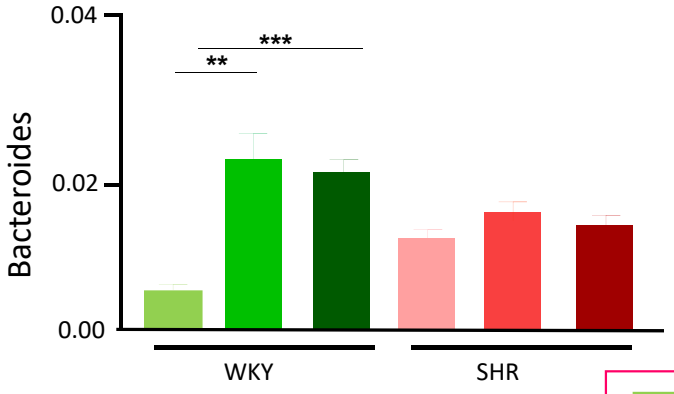
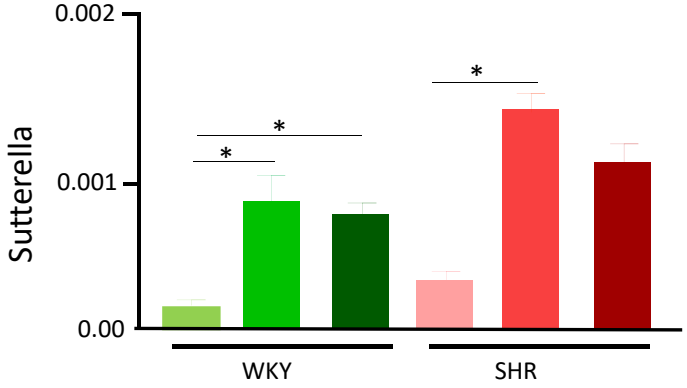
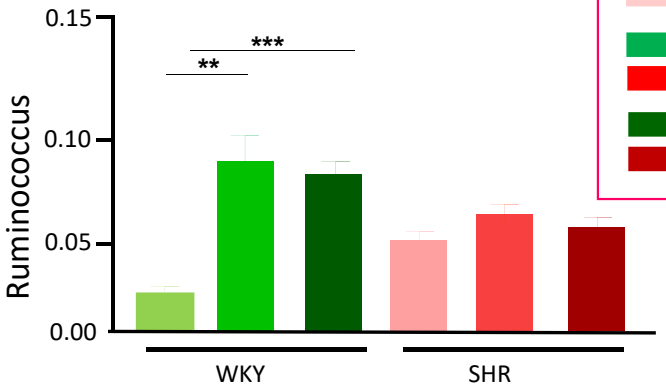
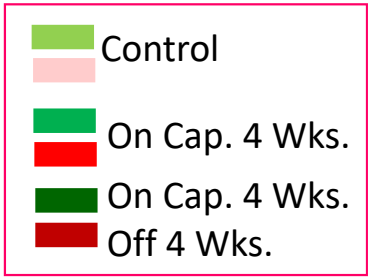
Captopril Increases Anaerobic Bacterial Genera in WKY and SHR











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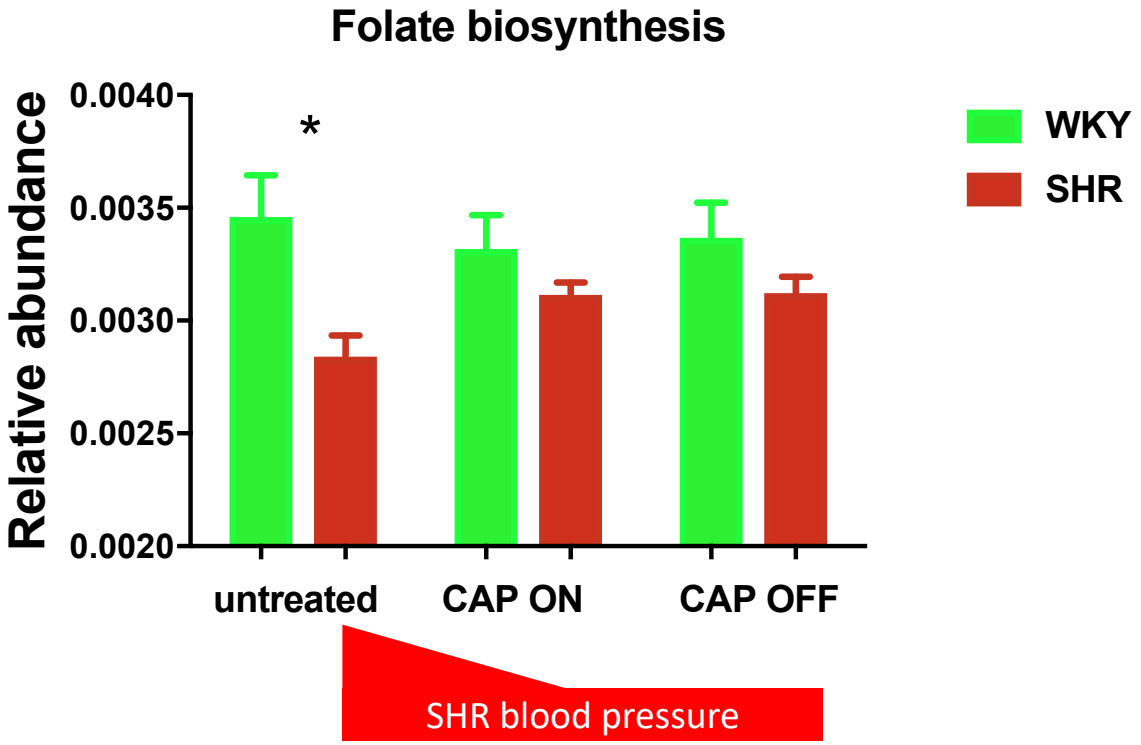
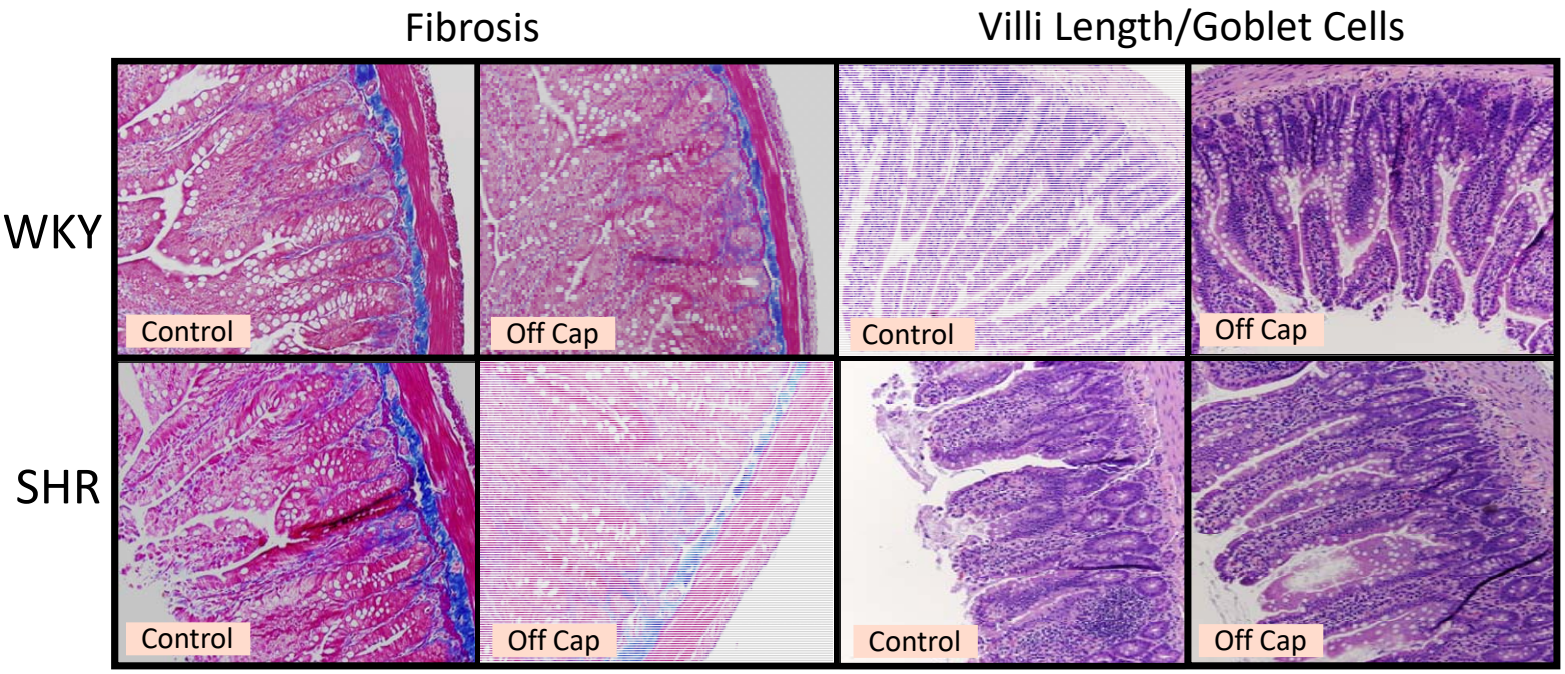
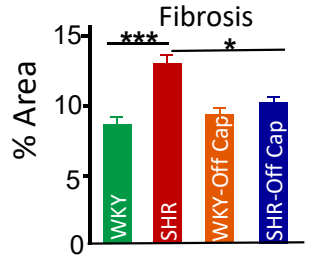
Gut Microbiota Folate Biosynthesis is Decreased in SHR and Corrected by Captopril

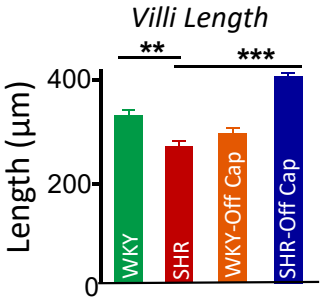


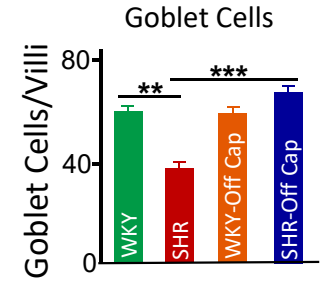
Phylogenetic Investigation of Communities by Reconstitution of Unobserved States (PICRUSt) of 16S rRNA gene sequencing

16 Weeks After Captopril Withdrawal Gut Pathophysiology was Still Reduced









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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

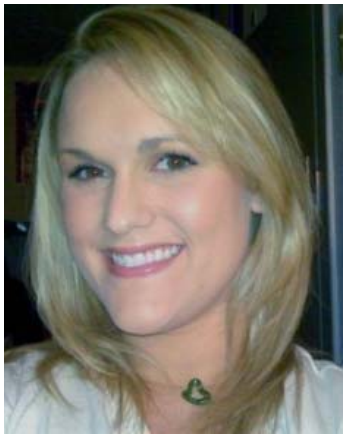
Carl Pepine, M.D.
Professor, Medicine

Eileen Handberg, Ph.D.
Assoc. Prof., Medicine

Mohan K Raizada, Ph.D.
Professor, Physiology

Sam Kim, Ph.D.
Postdoc. Associate

Yanfei Qi, M.D., Ph.D.
Asst. Prof. Medicine



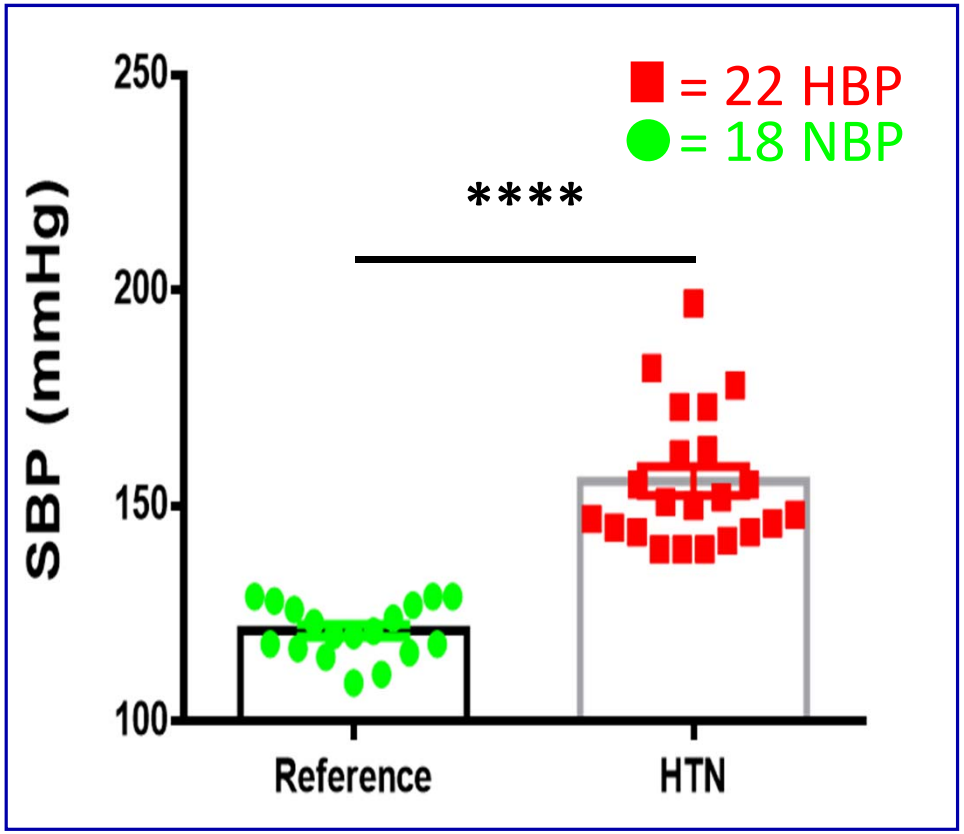
Sara Croft, B.S.
Coordinator

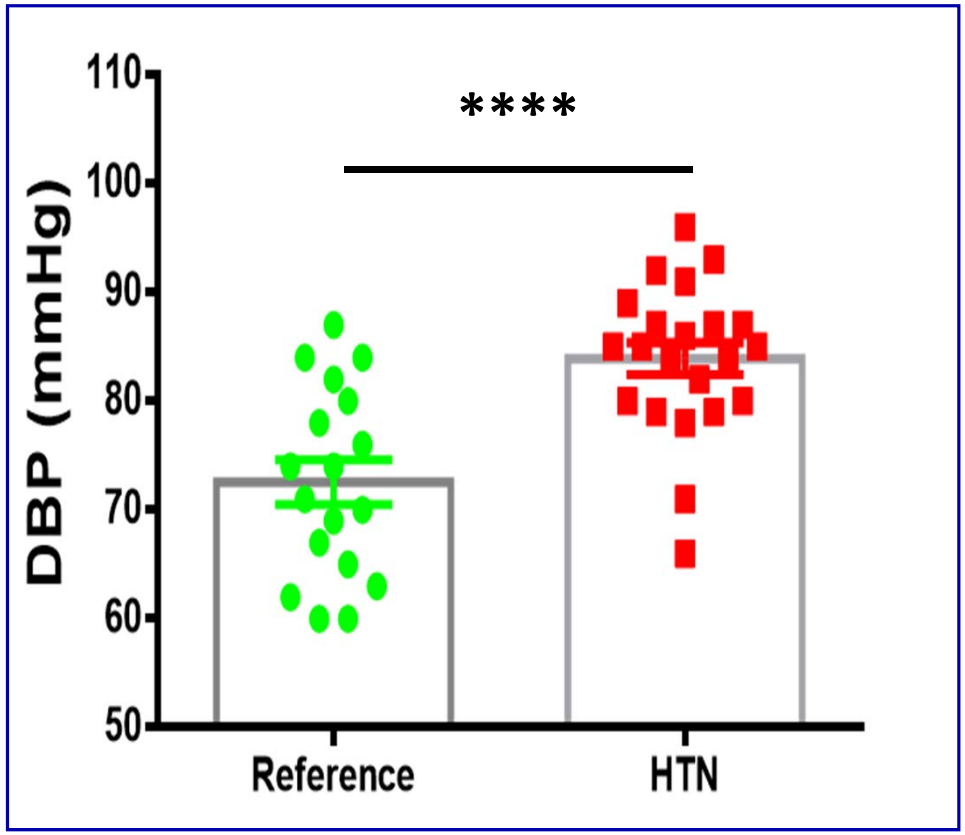
Clinicaltrials.gov: NCT02188381

Patients' Characteristics

Characteristics	Reference (Control)	High Blood Pressure
Number	18	22
Gender (% Female)	61%	63%
Age, years (SEM)	57.1 (3.5)	63.6 (3.3)
SBP, mmHg (SEM)	121.1 (1.5)	155.8 (3.4)
DBP, mmHg (SEM)	72.6 (2.1)	83.9 (1.5)
BMI, kg/m^2 (SEM)	31.5 (2.0)	35.7 (2.1)
Triglycerides, mg/dl (SEM)	122.8 (16.2)	143.7 (28.2)
Serum glucose, mg/dl (SEM)	128.0 (18.9)	132.0 (12.5)
LDL, mg/dl (SEM	80.2 (7.6)	99.1 (8.2)
Individuals not on Anti-HTN	33.3%	18.1%

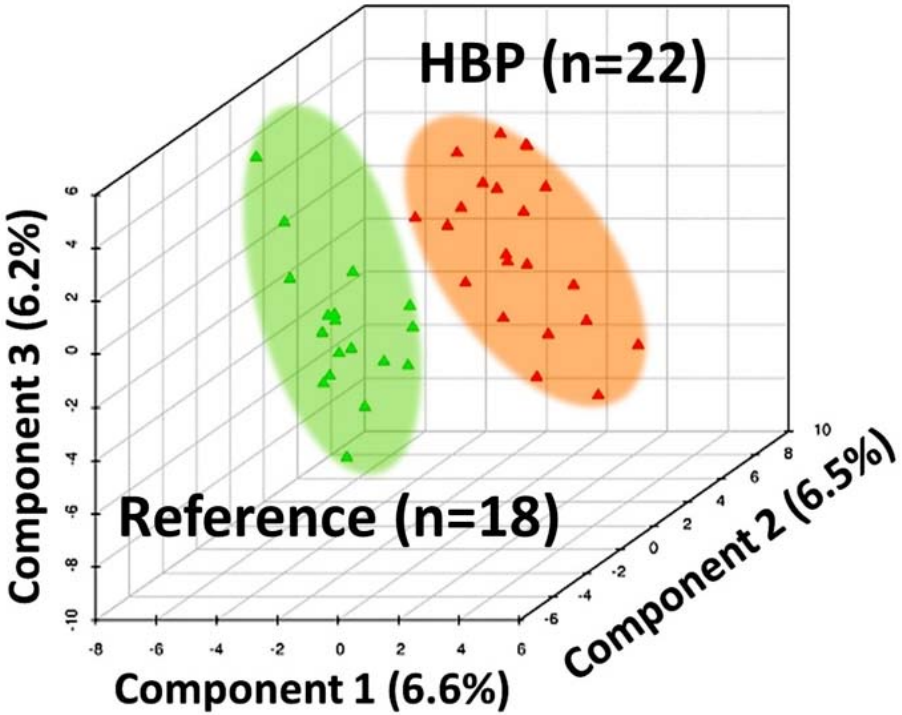
Blood Pressures Of Reference Subjects and Those with High BP



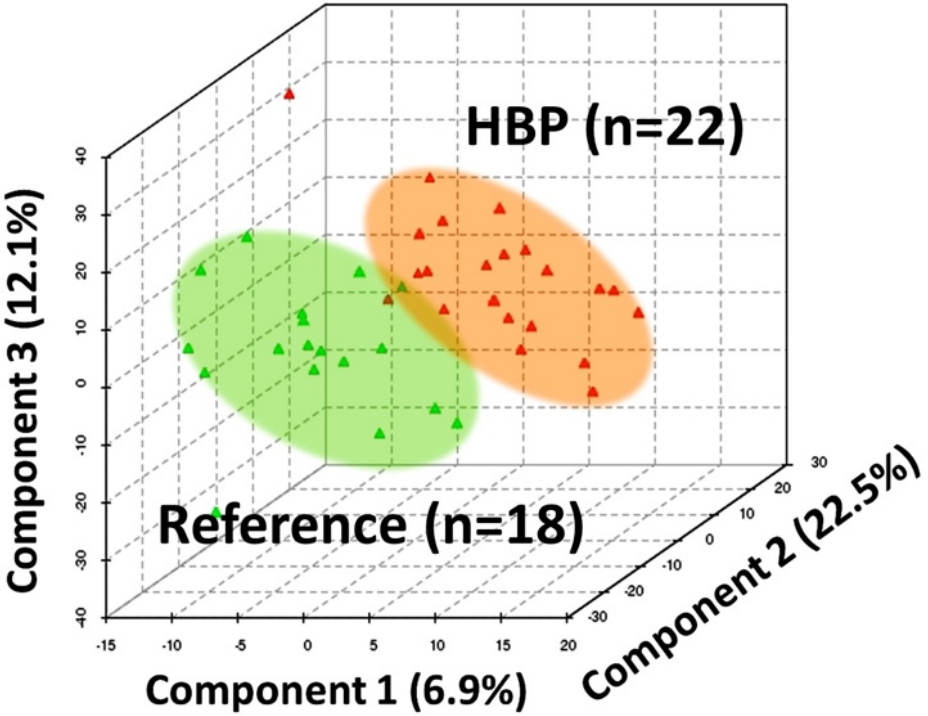


Microbiome is Functionally Rather than Taxonomically Changed with HBP (WGS)

PLS-DA Plot: Bacterial Taxonomy

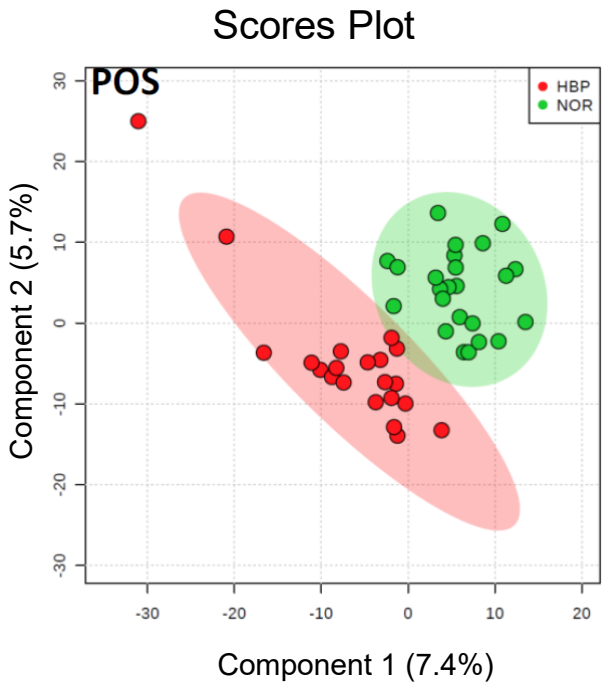


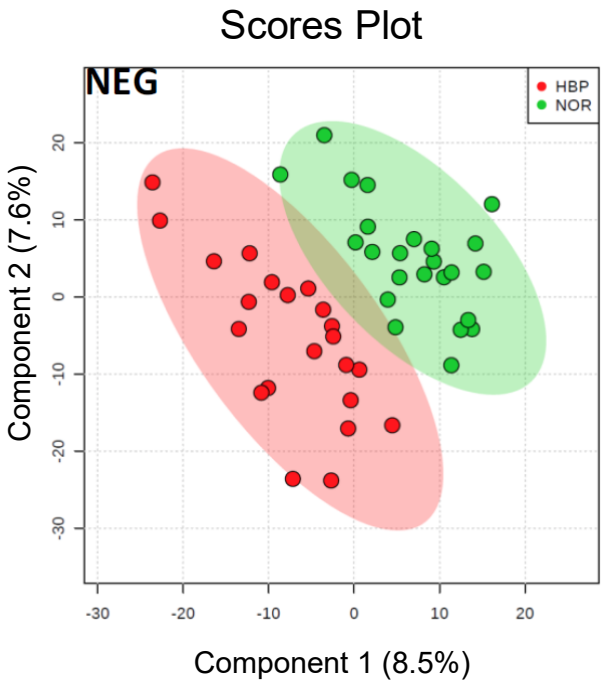
PLS-DA Plot: KEGG Orthology Function



Kim S. et al Clin Sci (Lond) 2018 Mar 30;132(6):701-718. doi: 10.1042/CS20180087.

Metabolome is Different in Hypertensive Patients





Global untargeted mass spectrometry of serum

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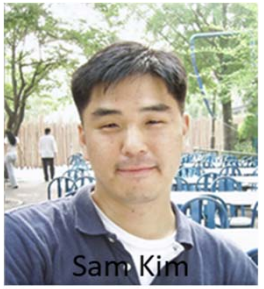
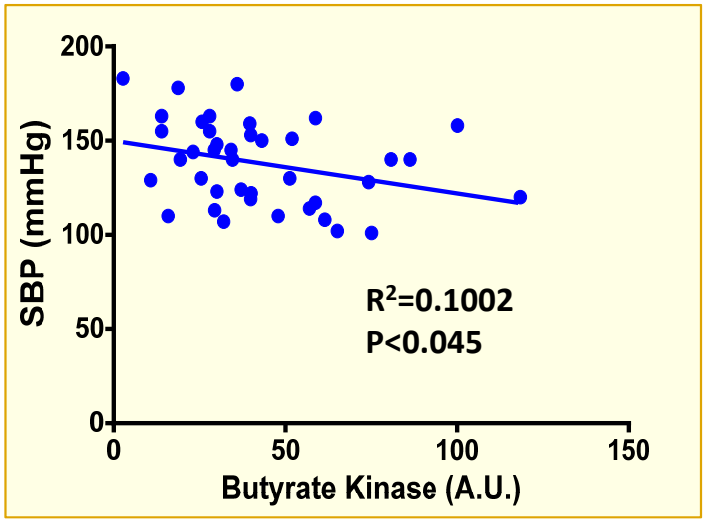
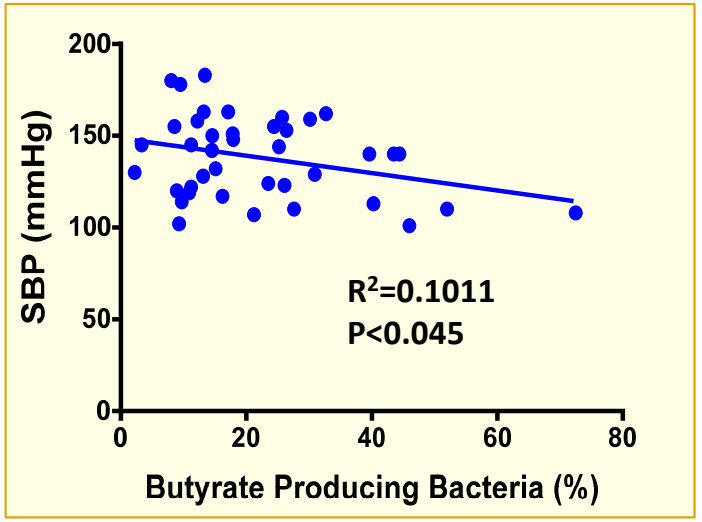
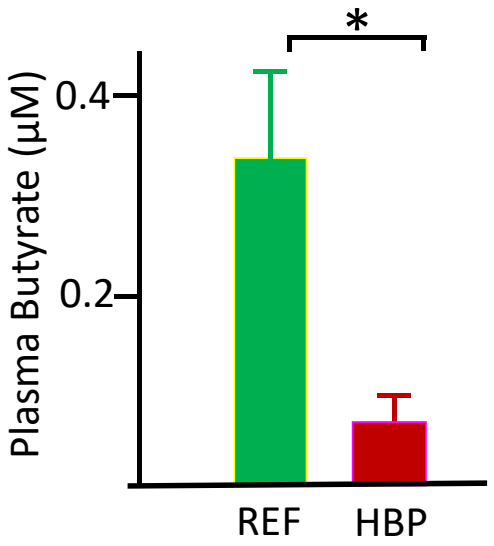
Butyrate Bacterial Communities and Plasma Butyrate Negatively Correlate with SBP



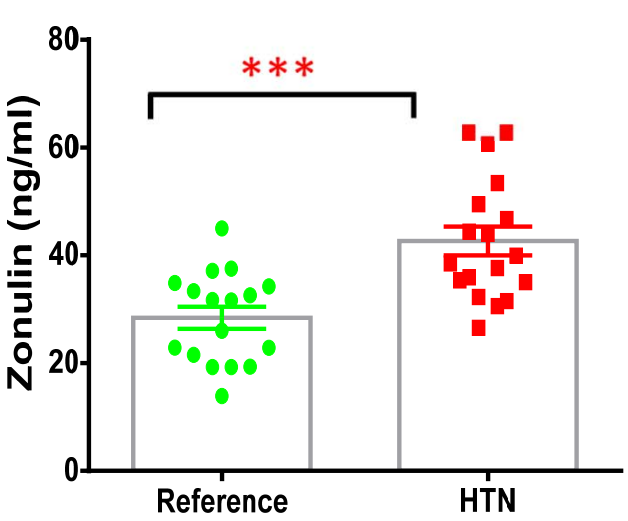


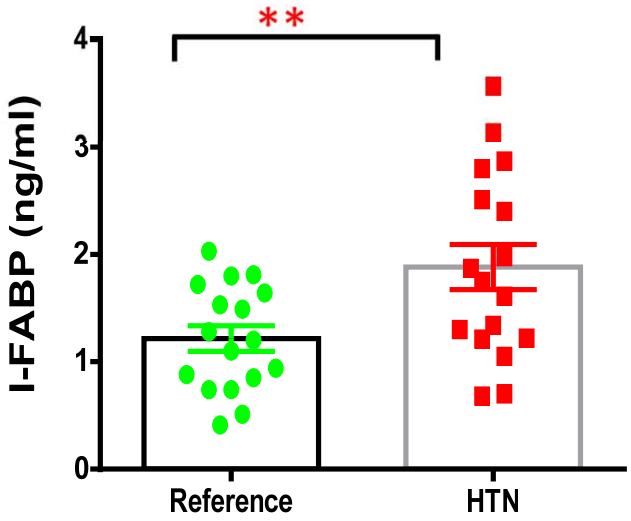


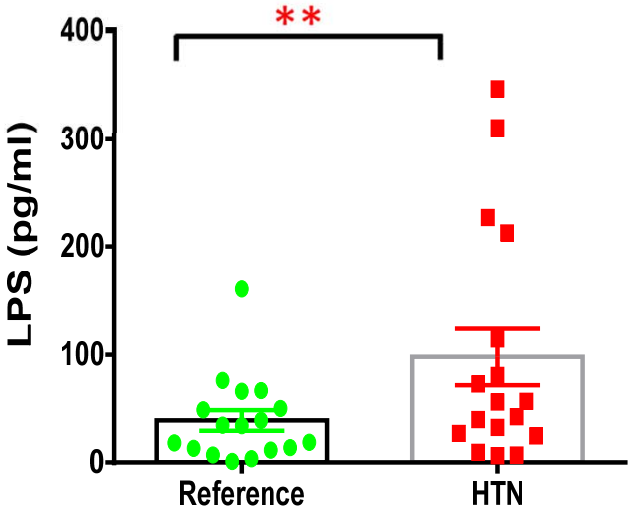


Kim S. et al Clin Sci (Lond) 2018 Mar 30;132(6):701-718. doi: 10.1042/CS20180087.

Gut Permeability Biomarkers Increased In HTN Patients







IFABP= Intestinal Fatty Acid Binding Protein: LPS= Lipopolysaccharides

Kim S. et al Clin Sci (Lond) 2018 Mar 30;132(6):701-718. doi: 10.1042/CS20180087.

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

Kim S. et al Clin Sci (Lond) 2018 Mar 30;132(6):701-718. doi: 10.1042/CS20180087.

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

Model 1: Zonulin:

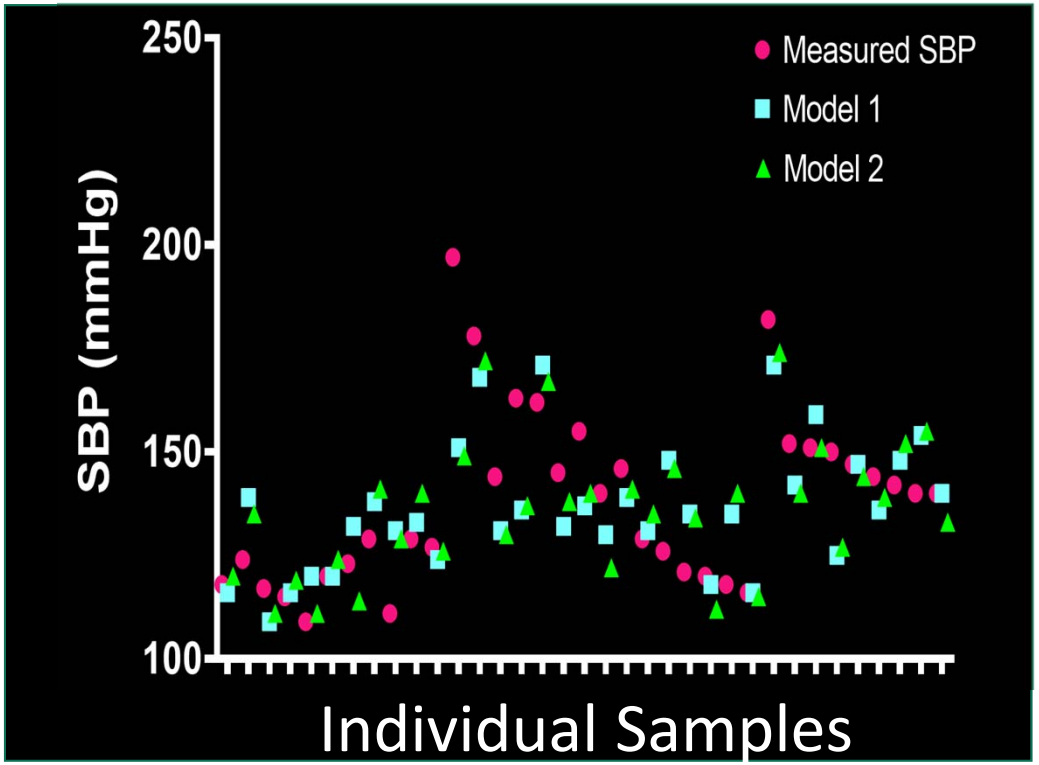
Estimated SBP = 90.1+1.27 (Zonulin)

[R=0.72, R^2 0.50, SD= 15.0]

Model 2: Zonulin + Butyrate:

Estimated SBP = 98.3 + 1.27 (Zonulin) - 0.35 (% Butyrate producer)

R=0.76, R^2 0.55, SD= 14.0]



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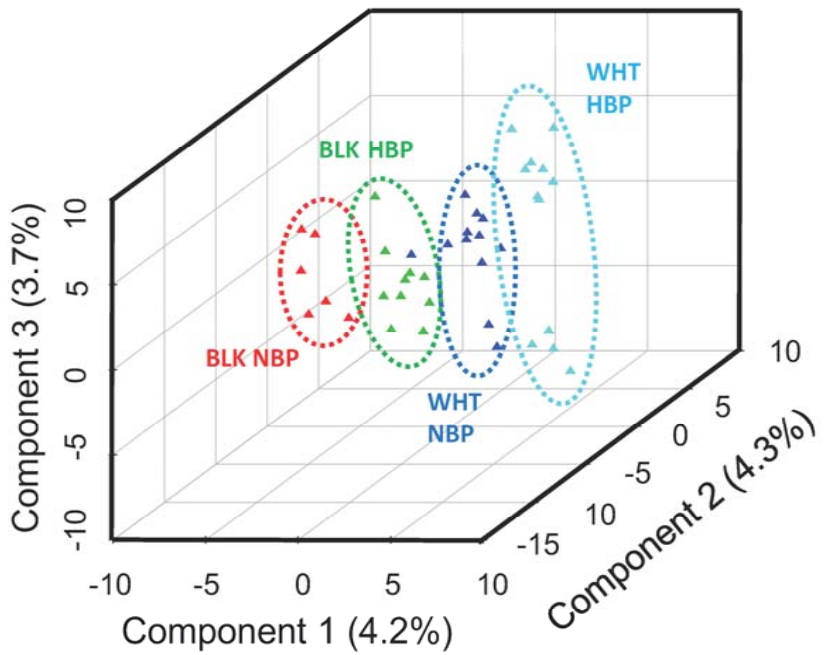
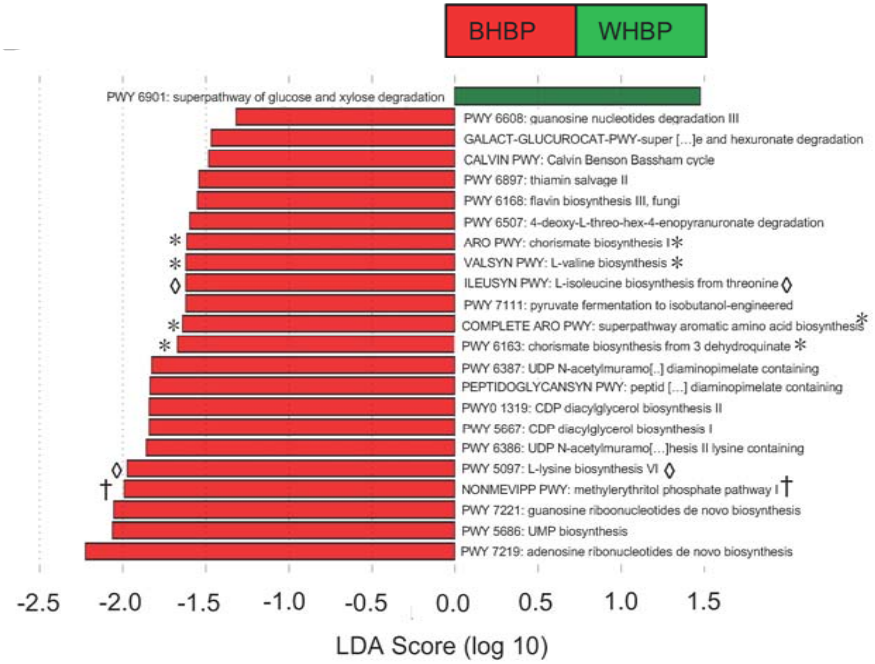
Gut Microbiome is Functionally Different in Black Compared to White Non-Hispanic Americans





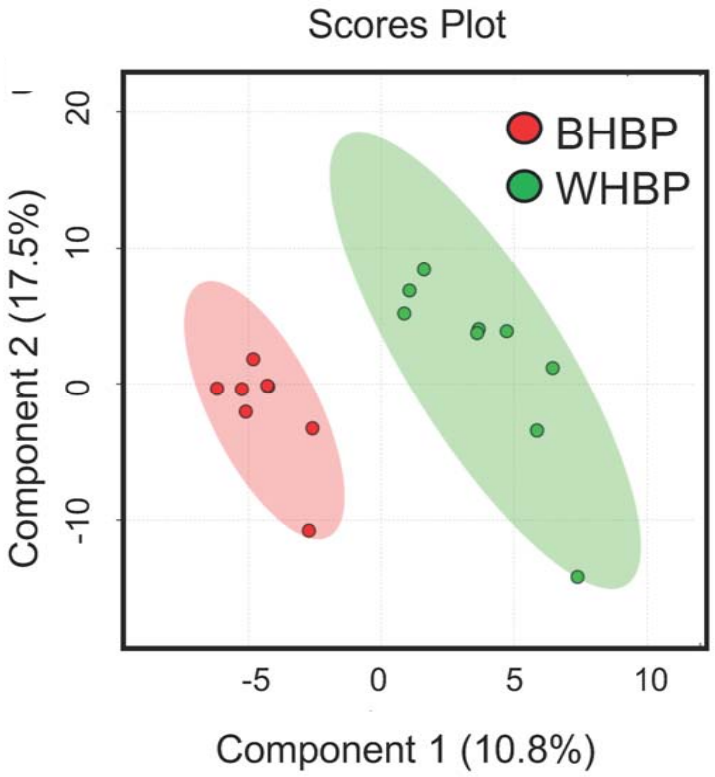
◇= de novo synthesis of amino acids

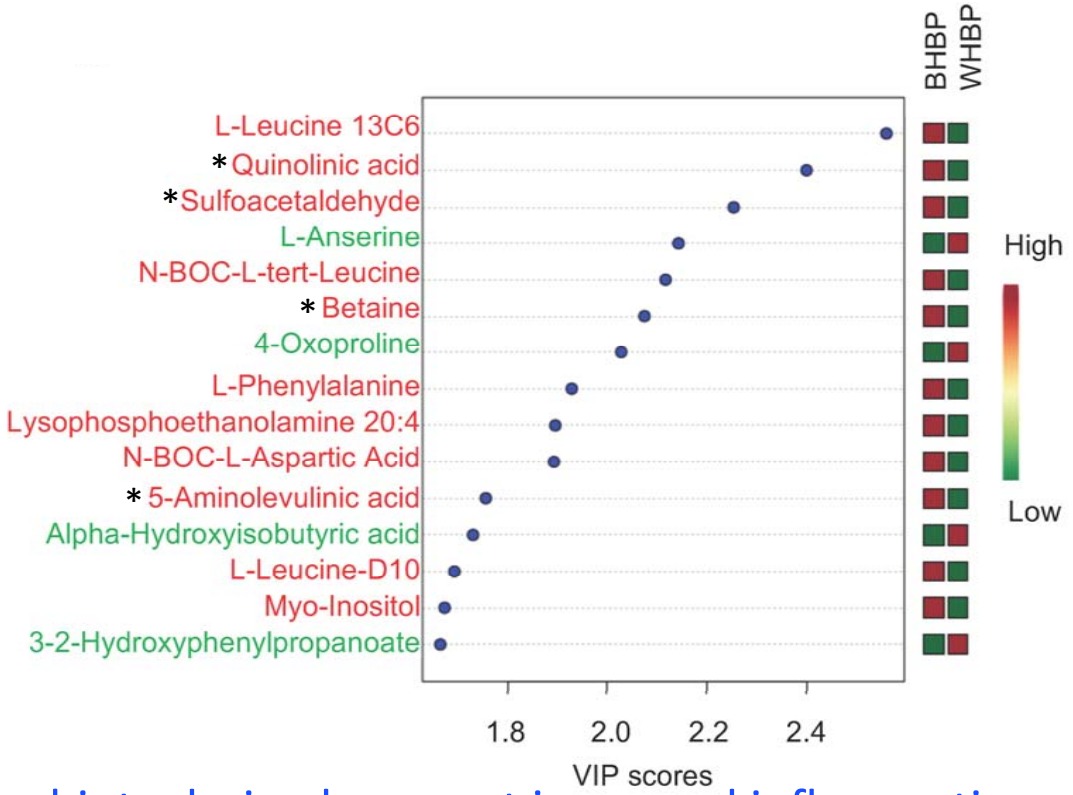
∗ =interconversion of amino acids

† = synthesis of inflammatory antigens

Walejko, JM, Kim,S. et al, Int. J. Cardiol (2018), in press

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

Walejko, JM, Kim,S. et al, Int. J. Cardiol (2018), in press

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).
- Raizada, MK et al: Report of the NHLBI Working Group on the Role of Microbiota in Blood Pressure Regulation: Current Status and Future Directions. Hypertension 2017 doi: 10.1161/HYPERTENSIONAHA.117.09699

Can We Fix Hypertension and Microbiome in People?

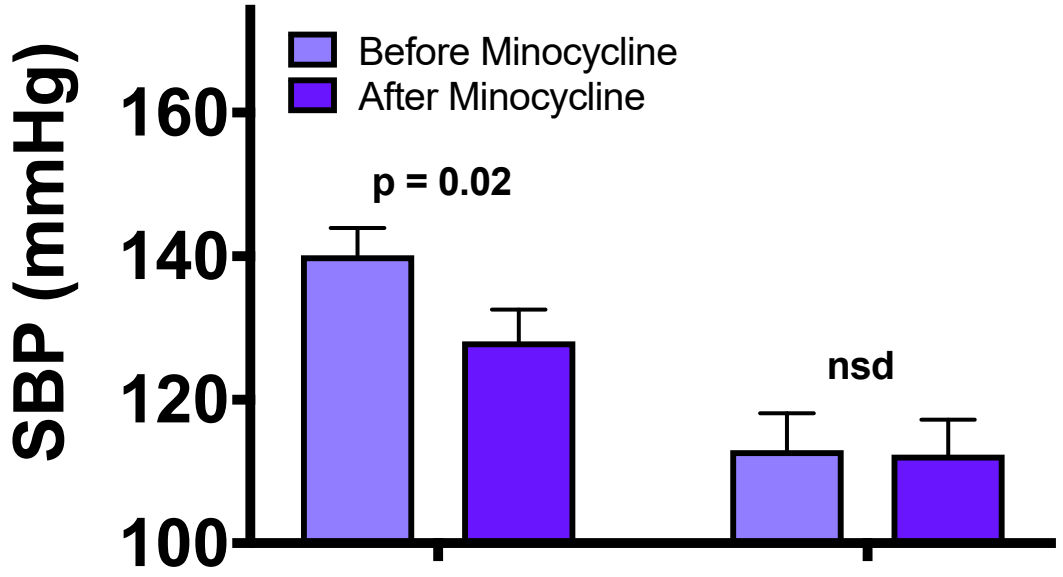
- Resistant hypertension, generally considered to have a neurogenic component, patients taking 3 or more antihypertensive drugs including a diuretic.
- Minocycline, an anti-inflammatory antibiotic.
- Safe and effective.
- Well-tolerated, minimal side effects.
- Crosses blood brain barrier.

- 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



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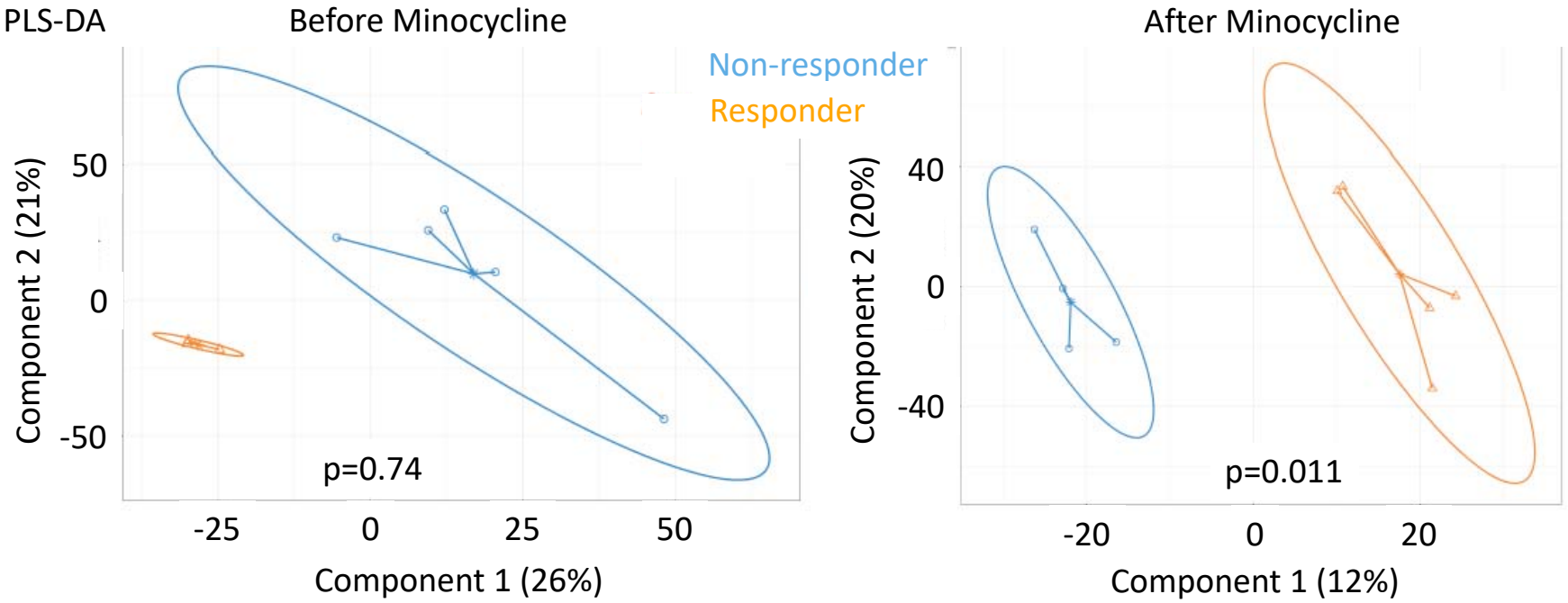
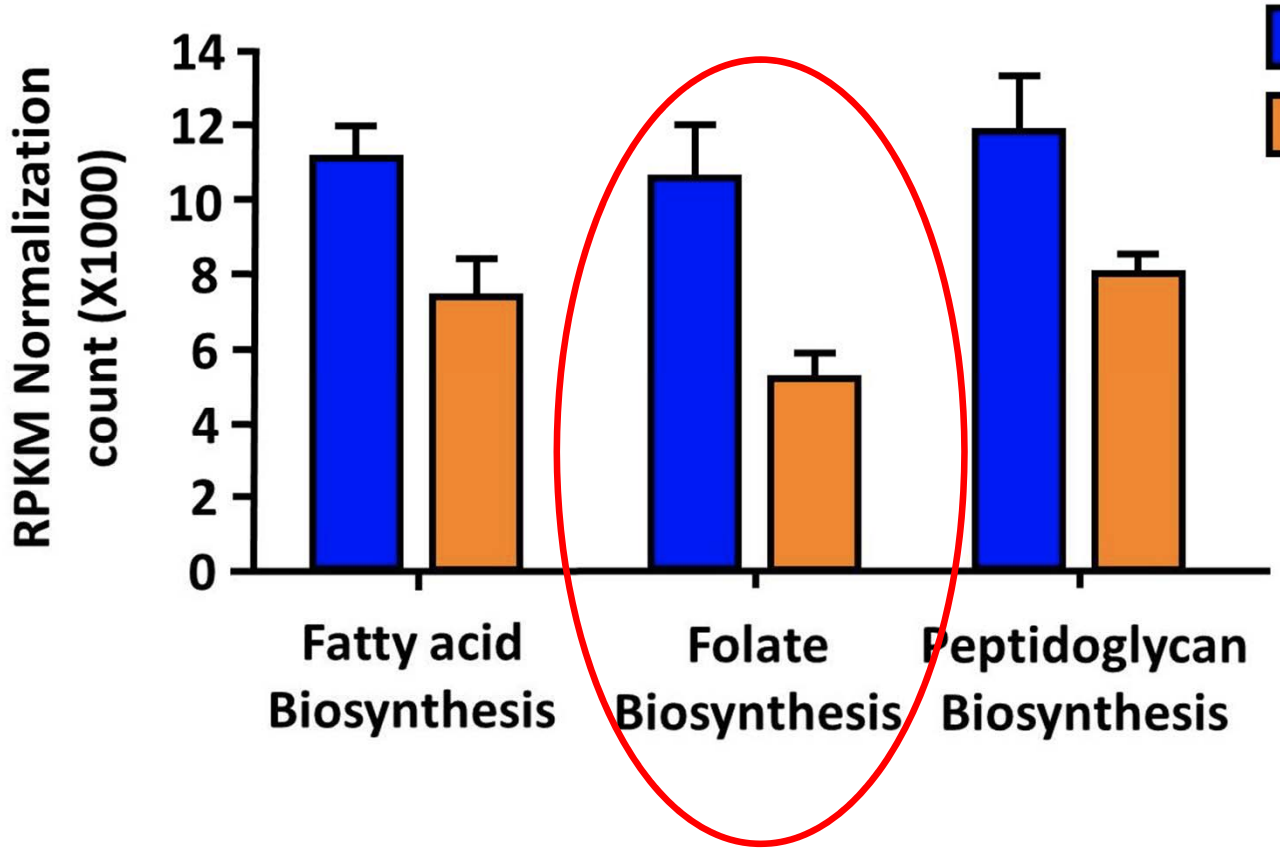
Gut microbiomes were not different before, but were after, minocycline treatment



Unpublished

Gut Bacterial Functions Enriched in Minocycline Responders



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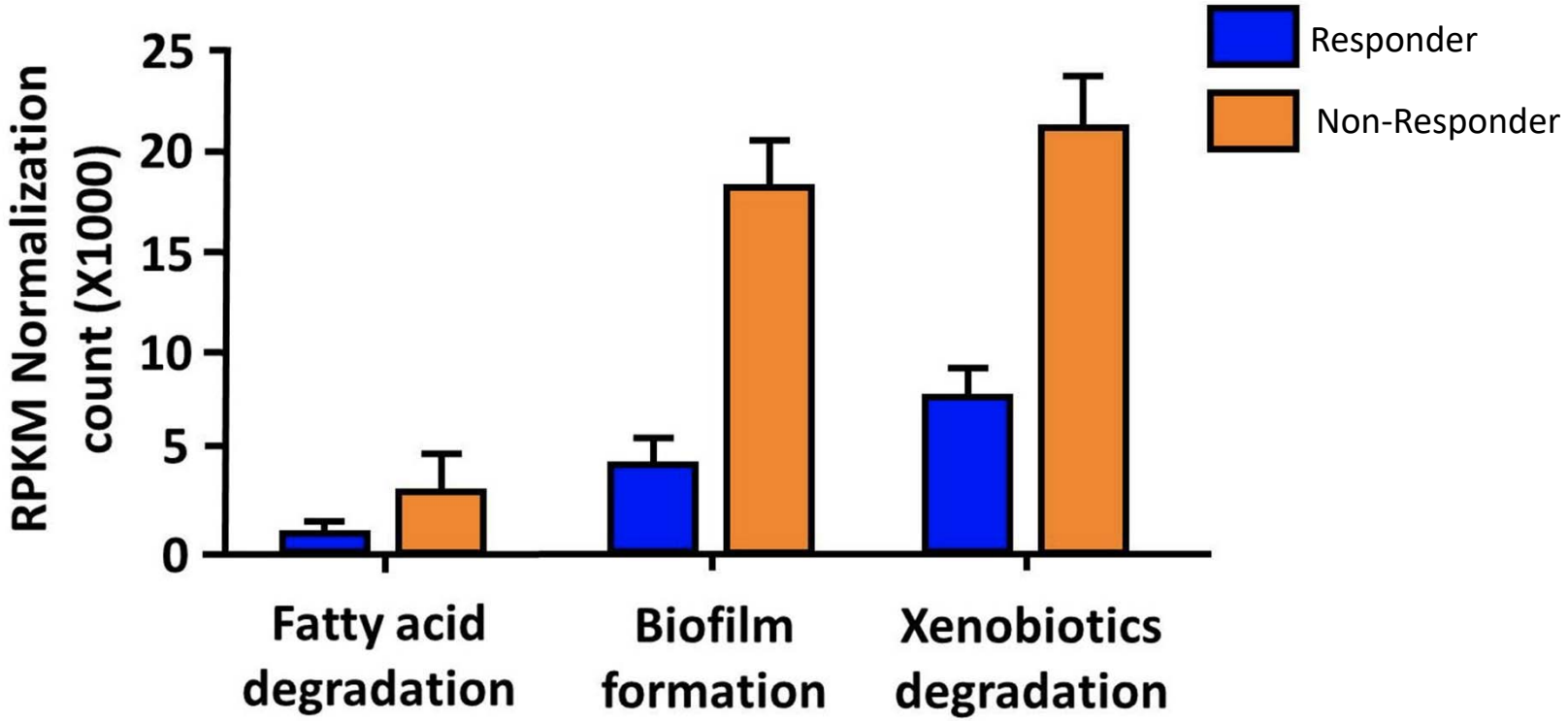
Unpublished

Gut Bacterial Functions Enriched in Minocycline Non-Responders



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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?

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- Elaine Richards
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- Tao Yang
- Jasenka Zubcevic
- Aline Oliveira
- Eric Krause
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- Victor Aquino
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2 R01 HL102033 06
1 R01 HL132448 01

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 preventionand 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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