Journal of the American Heart Association

ORIGINAL RESEARCH

Repurposing a Drug Targeting Inflammatory Bowel Disease for Lowering Hypertension

Xue Mei, MS; Blair Mell, BS; Ishan Manandhar, MS; Sachin Aryal, MS; Ramakumar Tummala, PhD; Jun Kyoung, BA, MS; Tao Yang, PhD; Bina Joe, PhD

BACKGROUND: The gut and gut microbiota, which were previously neglected in blood pressure regulation, are becoming increasingly recognized as factors contributing to hypertension. Diseases affecting the gut such as inflammatory bowel disease (IBD) present with aberrant energy metabolism of colonic epithelium and gut dysbiosis, both of which are also mechanisms contributing to hypertension. We reasoned that current measures to remedy deficits in colonic energy metabolism and dysbiosis in IBD could also ameliorate hypertension. Among them, 5-aminosalicylic acid (5-ASA; mesalamine) is a PPARγ (peroxisome proliferator-activated receptor gamma) agonist. It attenuates IBD by a dual mechanism of selectively enhancing colonic epithelial cell energy metabolism and ameliorating gut dysbiosis.

METHODS AND RESULTS: A total of 2 groups of 11- to 12-week-old male, hypertensive, Dahl salt-sensitive (S) rats were gavaged with (n=10) or without (n=10) 5-aminosalicylic acid (150 mg/kg) for 4 weeks. Rats receiving 5-aminosalicylic acid treatment had a lower mean blood pressure than controls (145±3 mm Hg versus 153±4 mm Hg; $P<0.0001$). This reduction in blood pressure was accompanied by increased activity of PPARγ, increased expression of energy metabolism–related genes, and lowering of the Firmicutes/Bacteroidetes ratio in the colon, the reduction of which is a marker for the correction of gut dysbiosis. Furthermore, these data were consistent with the American Gut Project wherein the Firmicutes/Bacteroidetes ratio of non-IBD (n=611) patients was significantly lower than patients with IBD (n=631).

CONCLUSIONS: 5-Aminosalicylic acid could be repurposed for hypertension by specifically enhancing the gut energy metabolism and correction of microbiota dysbiosis.

Key Words: anti-inflammatory ■ blood pressure ■ drug repurpose ■ mesalamine ■ microbiome

Hypertension is a global burden that affects >1.3 billion people worldwide, with increasing prevalence in both men and women.[1,2] Furthermore, this number is predicted to rise to 1.6 billion adults by 2025.[3] Hypertension is also often associated with metabolic diseases such as diabetes, obesity, and inflammatory bowel disease (IBD).[4–6] In recent years, it has been increasingly recognized that the gut is an important previously neglected organ that contributes to blood pressure (BP) regulation.[7–9] The gut microbiota has been a major research focus in hypertension. However, it remains unclear how the gut pathology may contribute to BP control, but such pathophysiological events in the gut contributing to IBD are well documented. Of notable interest to the current study is PPARγ (peroxisome proliferator-activated receptor gamma), which participates in multiple physiological mechanisms, including host energy metabolism[10,11] and gut microbiota homeostasis,[12,13] both of which are implicated in IBD as well as the regulation of BP.[14–18] Downregulation of PPARγ results in a dysregulated energy metabolism and gut dysbiosis. To rescue these defects in IBD, selective activators of

Correspondence to: Bina Joe, PhD, International Society of Hypertension Fellow Department of Physiology and Pharmacology, Center for Hypertension and Precision Medicine Program in Physiological Genomics, University of Toledo College of Medicine and Life Sciences, Block Health Science Bldg. Rm 237, 3000 Arlington Ave., Toledo, OH 43614. Email: bina.joe@utoledo.edu

Supplemental Material is available at https://www.ahajournals.org/doi/suppl/10.1161/JAHA.122.027893

For Sources of Funding and Disclosures, see page 10.

JAHA is available at: www.ahajournals.org/journal/jaha

CLINICAL PERSPECTIVE

What Is New?

- 5-Aminosalicylic acid, a drug approved by the U.S. Food and Drug Administration for inflammatory bowel disease, could be repurposed to target the gut and lower blood pressure in Dahl salt-sensitive rats.
- The newly identified antihypertensive effect of 5-aminosalicylic acid was linked to an increase in colonic energy metabolism and an improvement in gut microbiota homeostasis.

What Are the Clinical Implications?

- The anti-inflammatory bowel disease drug 5-aminosalicylic acid was found to be beneficial for lowering blood pressure in a hypertensive rat model, which is proof of concept for a potential new option for the clinical management of hypertension.

Nonstandard Abbreviations and Acronyms

5-ASA	5-aminosalicylic acid
IBD	inflammatory bowel disease
PPARγ	peroxisome proliferator-activated receptor gamma
S rats	Dahl salt-sensitive rats

PPARγ in the colon have been developed and are widely used.[19,20] 5-Aminosalicylic acid (5-ASA; mesalamine) is 1 such medication used in the clinical treatment of IBD.[21] It is the first-line drug for patients with mild to moderate ulcerative colitis.[22] 5-ASA can improve mitochondrial bioenergetics in the colonic epithelium and restore gut homeostasis.[23]

Given that the common converging pathophysiological pathways for IBD and hypertension occur in the gut, we hypothesized that the current medications used for IBD could be repurposed to ameliorate hypertension by targeting gut pathology. However, despite the use of 5-ASA in the treatment of IBD, to date this selective agonist of PPARγ has not been tested for its effects on hypertension.

In the current study, we tested the hypothesis that 5-ASA can be repurposed to lower BP in hypertensive Dahl salt-sensitive (S) rats. Our results support this hypothesis and demonstrate that treatment with 5-ASA has pleiotropic mechanistic effects of enhancing the energy metabolism and correcting microbiota dysbiosis in the gut to lower BP.

METHODS

All data and supporting materials have been provided with the published article.

Animals and Housing Conditions

All animal research protocols were approved by the University of Toledo's Institutional Animal Care and Use Committee. Experiments were carried out in accordance with the National Institutes of *Health Guide for the Care and Use of Laboratory Animals*, and results were reported per the Animal Research: Reporting of In Vivo Experiments guidelines. Inbred S rats were obtained from our colony. Rats were housed in cages with Carefresh paper bedding in the Department of Laboratory Animal Resources at the University of Toledo. The animal rooms were maintained at 70±2°F. The humidity in the animal rooms was maintained at 50%±20%.

BP Measurements and Drug Administration

A total of 2 groups of male S rats (10/group) were weaned at 4 weeks onto a low-salt diet (0.3% NaCl; Harlan Teklad, Teklad traditional diet [TD] 7034) for 5 weeks. All rats were implanted with radio-telemetry transmitters and allowed to recover from surgery for 1 week postimplantation before their baseline BP was monitored. They were then transferred onto a high-salt diet (2% NaCl; Harlan Teklad, TD 94217) and gavaged with 5-ASA (n=10) or the vehicle 0.5% sodium carboxymethyl cellulose (n=10) daily for 4 weeks. BP was recorded using the DSI software and equipment (https://www.datasci.com/). For data analysis, Dataquest A.R.T. 4.2 software was used to acquire the systolic, diastolic, and mean arterial pressures and heart rate. All BP values were collected at 5-minute intervals to obtain the average values for each hour. Final BP data reliably collected from the study had 7 in the control group and 9 in the 5-ASA–treatment group.

Collection of Tissues and Fecal Microbiota

Rats were euthanized, and proximal colon, heart, and kidneys were harvested at ≈4.5 months of age. Fecal samples were collected freshly after the rats were euthanized. One fecal pellet was collected for each animal. All samples were stored at −80 °C until further processing.

16S rRNA Gene Sequencing and Analysis of Microbiota Composition

16S Polymerase Chain Reaction Library Preparation, Clean-Up, Normalization, and Pooling

We followed the Illumina User Guide, *16S Metagenomic Sequencing Library Preparation—Preparing 16S Ribosomal RNA Gene Amplicons for the Illumina MiSeq*

System (*Part No. 15044223 Rev. B*).[24] The 16S rRNA gene targeting the V3 to V4 region was amplified by polymerase chain reaction (PCR) using the following Illumina sequencing primers: 5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG and 5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACHVGGGTWTCTAAT. For index PCR, the Nextera XT index kit (FC-131-1002) was used to attach dual indexes.

Each 25-μL reaction mixture contained 2.5 μL of 10X reaction buffer (Invitrogen, Thermo Fisher Scientific, Waltham, MA), 0.5 μL of 10 mmol/L deoxynucleotide triphosphate, 0.75 (for target PCR)/1 μL (for index PCR) of 50 mmol/L $MgCl_2$, 0.1 μL of 5 U/μL of HotTaq polymerase (Invitrogen), 1 μL of each primer (5 μmol/L), and 2.5 μL of 5 ng/μL DNA. All samples were reconstituted in water for a final volume of 25 μL.

Thermocycling was performed in a BioRad T100TM thermal cycler (Hercules, CA), and the cycling conditions were as follows: initial denaturation at 95 °C for 5 minutes followed by 25 cycles of 95 °C for 30 seconds, 58 °C for 30 seconds, 72 °C for 30 seconds, and a final extension at 72 °C for 5 minutes for target PCR. Index PCR was carried out in 8 cycles, with an initial denaturation at 95 °C for 3 minutes followed by 95 °C for 30 seconds, 55 °C for 30 seconds, 72 °C for 30 seconds, and a final extension at 72 °C for 5 minutes. Each PCR amplicon sample was purified in 2 rounds using AMPure XP beads (Beckman Coulter Inc., Brea, CA). Each concentration of purified index PCR products was measured using the Qubit dsDNA HS Assay kit with Qubit 3.0 fluorometer (Life Technologies, Carlsbad, CA). The 4 nmol/L of each amplicon was pooled equally. The pooled library was checked for quality using a 2100 Bioanalyzer (Agilent, Santa Clara, CA) before sequencing.

Library Denaturing and MiSeq Sample Loading

Following the Illumina User Guide Illumina MiSeq System, the aforementioned amplicon was loaded on the Illumina MiSeq V3 flow cell kit for sequencing for 2×300 cycles.

Quality Filtering, Amplicon Sequence Variant Picking, and Data Analysis

Chimeric sequences were identified and filtered using the Quantitative Insights Into Microbial Ecology version 2 software package (version 2021.11).[25] The amplicon sequence variants were subsequently picked using Quantitative Insights Into Microbial Ecology version 2, and taxonomy assignment was performed using Silva[26] as the reference database. LEfSe (https://huttenhower.sph.harvard.edu/galaxy/) was used to visualize the differential enrichment of gut microbiota between groups.

Reverse Transcription–Quantitative PCR

RNA was extracted from the proximal colon, kidney, and heart samples from animals using the TRIzol method as described.[27] RNA concentration was measured using a NanoDrop, and reverse transcription–PCR was performed to obtain cDNA using the SuperScript III kit (Invitrogen). The resultant cDNA was diluted, and real-time PCR mixtures were prepared using the SYBR Green master mix (Applied Biosystems). Following PCR amplification, *Pparg* (peroxisome proliferator-activated receptor gamma) and transcripts of various genes were tested. These included the following: *Acss1* (acyl-CoA synthetase short-chain family member 1), *Acadl* (acyl-CoA dehydrogenase long chain), *Cpt2* (carnitine palmitoyltransferase 2), *Sirt3* (sirtuin 3), *Crat* (carnitine O-acetyltransferase), *Hadh* (hydroxyacyl-CoA dehydrogenase), *Gk* (glycerol kinase), *Cat* (catalase), *Aldob* (aldolase, fructose-bisphosphate B), *Egln3* (egl-9 family hypoxia-inducible factor 3), *Bnip3* (BCL2 interacting protein 3), *Arbp* (acidic ribosomal phosphoprotein), *Hif1a* (hypoxia inducible factor 1 subunit alpha), and *Hprt* (hypoxanthine guanine phosphoribosyl transferase). Expressions of these genes were quantitated for their relative expression to 18S ribosomal RNA using the $2^{-\Delta\Delta Ct}$ method.[28]

PPARγ Transcriptional Activity

The Nuclear Extraction Kit (No. 10009277, Cayman Chemical) and PPARγ Transcription Factor Assay Kit (No. 10006855, Cayman Chemical) were used to isolate nuclear proteins and measure PPARγ transcriptional activity, respectively.

Statistical Analysis

GraphPad Prism version 9.1.1 was used for statistical analyses. Unpaired *t* test was used to compare relative gene expression. Quantitative Insights Into Microbial Ecology version 2 was used to delineate the abundance percentages of microbiota between the control and 5-ASA–treated groups followed by unpaired *t* tests. The analysis of similarities statistical method was used to calculate the *P* value of unweighted β diversity. A 1-way ANOVA with the Fisher least significant difference test was used for BP comparisons between the 2 groups for 24 hours. Statistically significant values were represented as $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$, and $^{****}P<0.0001$. All the figures with scattered dots are expressed as mean±SEM.

RESULTS

5-ASA Significantly Attenuated BP

Compared with the control group, the rats gavaged with 5-ASA for 4 weeks showed lower mean

arterial BP (control group, 153±4 mm Hg; 5-ASA group, 145±3 mm Hg; $P<0.0001$), systolic BP (control group, 176±5 mm Hg; 5-ASA group, 173±3 mm Hg; $P<0.01$), and diastolic BP (control group, 131±4 mm Hg; 5-ASA group, 121±3 mm Hg; $P<0.0001$). The BP-lowering effect of 5-ASA was mainly attributed to the lowering of diastolic BP (Figure 1).

5-ASA Increased Colonic Expression of Energy Metabolism–Related Genes

5-ASA functions as a PPARγ agonist in the gut epithelial cells.[29] Beyond the gut, because of its metabolism, 5-ASA is not available to exert such effects on PPARγ elsewhere.[29] Therefore, to confirm the specificity of 5-ASA targeting the colon, we measured *Pparg* gene expression in the proximal colon, heart, and kidney. *Pparg* gene expression was significantly increased in the proximal colon (Figure 2A) but not in the heart or kidney (Figure S1). For further confirmation, the PPARγ transcriptional activity was compared between treated and untreated rats. As seen in Figure 2B, the transcriptional activity of PPARγ was significantly higher in the colon of the 5-ASA–treated group compared with the control group.

Next, as the functional PPARγ agonist affects energy metabolism, we tested if colonic energy metabolism was altered by 5-ASA. To test this, several genes in the mitochondrial β-oxidation and glycolysis pathways were examined for their expression in the 5-ASA–treated and untreated rats. As seen in Figure 3, multiple energy metabolism–related genes, including

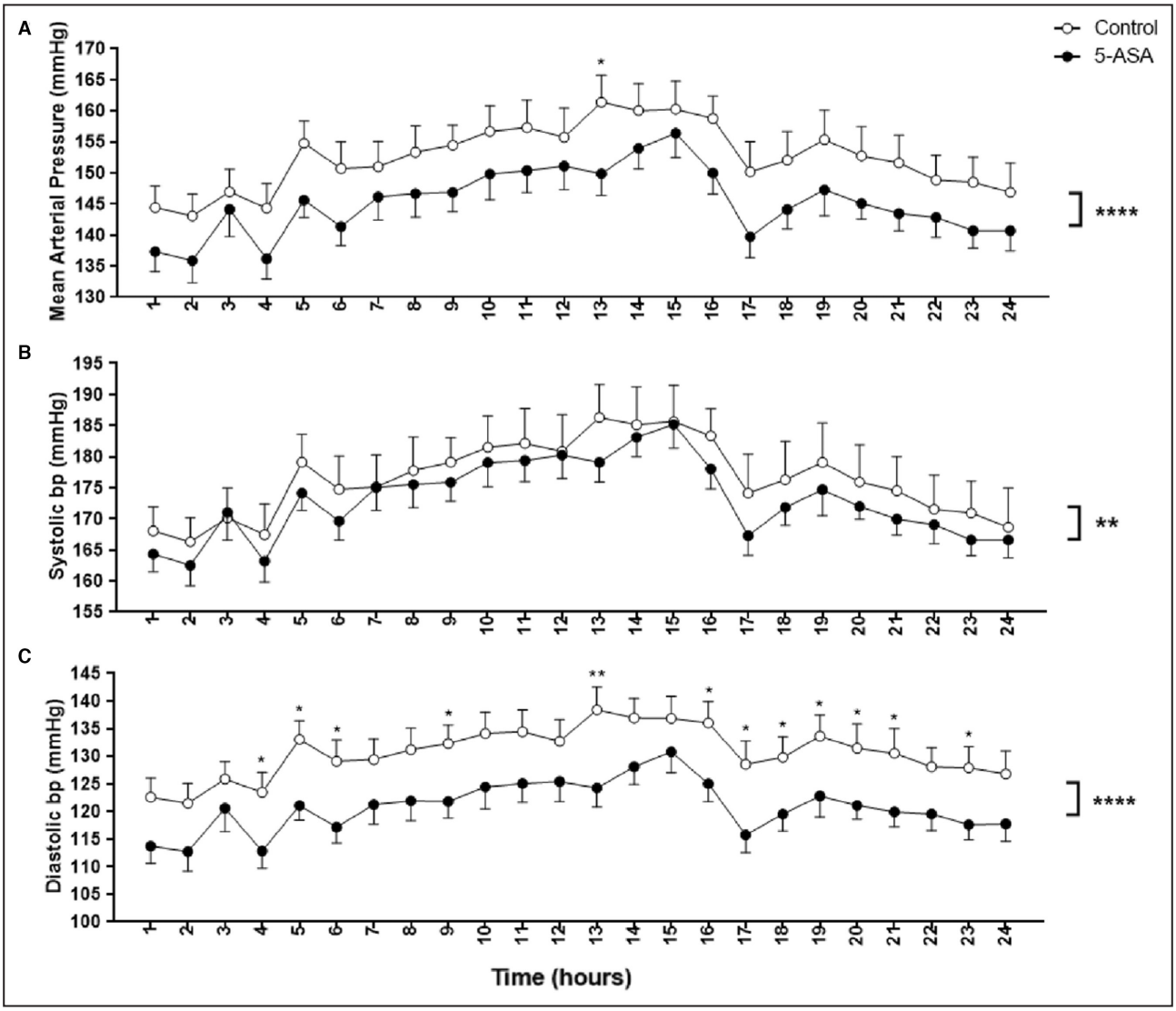


Figure 1. Mean arterial (A), systolic (B), and diastolic (C) BP comparison between the control group (n=7) and 5-ASA group (n=9).

Data were analyzed by 2-way ANOVA followed by Fisher's least significant difference test. *$P<0.05$; **$P<0.01$; ****$P<0.0001$. 5-ASA indicates 5-aminosalicylic acid; and BP, blood pressure.

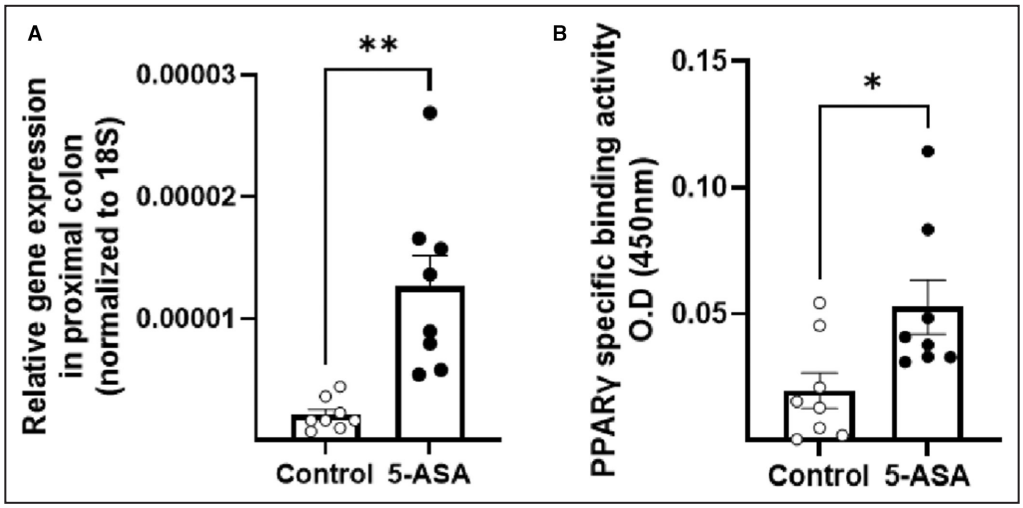


Figure 2. ***Pparg*** **(peroxisome proliferator-activated receptor gamma) gene expression (A) and the PPARγ transcriptional activity (B) comparison between the control (n=8) and 5-ASA (n=8) treated groups in the proximal colon.**
Data were analyzed by unpaired *t* test. PPARγ-specific binding activity in the colon was compared with the optical density 450 nm value in the 20-μg protein between groups. *$P<0.05$; **$P<0.01$. 5-ASA indicates 5-aminosalicylic acid; and PPARγ, peroxisome proliferator-activated receptor gamma protein.

Acss1, *Acadl*, *Cpt2*, *Sirt3*, *Crat*, *Hadh*, *Gk*, *Cat*, and *Aldob*, were significantly upregulated in the colon of the S rats treated with 5-ASA but not in the heart or kidney. Among these 9 genes, 7 genes (*Acss1*, *Acadl*, *Cpt2*, *Sirt3*, *Crat*, *Hadh*, and *Cat*) were involved in the β-oxidation pathway, and 2 genes (*Gk* and *Aldob*) belonged to the glycolysis pathway, both of which contribute to the catabolism of fatty acids and glucose, respectively, to produce energy. Meanwhile, we also tested the proinflammatory genes expression in the colon. However, the representative proinflammatory genes tested, including *Tnf-α* (tumor necrosis factor-α), *interleukin 17 alpha*, and *Il-22* (interleukin-22), showed no differences in colonic expression between the 5-ASA–treated and control groups (data not shown). Collectively, these data suggest that the function of 5-ASA to increase colonic energy metabolism is more likely contributing to the observed lowering of BP compared with the function of 5-ASA as an anti-inflammatory agent.

Significant Remodeling of Fecal Microbiota Composition by 5-ASA

As gut dysbiosis is linked to impaired mitochondrial function in the colonic epithelium, which disrupts epithelial hypoxia[23] as well as the signaling axis via hypoxia inducible factor 1,[30] we additionally tested several genes linked to hypoxia, including *Egln3*, *Bnip3*, *Arbp*, *Hif1a*, and *Hprt*. The expression of 2 genes, *Egln3* and *Bnip3*, was significantly upregulated in the colon of 5-ASA–treated rats, whereas the expression of the remaining genes showed no significant differences (Figure S2). Next, because impaired energy metabolism in the colonic epithelium is reported to induce microbiota dysbiosis,[31] we examined if this was the case in our study. As is shown in Figure 4, significantly different unweighted β diversity of the fecal microbiota was observed between the control and 5-ASA–treated groups. Firmicutes and Bacteroidetes were differentially enriched in the control and 5-ASA groups, respectively (Figure 5). Specifically, a lower abundance of Firmicutes and a higher Bacteroidetes level were noted in the 5-ASA–treated group compared with the control group (Figure 6A and 6B). Accordingly, the Firmicutes/Bacteroidetes ratio, which is a gut microbiota dysbiosis marker, was significantly lower in the 5-ASA–treated group compared with the control (Figure 6C). To examine the translational relevance of this observation, we compared the Firmicutes/Bacteroidetes ratio of patients with IBD and non-IBD patients from the American Gut Project.[32] Non-IBD patients showed a similar pattern as S rats treated with 5-ASA as evidenced by a lower abundance of Firmicutes, a higher abundance of Bacteroidetes, and a lower Firmicutes/Bacteroidetes ratio in the non-IBD patients compared with patients with IBD (Figure 6D through 6F).

In addition, the abundances of several microbiota previously associated with BP regulation were also associated with BP in our study. These include Prevotellaceae, Bacillales, Lachnospiraceae, and Allobaculum. Prevotellaceae was significantly increased in rats treated with amoxicillin, which lowered their BP.[33] When the fecal content from hypertensive

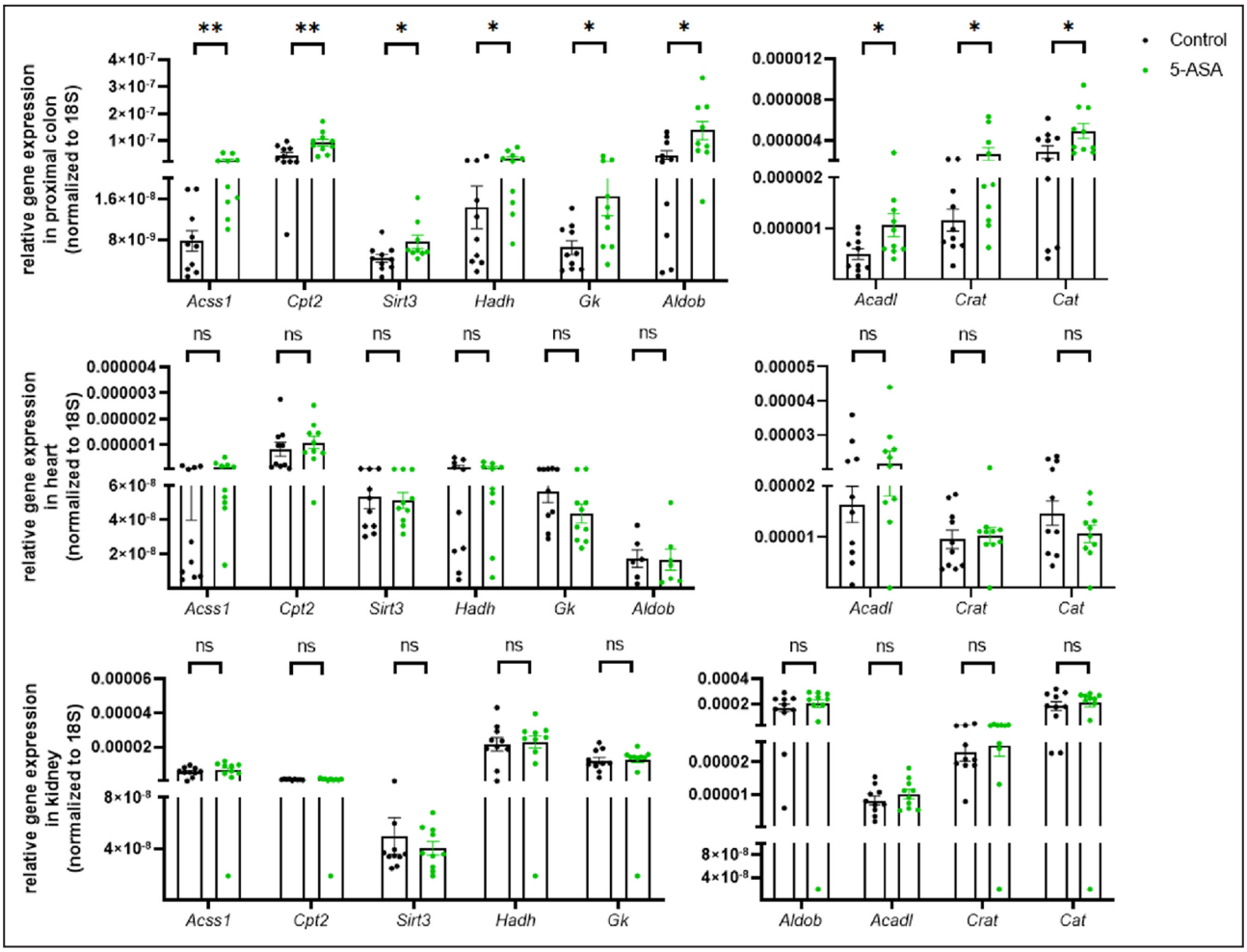


Figure 3. Energy metabolism–related gene expression comparison between the control (n=6–10) and 5-ASA–treated groups (n=7–10) in the proximal colon, heart, and kidney.
Data were analyzed by unpaired *t* test. **P*<0.05; ***P*<0.01. 5-ASA indicates 5-aminosalicylic acid; *Acadl*, acyl-CoA dehydrogenase long chain; *Acss1*, acyl-CoA synthetase short-chain family member 1; *Aldob*, aldolase, fructose-bisphosphate B; *Cpt2*, carnitine palmitoyltransferase 2; *Cat*, catalase; *Crat*, carnitine O-acetyltransferase; *Gk*, glycerol kinase; *Hadh*, hydroxyacyl-CoA dehydrogenase; ns, nonsignificant; and *Sirt3*, sirtuin 3.

rats was transplanted into normotensive rats, Bacillales were reduced, which was accompanied by an elevation in BP.[34] Both in rats and humans, Lachnospiraceae is reported to positively associate with BP.[34,35] In humans, a lower level of the taxon Allobaculum is associated with hypertension.[36]

Gut Microbiota Firmicutes/Bacteroidetes Ratio Was Positively Correlated With Systolic BP in S Rats, and This Correlation Was Subdued by 5-ASA

Lastly, we asked whether the effect on the Firmicutes/Bacteroidetes ratio was related to the BP regulation by 5-ASA. As shown in Figure 7, systolic BP was positively correlated with the abundance of Firmicutes and negatively correlated with the abundance of Bacteroidetes and resulted in an overall positive correlation of Firmicutes/Bacteroidetes ratio and systolic BP in control S rats (Figure 7A through 7C). Importantly, these correlations were lost in the 5-ASA group, as we did not observe the similar pattern of positive correlation between systolic BP and Firmicutes/Bacteroidetes ratio in these treated rats (Figure 7D through 7F). Thus, we concluded that this loss of correlation was caused by 5-ASA treatment, and the tempering effect of 5-ASA on the composition of Firmicutes and Bacteroidetes was sufficient to lower BP.

DISCUSSION

Our study demonstrated that 5-ASA, the U.S. Food and Drug Administration–approved drug for IBD, is beneficial for attenuating hypertension. The increased expression of energy metabolism-related genes and

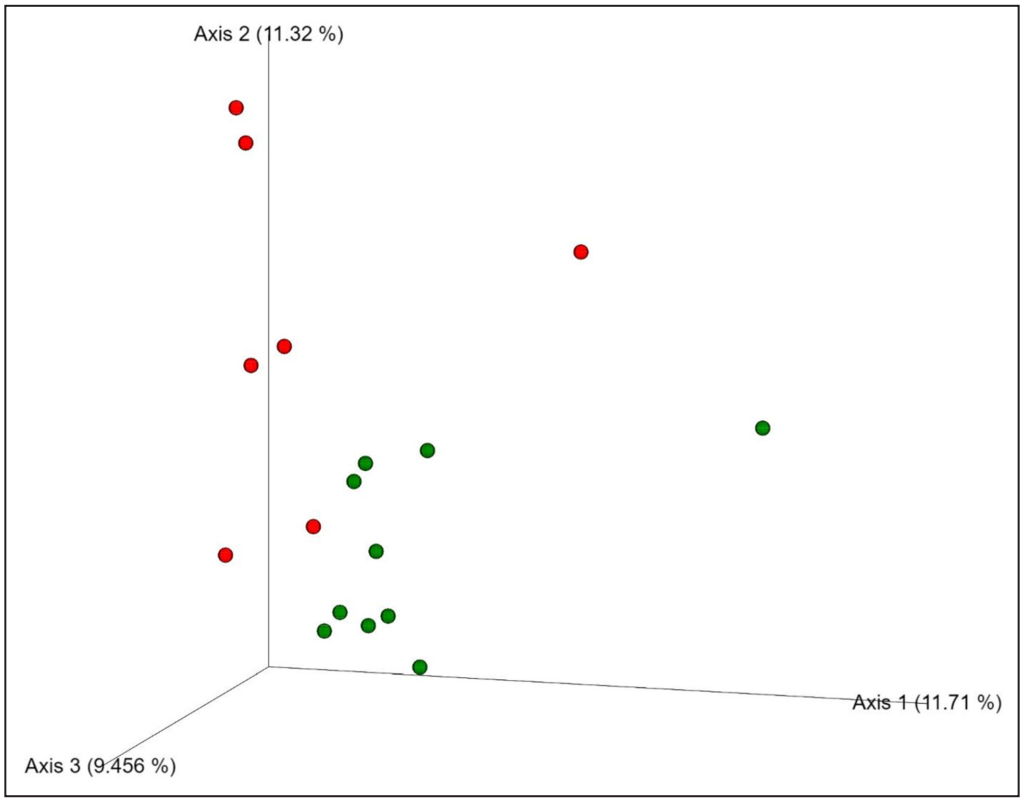


Figure 4. Unweighted β diversity of Dahl salt-sensitive rats with or without 5-aminosalicylic acid, *P*<0.01.
The unique fraction metric distance was used as the metric to explain the significant difference among microbial communities in the control (n=7) and 5-aminosalicylic acid–treated groups (n=10). Analysis of similarities *P* value was calculated using the Quantitative Insights Into Microbial Ecology version 2 script. Colors: red, control group; green, 5-aminosalicylic acid group.

the corrected Firmicutes/Bacteroidetes ratio (a gut dysbiosis marker) in the gut most likely contributed to the 5-ASA BP downregulation. Specifically, treatment with 5-ASA was sufficient to enhance the expression of colonic *Pparg* and PPARγ activity. The BP downregulation by 5-ASA was likely contributed by the increased gut energy metabolism and amelioration of gut dysbiosis. Overall, this is the first study to test and report that targeting the gut and improving energy metabolism mediated by colonic PPARγ is beneficial for lowering BP.

Current clinical management of hypertension targets select organs such as the kidney, vasculature, or heart. The involvement of the gut as an important organ in the pathogenesis of hypertension has been recently recognized, whereby targeting the mechanisms operating in the gut is an attractive new opportunity. In the gut, there are 2 therapeutic components. The first is the gut itself, which is the host component, and the second is its content, which is mainly the gut microbiota. Our previous studies[37,38] that used a variety of antibiotics as well as gut microbiota transplantation clearly demonstrated that elimination or replacement of gut microbiota does not help but further aggravated hypertension in the S rats. Therefore, an indirect method to reset gut microbiota is needed.

In this study, we used 5-ASA to target gut energy metabolism and impact gut microbiota composition and demonstrated that by increasing colonic energy metabolism, the desired result of resetting the Firmicutes/Bacteroidetes ratio was achieved. Because both mechanisms operate simultaneously, it is difficult to pinpoint whether upregulation of PPARγ in the proximal colon or reduction in the Firmicutes/Bacteroidetes ratio caused the BP-lowering effect. Nevertheless, the following 2 observations are worth noting: (1) diastolic BP is contributing largely to the observed overall lowering of mean arterial pressure in S rats treated with 5-ASA, and (2) systolic BP, but not diastolic BP, was positively correlated with the Firmicutes/Bacteroidetes ratio in S rats.

Importantly, we discovered an overall positive correlation between the Firmicutes/Bacteroidetes ratio and systolic BP in control S rats (Figure 7A through 7C), and these correlations were tempered in the 5-ASA group, where the observation of a positive correlation

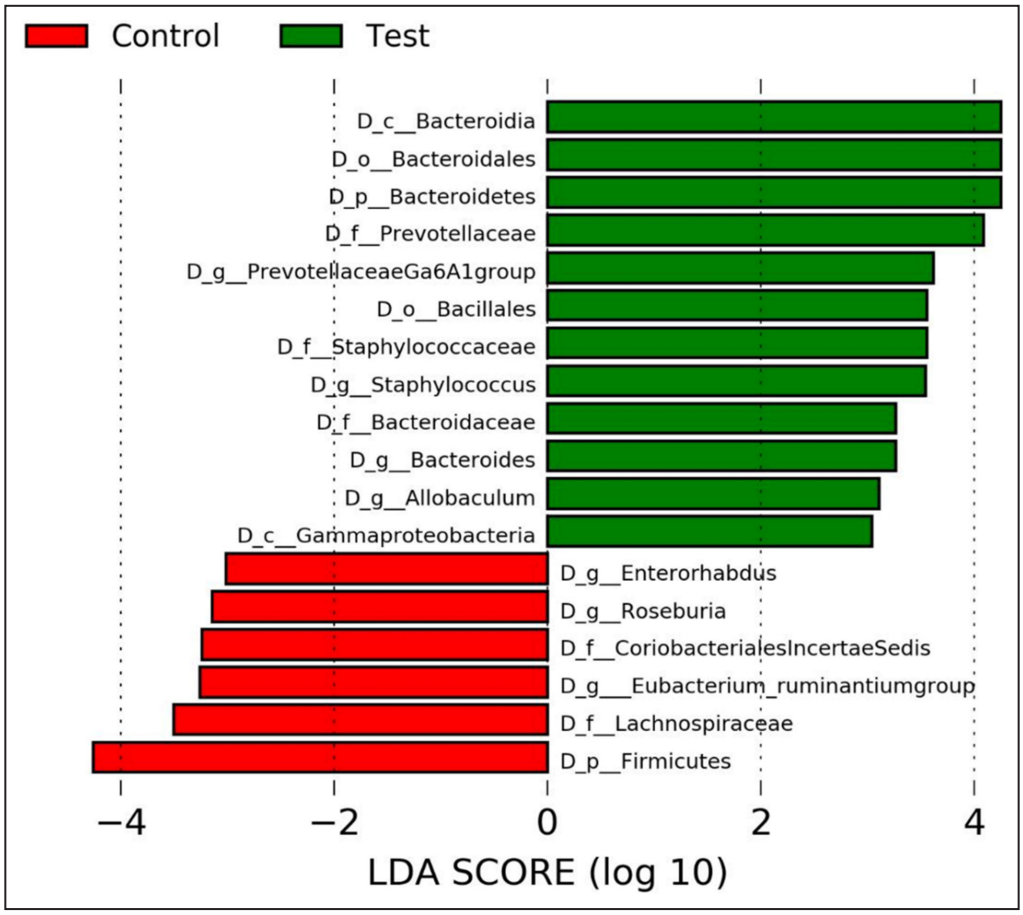


Figure 5. Differential bacterial taxa between the control and test groups.
Linear discriminant analysis effect size bar plot (LDA>3.0) showing enriched taxa in the control (n=7) and test groups (n=10). Taxa labels: D, dominant representative bacterial; f, family; g, genus; c, class; o, order; p, phylum. 5-ASA indicates 5-aminosalicylic acid; and LDA, linear discriminant analysis.

between systolic BP and Firmicutes/Bacteroidetes ratio was lost (Figure 7D through 7F). Based on these data, we concluded that 5-ASA treatment was responsible for this loss of correlation and that the tempering effect of 5-ASA on the Firmicutes and Bacteroidetes composition was sufficient to lower BP.

Taken together, these observations indicate that the direct effect of 5-ASA on BP is through its action on the host (increased PPARγ activity and gut energy metabolism), whereas the effect of 5-ASA on decreasing the microbiota Firmicutes/Bacteroidetes ratio is more notable on systolic BP. Thus, both effects of 5-ASA on the host colon as well as the microbiota contribute to the overall reduction in BP.

The mechanism by which 5-ASA preferentially lowers diastolic BP is unknown. Some evidence was notable in the form of a lower heart rate and heart *interleukin 17 alpha* expression in the S rats treated with 5-ASA (Figure S3, S4). Interestingly, resting heart rate is positively associated with diastolic BP in humans.[39] Also, it is reported that heart IL-17A triggers cardiac injury in hypertensive mice and plays an essential role in human heart failure.[40,41] The lower heart rate and expression of *interleukin 17 alpha* in the 5-ASA–treated group may thus contribute to the observed lowering of diastolic BP.

Several groups have reported that a dampened energy metabolism is 1 of the features in hypertension. Lack of energy in cells and tissues is directly causally related to the steady rise in systemic BP.[18] Also, the elevation of fumarase, the enzyme that produces energy in the form of reduced nicotinamide adenine dinucleotide, attenuates hypertension in S rats.[42] In addition, we and others have suggested that alternate fuels such as β-hydroxybutyrate as well as microbial metabolites, which confer energy to the host, such as short-chain fatty acids, are also deficient in the S rats.[43,44] Although these observations support the suggestion that rescuing overall energy metabolism might lower hypertension in the S rats, our study is the first to demonstrate that specifically targeting and improving PPARγ-mediated energy metabolism in the gut is sufficient to lower the BP of the S rats. The specificity of

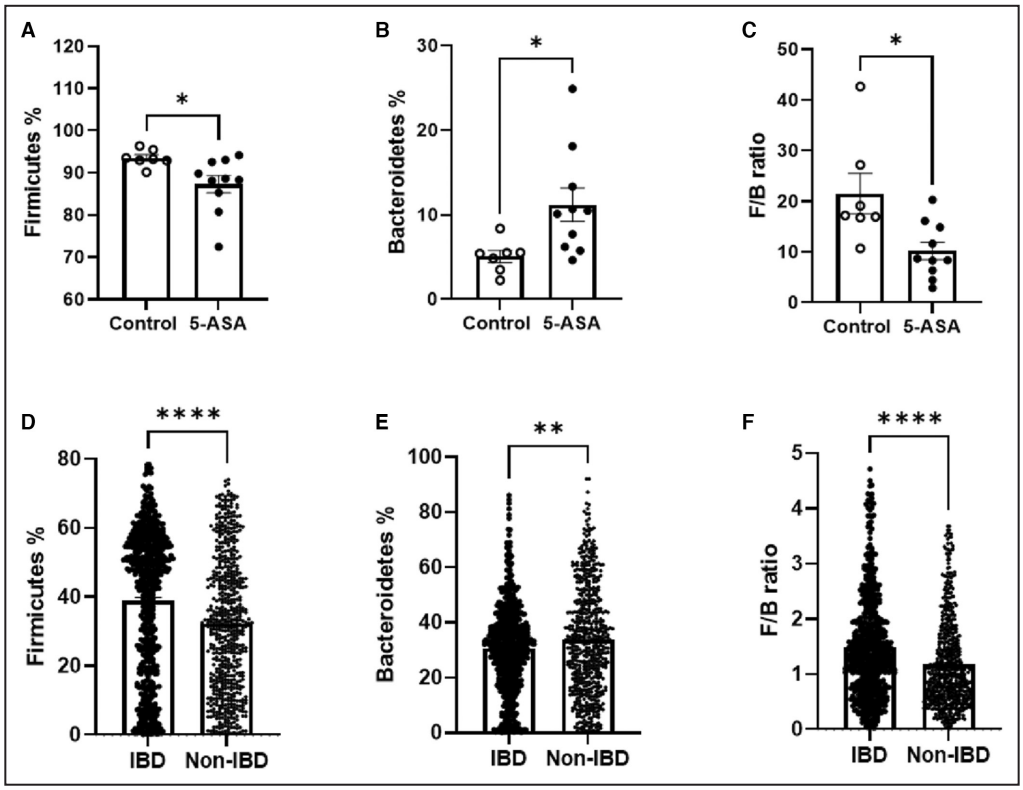


Figure 6. The percentages of Firmicutes, Bacteroidetes, and Firmicutes/Bacteroidetes ratio comparisons between the control group (n=7), 5-ASA group (n=10), patients with IBD (n=631), and non-IBD patients (n=611).

Data were analyzed by unpaired *t* test. The data of patients with IBD and non-IBD patients were collected from the American Gut Project as previously described.[32] (A) the abundance of *Firmicutes* comparison between S rats treated with or without 5-ASA; (B) the abundance of *Bacteroidetes* comparison between S rats treated with or without 5-ASA; (C) the *Firmicutes*/*Bacteroidetes* ratio comparison between S rats treated with or without 5-ASA; (D),the abundance of *Firmicutes* comparison between patients with IBD and non-IBD patients; (E), the abundance of *Bacteroidetes* comparison between patients with IBD and non-IBD patients; (F), the *Firmicutes*/*Bacteroidetes* ratio comparison between IBD and non-IBD patients. **P*<0.05; ***P*<0.01; *****P*<0.0001. 5-ASA indicates 5-aminosalicylic acid; F/B, Firmicutes/Bacteroidetes; and IBD, inflammatory bowel disease.

this rescue by 5-ASA in the colon is because of the reports that after its action in the colonic epithelial cells, 5-ASA is N-acetylated and not available for binding to activate PPARγ in other tissues.[29]

5-ASA is also known to lower the expression of proinflammatory genes in the context of IBD.[45] However, we did not observe this effect in our study, which is likely because the extent of inflammation in the hypertensive rat is low grade and not as exaggerated in IBD. We acknowledge that this conclusion is based on limited examination of a panel of anti-inflammatory genes. Further studies are clearly warranted to test the role of anti-inflammatory mechanisms of 5-ASA in the context of BP regulation.

Gut dysbiosis is associated with impaired mitochondrial function in the colonic epithelium, which disrupts the epithelial hypoxia[31] and signaling axis via hypoxia inducible factor 1.[30] Therefore, gene expression data were collected from 5 genes, of which only 2, *Egln3* and *Bnip3*, but not *Hif1a*, *Arbp*, and *Hprt*, were significantly upregulated in the colon of S rats treated with 5-ASA (Figure S2). These disparate data are insufficient to conclude that enhancing hypoxia is a mechanism by which 5-ASA protects from hypertension. The limitation of this conclusion is that monitoring gene expression is an indirect reporting of hypoxia. Further studies using direct measurements such as using the hypoxyprobe[30] will be needed to overcome this limitation.

Nevertheless, these data further strengthen the conclusion that enhancing energy metabolism coupled with resetting of gut microbiota is more likely contributing to the BP-lowering effect of 5-ASA.

Perspectives

The use of gut-target therapies such as antibacterial antibiotics, probiotics, bacteriophages, or fecal

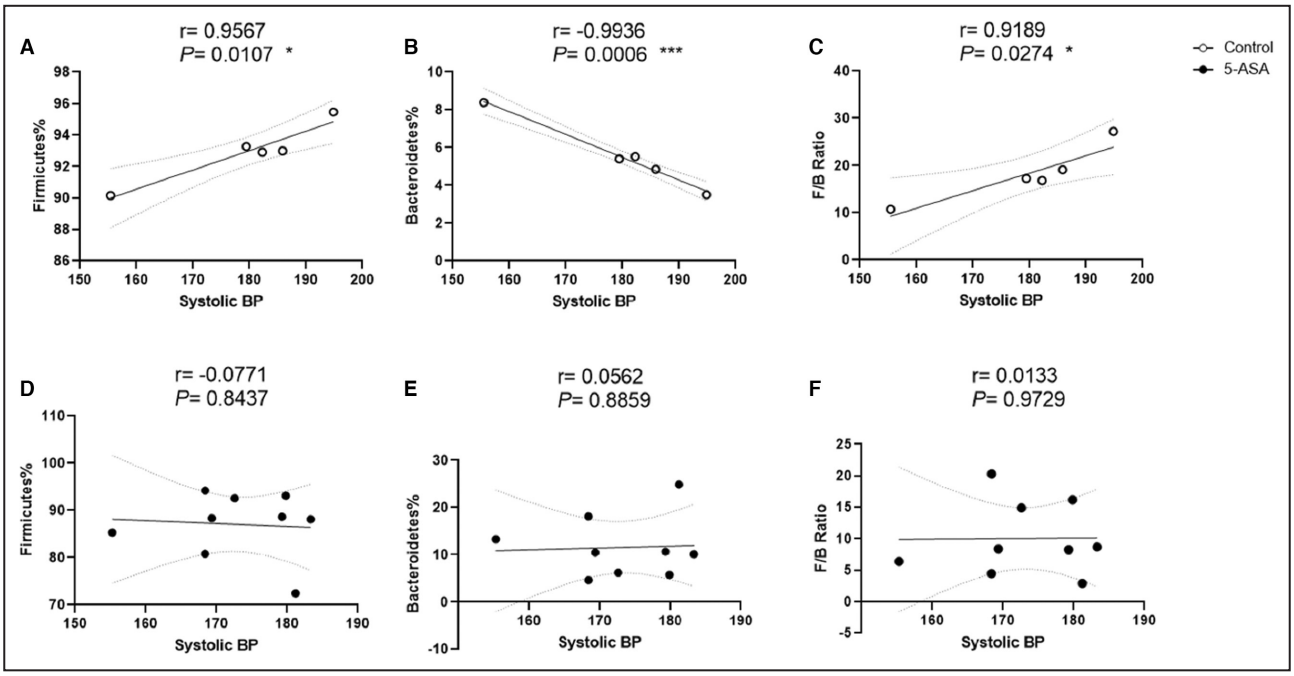


Figure 7. Correlation between gut microbiota and systolic BP in the control and 5-aminosalicylic acid groups.
Control group, n=5; 5-aminosalicylic acid group, n=9. Only animals with matched data sets for both BP and microbiota were used for the correlation analysis. (A) the correlation of *Firmicutes* aboundance and systolic BP in control S rats; (B) the correlation of *Bacteroidetes* aboundance and systolic BP in control S rats; (C) the correlation of *Firmicutes/Bacteroidetes* ratio and systolic BP in control S rats; (D) the correlation of *Firmicutes* aboundance and systolic BP in S rats treated with 5-ASA; (E) the correlation of Bacteroidetes aboundance and systolic BP in S rats treated with 5-ASA; (F) the correlation of Firmicutes/Bacteroidetes ratio and systolic BP in S rats treated with 5-ASA. **P*<0.05; ****P*<0.001. BP indicates blood pressure; and F/B, Firmicutes/Bacteroidetes.

transplants as well as science-influenced diets are being investigated for the treatment of hypertension. However, it remains unclear how the gut pathology may contribute to BP control.

In this context, our study is the first to report that 5-ASA, the U.S. Food and Drug Administration–approved drug for IBD, can be repurposed to target the gut and lower BP in S rats. The newly described antihypertensive effect of 5-ASA was traced as being operational because of an increase in colonic energy metabolism and an improvement in gut microbiota homeostasis.

ARTICLE INFORMATION

Received August 20, 2022; accepted November 10, 2022.

Affiliation

Program in Physiological Genomics, Center for Hypertension and Precision Medicine, Department of Physiology and Pharmacology, College of Medicine and Life Sciences, University of Toledo, OH

Sources of Funding

Funding from the National Heart Lung and Blood Institute of the National Institutes of Health (R01HL1430820) to B. Joe is gratefully acknowledged.

Disclosures

None.

Supplemental Material

Figures S1–S4

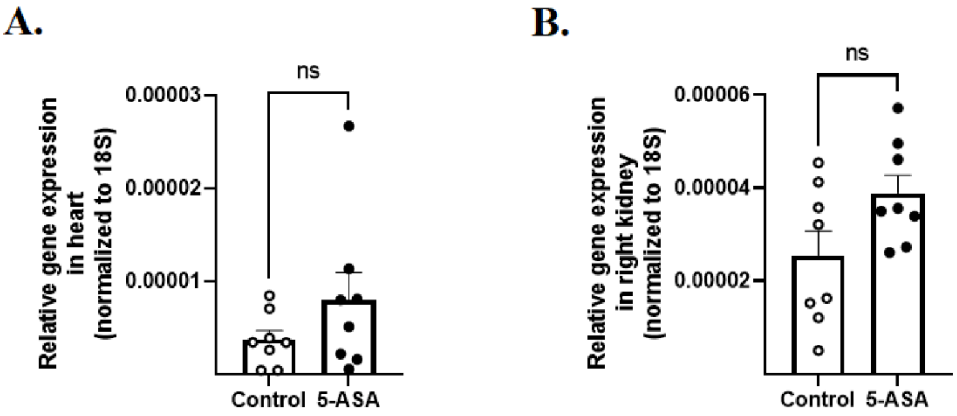
REFERENCES

1. Mills KT, Stefanescu A, He J. The global epidemiology of hypertension. *Nat Rev Nephrol*. 2020;16:223–237. doi: 10.1038/s41581-019-0244-2
2. Ramirez LA, Sullivan JC. Sex differences in hypertension: where we have been and where we are going. *Am J Hypertens*. 2018;31:1247–1254. doi: 10.1093/ajh/hpy148
3. Kearney PM, Whelton M, Reynolds K, Muntner P, Whelton PK, He J. Global burden of hypertension: analysis of worldwide data. *Lancet*. 2005;365:217–223. doi: 10.1016/S0140-6736(05)17741-1
4. Elliott WJ. Hypertension and diabetes. In: Lerma EV, Batuman V, eds. *Diabetes and Kidney Disease*. New York, NY: Springer; 2022:263–291.
5. Shams E, Kamalumpundi V, Peterson J, Gismondi RA, Oigman W, de Gusmão Correia ML. Highlights of mechanisms and treatment of obesity-related hypertension. *J Hum Hypertens*. 2022;36:785–793.
6. Zanoli L, Rastelli S, Inserra G, Castellino P. Arterial structure and function in inflammatory bowel disease. *World J Gastroenterol: WJG*. 2015;21:11304–11311. doi: 10.3748/wjg.v21.i40.11304
7. Li J, Yang X, Zhou X, Cai J. The role and mechanism of intestinal flora in blood pressure regulation and hypertension development. *Antioxid Redox Signal*. 2021;34:811–830. doi: 10.1089/ars.2020.8104
8. Mishima E, Abe T. Role of the microbiota in hypertension and antihypertensive drug metabolism. *Hypertens Res*. 2022;45:246–253. doi: 10.1038/s41440-021-00804-0
9. Xiong Y, Xiong Y, Zhu P, Wang Y, Yang H, Zhou R, Shu Y, Zhou H, Li Q. The role of gut microbiota in hypertension pathogenesis and the efficacy of antihypertensive drugs. *Curr Hypertens Rep*. 2021;23:1–14. doi: 10.1007/s11906-021-01157-2
10. Hong F, Pan S, Guo Y, Xu P, Zhai Y. Ppars as nuclear receptors for nutrient and energy metabolism. *Molecules*. 2019;24:2545. doi: 10.3390/molecules24142545
11. Wang Y-X. Ppars: diverse regulators in energy metabolism and metabolic diseases. *Cell Res*. 2010;20:124–137. doi: 10.1038/cr.2010.13
12. Byndloss MX, Olsan EE, Rivera-Chávez F, Tiffany CR, Cevallos SA, Lokken KL, Torres TP, Byndloss AJ, Faber F, Gao Y. Microbiota-activated

ppar-γ signaling inhibits dysbiotic enterobacteriaceae expansion. *Science*. 2017;357:570–575. doi: 10.1126/science.aam9949
13. Hasan AU, Rahman A, Kobori H. Interactions between host ppars and gut microbiota in health and disease. *Int J Mol Sci*. 2019;20:387. doi: 10.3390/ijms20020387
14. Tamboli CP, Neut C, Desreumaux P, Colombel JF. Dysbiosis in inflammatory bowel disease. *Gut*. 2004;53:1–4. doi: 10.1136/gut.53.1.1
15. Yang T, Santisteban MM, Rodriguez V, Li E, Ahmari N, Carvajal JM, Zadeh M, Gong M, Qi Y, Zubcevic J. Gut dysbiosis is linked to hypertension. *Hypertension*. 2015;65:1331–1340. doi: 10.1161/HYPERTENSIONAHA.115.05315
16. Özsoy M, Stummer N, Zimmermann FA, Feichtinger RG, Sperl W, Weghuber D, Schneider AM. Role of energy metabolism and mitochondrial function in inflammatory bowel disease. *Inflamm Bowel Dis*. 2022;28:1443–1450. doi: 10.1093/ibd/izac024
17. Weisshof R, Chermesh I. Micronutrient deficiencies in inflammatory bowel disease. *Curr Opin Clin Nutr Metab Care*. 2015;18:576–581. doi: 10.1097/MCO.0000000000000226
18. Iuv P. Energy-dependent pathogenesis in chronic hypertension. *Arkh Patol*. 2009;71:3–11.
19. Annese V, Rogai F, Settesoldi A, Bagnoli S. Pparγ in inflammatory bowel disease. *PPAR Res*. 2012;2012:1–9. doi: 10.1155/2012/620839
20. Thompson EA. Pparγ physiology and pathology in gastrointestinal epithelial cells. *Mol Cells*. 2007;24:167–176.
21. Williams C, Panaccione R, Ghosh S, Rioux K. Optimizing clinical use of mesalazine (5-aminosalicylic acid) in inflammatory bowel disease. *Ther Adv Gastroenterol*. 2011;4:237–248. doi: 10.1177/1756283X11405250
22. Ham M, Moss AC. Mesalamine in the treatment and maintenance of remission of ulcerative colitis. *Expert Rev Clin Pharmacol*. 2012;5:113–123. doi: 10.1586/ecp.12.2
23. Lee JY, Tsolis RM, Bäumler AJ. The microbiome and gut homeostasis. *Science*. 2022;377:eabp9960. doi: 10.1126/science.abp9960
24. Amplicon PCR, Clean-Up PCR, Index PCR. *16s Metagenomic Sequencing Library Preparation*. San Diego, CA: Illumina; 2013.
25. Bolyen E, Rideout JR, Dillon MR, Bokulich NA, Abnet CC, Al-Ghalith GA, Alexander H, Alm EJ, Arumugam M, Asnicar F. Reproducible, interactive, scalable and extensible microbiome data science using qiime 2. *Nat Biotechnol*. 2019;37:852–857.
26. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peplies J, Glöckner FO. The silva ribosomal RNA gene database project: improved data processing and web-based tools. *Nucleic Acids Res*. 2012;41:D590–D596. doi: 10.1093/nar/gks1219
27. Rio DC, Ares M, Hannon GJ, Nilsen TW. Purification of RNA using trizol (tri reagent). *Cold Spring Harb Protoc*. 2010;2010:pdb.prot5439. doi: 10.1101/pdb.prot5439
28. Schmittgen TD, Livak KJ. Analyzing real-time PCR data by the comparative ct method. *Nat Protoc*. 2008;3:1101–1108. doi: 10.1038/nprot.2008.73
29. Desreumaux P. Understanding the mechanism of 5-ASA in treating colonic inflammation. *Gastroenterol Hepatol*. 2008;4:319–320.
30. Kelly CJ, Zheng L, Campbell EL, Saeedi B, Scholz CC, Bayless AJ, Wilson KE, Glover LE, Kominsky DJ, Magnuson A. Crosstalk between microbiota-derived short-chain fatty acids and intestinal epithelial hif augments tissue barrier function. *Cell Host Microbe*. 2015;17:662–671. doi: 10.1016/j.chom.2015.03.005
31. Venable AH, Lee LE, Feola K, Santoyo J, Broomfield T, Huen SC. Fasting-induced HMGCS2 expression in the kidney does not contribute to circulating ketones. *Am J Physiol Renal Physiol*. 2022;322:F460–F467. doi: 10.1152/ajprenal.00447.2021
32. Manandhar I, Alimadadi A, Aryal S, Munroe PB, Joe B, Cheng X. Gut microbiome-based supervised machine learning for clinical diagnosis of inflammatory bowel diseases. *Am J Physiol Gastrointest Liver Physiol*. 2021;320:G328–G337.
33. Galla S, Chakraborty S, Cheng X, Yeo JY, Mell B, Chiu N, Wenceslau CF, Vijay-Kumar M, Joe B. Exposure to amoxicillin in early life is associated with changes in gut microbiota and reduction in blood pressure: findings from a study on rat dams and offspring. *J Am Heart Assoc*. 2020;9:e014373. doi: 10.1161/JAHA.119.014373
34. Adnan S, Nelson JW, Ajami NJ, Venna VR, Petrosino JF, Bryan RM Jr, Durgan DJ. Alterations in the gut microbiota can elicit hypertension in rats. *Physiol Genomics*. 2017;49:96–104. doi: 10.1152/physiolgenomics.00081.2016
35. Calderón-Pérez L, Gosalbes MJ, Yuste S, Valls RM, Pedret A, Llauradó E, Jimenez-Hernandez N, Artacho A, Pla-Pagà L, Companys J. Gut metagenomic and short chain fatty acids signature in hypertension: a cross-sectional study. *Sci Rep*. 2020;10:1–16. doi: 10.1038/s41598-020-63475-w
36. Richards EM, Li J, Stevens BR, Pepine CJ, Raizada MK. Gut microbiome and neuroinflammation in hypertension. *Circ Res*. 2022;130:401–417. doi: 10.1161/CIRCRESAHA.121.319816
37. Waghulde H, Cheng X, Galla S, Mell B, Cai J, Pruett-Miller SM, Vazquez G, Patterson A, Vijay Kumar M, Joe B. Attenuation of microbiotal dysbiosis and hypertension in a CRISPR/Cas9 gene ablation rat model of GPER1. *Hypertension*. 2018;72:1125–1132. doi: 10.1161/HYPERTENSIONAHA.118.11175
38. Mell B, Jala VR, Mathew AV, Byun J, Waghulde H, Zhang Y, Haribabu B, Vijay-Kumar M, Pennathur S, Joe B. Evidence for a link between gut microbiota and hypertension in the Dahl rat. *Physiol Genomics*. 2015;47:187–197. doi: 10.1152/physiolgenomics.00136.2014
39. Christofaro DGD, Casonatto J, Vanderlei LCM, Cucato GG, Dias RMR. Relationship between resting heart rate, blood pressure and pulse pressure in adolescents. *Arq Bras Cardiol*. 2017;108:405–410. doi: 10.5935/abc.20170050
40. Li Y, Wu Y, Zhang C, Li P, Cui W, Hao J, Ma X, Yin Z, Du J. γδT cell–derived interleukin-17a via an interleukin-1β-dependent mechanism mediates cardiac injury and fibrosis in hypertension. *Hypertension*. 2014;64:305–314. doi: 10.1161/HYPERTENSIONAHA.113.02604
41. Sandip C, Tan L, Huang J, Li Q, Ni L, Cianflone K, Wang DW. Common variants in IL-17a/IL-17RA axis contribute to predisposition to and progression of congestive heart failure. *Medicine*. 2016;95:e4105. doi: 10.1097/MD.0000000000004105
42. Usa K, Liu Y, Geurts AM, Cheng Y, Lazar J, Baker MA, Grzybowski M, He Y, Tian Z, Liang M. Elevation of fumarase attenuates hypertension and can result from a nonsynonymous sequence variation or increased expression depending on rat strain. *Physiol Genomics*. 2017;49:496–504. doi: 10.1152/physiolgenomics.00063.2017
43. Chakraborty S, Galla S, Cheng X, Yeo JY, Mell B, Singh V, Yeoh B, Saha P, Mathew AV, Vijay-Kumar M. Salt-responsive metabolite, β-hydroxybutyrate, attenuates hypertension. *Cell Rep*. 2018;25:677–689.e4.
44. Chakraborty S, Mandal J, Yang T, Cheng X, Yeo JY, McCarthy CG, Wenceslau CF, Koch LG, Hill JW, Vijay-Kumar M. Metabolites and hypertension: insights into hypertension as a metabolic disorder: 2019 harriet dustan award. *Hypertension*. 2020;75:1386–1396. doi: 10.1161/HYPERTENSIONAHA.120.13896
45. Mbodji K, Charpentier C, Guérin C, Querec C, Bole-Feysot C, Aziz M, Savoye G, Déchelotte P, Marion-Letellier R. Adjunct therapy of n-3 fatty acids to 5-ASA ameliorates inflammatory score and decreases NF-κb in rats with TNBS-induced colitis. *J Nutr Biochem*. 2013;24:700–705. doi: 10.1016/j.jnutbio.2012.03.022

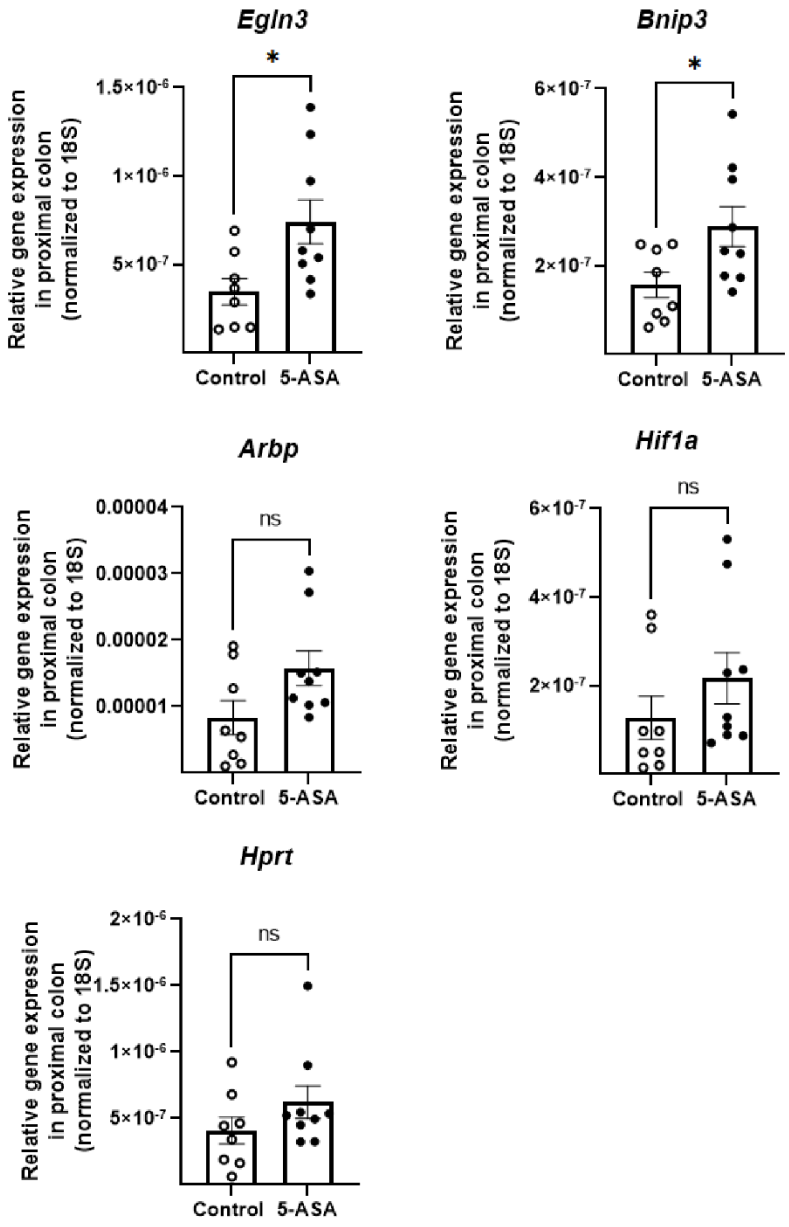
SUPPLEMENTAL MATERIAL

Figure S1. The *Pparg* gene expression comparison between 5-ASA and control group in heart (panel A) and kidney (panel B)[1].



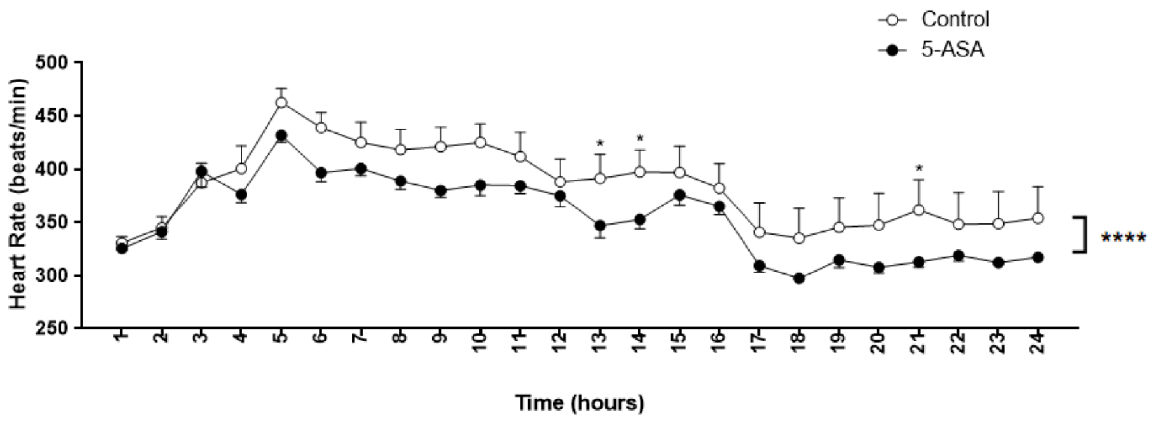
Data were analyzed by unpaired t-test, ns, non-significant. Control group (n=8); test group (n=8). *Pparg,* peroxisome proliferator activated receptor gamma gene.

Figure S2. Hypoxia indicator genes expression comparison between control and 5-ASA treated group in proximal colon.



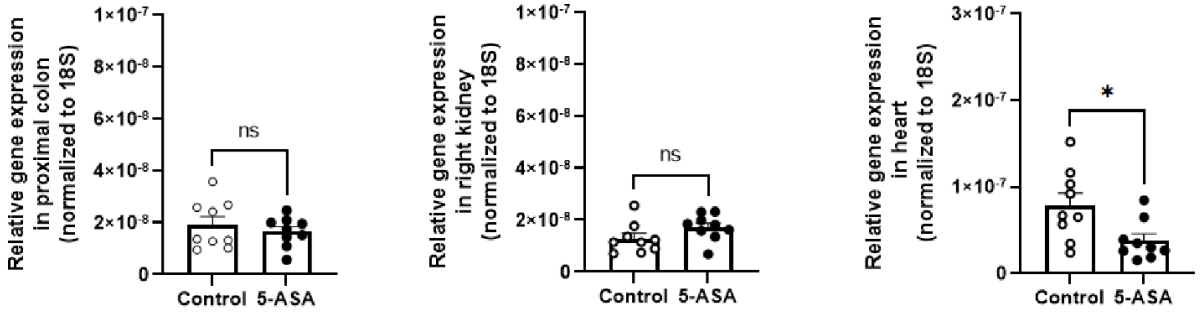
Data were analyzed by unpaired t-test, *p<0.05, ns, non-significant. Control group (n=8); 5-ASA group (n=9). 5-ASA, 5-aminosalicylic acid.

Figure S3. The heart rate comparison between the control (n=7) and 5-ASA groups (n=9).



Data were analyzed by 2-way ANOVA followed by Fisher's LSD test, *p<0.05, ****p<0.0001. ANOVA, analysis of variance; LSD, least significant difference.

Figure S4. The *Il-17α* gene expression comparison between control (n=9) and 5-ASA treated group (n=9) in proximal colon, heart, and kidney.



Data were analyzed by unpaired t-test, *p<0.05; ns, non-significant. *Il-17α,* interleukin 17 alpha.