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GUT MICROBIOME, SYMPATHETIC NERVOUS SYSTEM, AND HYPERTENSION (MK RAIZADA AND EM RICHARDS, SECTION EDITORS)

The Gut, Its Microbiome, and Hypertension

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Abstract
Purpose of the Review Evidence is rapidly accumulating implicating gut dysbiosis in hypertension (HTN). However, we are far from understanding whether this is a cause or consequence of HTN, and how to best translate this fundamental knowledge to advance the management of HTN. This review aims to summarize recent advances in the field, illustrate the connections between the gut and hypertension, and establish that the gut microbiota (GM)-gut interaction is centrally positioned for consideration as an innovative approach for HTN therapeutics.
Recent Findings Animal models of HTN have shown that gut pathology occurs in HTN, and provides some clues to mechanisms linking the dysbiosis, gut pathology, and HTN.
Summary Circumstantial evidence links gut dysbiosis and HTN. Gut pathology, apparent in animal HTN models, has not been fully investigated in hypertensive patients. Objective evidence and an understanding of mechanisms could have a major impact for new antihypertensive therapies and/or improved applications of current ones.

Keywords Hypertension · Gut microbiota · Gut pathology · Renin angiotensin aldosterone system · Brain-gut-immune axis

This article is part of the Topical Collection on *Gut Microbiome, Sympathetic Nervous System, and Hypertension*

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Introduction

The latest American Heart Association statistics for 2009–2012 note approximately 80 million US adults or a third of Americans over 20 years old have hypertension (HTN) [1]. These hypertensive patients are at a greater risk for cardiovascular disease, kidney disease, stroke, and death than normotensive subjects. Indeed, 34% of deaths in 10 years between 2003 and 2013 were attributable to high blood pressure [1]. HTN creates an enormous health and economic burden, not just in the USA, but globally. Unfortunately, despite lifestyle modifications, new therapies, and intensive medical interventions, approximately a third of hypertensive patients do not achieve blood pressure control when prescribed ≥3 medications, and have "treatment-resistant" hypertension (RHTN) [1]. Black Americans have some of the highest rates of HTN in the world, especially black women where almost half have hypertension. This disparity is worsened by earlier onset, higher blood pressures, and higher rates of RHTN [1]. Effective treatment paradigms are urgently needed for HTN, especially for RHTN, and the causes of HTN are poorly understood in many, particularly those with RHTN. This review will highlight recent developments in our understanding of the pathogenesis of HTN and will focus on a role for the gut.

Potential for the Involvement of the Gut in Blood Pressure Control and HTN

Gastrointestinal Tract Anatomy and Ecology

The gastrointestinal tract has distinct structures, functions, motility and mucin layers along its length. It is also one of the few organs where the epithelial cell lining is capable of regeneration every 5 days. These properties provide a

dynamic environment and varied ecological niches for bacteria to exploit along the length of the tract. This results in different types and communities of bacteria residing at different levels. There are not only bacteria inhabiting these ecological niches but also viruses and in some people fungi and parasites. The complexity of the mix is influenced by the gut itself which secretes antimicrobial proteins, such as defensins, to favor certain bacteria over others. A healthy gut maintains a balance of these, often competing, elements. Thus, the gut microbial community plays a key role in maintaining normal physiological homeostasis in the host.

Gut epithelium has a vast surface area, necessary for efficient absorption and secretion. This large surface area presents a challenge to maintain its epithelial barrier function and it is estimated that ~70% of the body's immune cells reside in the gut, constituting the "gut associated lymphatic tissue" (GALT). The GALT moderates the continuous interactions of the bacterial communities and their byproducts with the gut [2]. The gut microbiome (GM) interacts with the immune system of the host to "educate" it, as germ-free mice have a defective immune system [3]. This "education", begins perinatally and continues until about 3 years of age in humans. The result is a symbiosis between the immune status and the GM that is beneficial and balanced to both sides, e.g., eubiosis. This eubiosis is very stable such that perturbations to the system are generally only short term, [4]. There are circadian and seasonal rhythms to the GM, but in general, the GM is considered imperturbable. The GM of an individual is stable, but there are differences between individuals, and even greater differences between individuals living in different communities. Occasionally, this eubiosis is thrown into disarray, resulting in dysbiosis with major pathophysiological consequences to the host.

The notion that the gut and its contents influence blood pressure has been in existence for more than half a century based on epidemiological studies. These studies identified salt [5], and alcohol [6] intake, the hyperglycemia related to increased carbohydrate consumption [7], and a lack of fiber in the diet [8] as risk factors for HTN. Their common element is that the gastrointestinal tract is the initial point of contact of these dietary elements with the body. Connections between the gut and HTN are further suggested by the important interactions of the gut and its contents with the immune system, the afferent and efferent portions of the autonomic nervous system, pre-autonomic brain areas and the enteric nervous system (ENS), and the renal axis [9]; all of which become dysregulated in HTN. Life-style choices and illnesses that affect the risk for developing HTN, impact the GM and gut motility. Finally, dysbiosis of the GM has recently been linked to metabolic HTN [10], HTN during obese pregnancies [11], lifetime risk of developing HTN [12••], and found in prehypertensive as well as hypertensive populations of Asian descent [13••]. These connections and their link to HTN is the focus of this review.

Gut Microbiota Metabolites and Their Effects on Blood Pressure

GM produce unique metabolites that are potentially important in BP control. Some of these metabolites with clear links to blood pressure regulation are discussed. The bacteria of the GM are the only source of short chain fatty acids (SCFA) for the body. They are predominantly acetate, proprionate and butyrate, and are derived from the digestion of dietary fiber by the GM. SCFA are an energy source for colonic epithelium, influencing cell growth, gut motility [14] and proliferation to maintain the gut barrier [15]. They epigenetically alter epithelial cells to effect transcription, for example acting on histone deacetylases [16]. They bind to the aryl hydrocarbon receptor (AHR) to increase transcription of IL10R via an AHR element in the IL10R promotor to reduce inflammation in ileal epithelial cells [17]. Furthermore, intestinal immunity is under regulation of AHR signaling [18]. Polymorphisms of AHR are associated, with other genes in its signaling pathway, with essential hypertension [19]. SCFA bind the olfactory receptors gpr41, gpr43, and olfO79 in the kidney, heart, sympathetic ganglia, and blood vessels to modulate blood pressure [20, 21]. SCFA modify gut motility by actions on the ENS [22]. And finally, the lack of butyrate due to depletion of butyrate producing bacteria in the GM was identified as a factor increasing blood pressure in obese pregnant women. SCFA maintain the epithelial barrier to reduce inflammation, directly affect immune cells and reduce sympathetic nerve activity. Thus, the GM directly mediates the effects of dietary fiber, a known modulator of risk for HTN, as well as blood pressure per se. *Collectively, these findings support the hypothesis that SCFA, the products of GM digestion of dietary fiber, affect immune-, epithelial-, nervous system-, and blood vessel function to modulate blood pressure and mediate the decrease in the risk of HTN due to a fiber rich diet.*

Formate and alanine serve as urinary markers of blood pressure and cardiovascular disease risk, especially as related to diet and the amount of animal protein consumed, [23]. Toxic byproducts of the microbiota produced by the metabolism of phosphatidylcholine to trimethylamine N-oxide also increase blood pressure [24]. Products of the GM, such as hydrogen sulfate, can directly act on blood vessels to modulate blood pressure [25]. *Thus, by-products of gut bacterial metabolism have direct effects on blood pressure.*

Tryptophan and its metabolites are important in host-GM communication. Firstly, they are precursors of neurotransmitters and directly affect the autonomic and ENS. Tryptophan metabolites can readily traverse the blood brain barrier (BBB) to influence the inflammatory status in the brain, as can SCFA and neuroactive steroids made by the GM. Neuroinflammation and its link to the GM have been demonstrated in many neurological diseases/disorders, for example multiple sclerosis [26], and hypertension, [27]. Therefore, GM

dysbiosis may modulate centrally acting GM metabolites to promote neuroinflammation, a state strongly associated with HTN in animal models. The finding that a centrally penetrating anti-inflammatory antibiotic, minocycline, produced long-lasting blood pressure reduction in a hypertensive individual [28] suggests that GM metabolites may also promote neuroinflammation, and the related human pathology. Secondly, the tryptophan metabolite kynurenine acts on ileal epithelial cells to promote wound healing [17], while another metabolite, tryptophol, modulates release of IFN gamma after LPS stimulation [29]. GM metabolites can influence mitochondrial function. For example, pieces of mitochondrial DNA in the CSF cause HTN through activation of TGFß-mediated pathways and neuroinflammation [30]; propionate influences the TCA cycle and secondarily carnitine metabolism in mitochondria to cause dysfunction [31]. *To summarize, GM-mediated tryptophan metabolism supports the epithelia of the ileum by increasing expression of receptors for anti-inflammatory cytokines and modulates immune responses to stimuli such as LPS. Microbiota-derived metabolites such as SCFA, neurosteroids and of tryptophan modulate central glial activation, a hallmark of HTN in animal models and possibly some humans.*

Gut-Immune Interactions

Germ-free mice have an altered microglial population in their brains that is associated with defects in innate immune responses [3]. These deficits were recapitulated by conventional mice treated with antibiotics and mice raised with reduced gut microbial diversity, [3], suggesting that there must be a continuous conversation between the immune system and GM metabolites to maintain a normally responsive immune system. GM dysbiosis accompanies HTN in rodents [32•, 33, 34] and alterations in the human GM occur in the prehypertensive state [13••]. The microbiome of the human prehypertensive subject is very similar to that of the hypertensive patient. This suggests that changes in the GM precede the onset of HTN. Furthermore, FMT from hypertensive humans to germfree mice resulted in increased blood pressure [13••]. It is not clear whether GM metabolites increase blood pressure, whether the gut needs to be colonized with HTN-associated bacteria to effect HTN, or if the FMT included bacteria harboring lysogenic viruses that infected the host to increase gut inflammation and permeability. However, the implication is that GM would have a different interaction with the immune system in the prehypertensive and hypertensive states compared to the normotensive. Systemic immune system dysregulation is a hallmark of HTN in animal models and humans [35] and neuroinflammation in blood pressure-relevant brain areas has been demonstrated in animal models of HTN [27]. This results from both resident microglial activation and recruitment of precursors from bone marrow that differentiate once in place in the brain [27]. Whether this inflammation results from a prohypertensive signal from the brain, the gut or the bone marrow, or a combination of these is unknown. Humans known to have "leaky guts" with stimulated immune responses, gut fibrosis, etc. for example inflammatory bowel disease sufferers, are less likely than the general population to be hypertensive [36]. So, the simple explanation of HTN resulting from immune stimulation by bacteria leaking into the host is neither accurate nor does it arise directly from the signal of gut inflammation. *Taken together, these studies indicate that chronic systemic inflammation resulting from immune activation is associated with HTN; dysbiosis of the gut microbiota and the consequent immune responses likely contribute to this inflammation.*

The GM is remarkably stable in the adult, [4, 37], but is malleable in early childhood [38], so environmental or lifestyle changes in adulthood are unlikely to manipulate the GM to cause a long-term outcome like HTN. So how could this GM dysbiosis-linked inflammation arise? The DOHaD (developmental origins of health and disease) hypothesis, that posits that non-communicable diseases result from early life influences, was initially based on epidemiological studies showing that low birth weight infants were more likely to die from CAD and CVD in later life than normal birth weight infants [39, 40]. The intrauterine environment coupled with early postnatal exposures contribute to this susceptibility [41]. It is tempting to suggest that the GM-gut-immune-brain axis is incorrectly established in early life in these small-for-gestational age infants resulting in a predisposition towards HTN, but this hypothesis remains to be tested. A recent study found the lifetime risk of cardiovascular disease to be associated with six bacterial genera and overall microbial richness, [12••]. It would be very helpful to better understand when these GM conditions are achieved in the lifespan and their stability, to be able to propose new HTN treatment options. Could, for example, an undernourished mother pass epigenetic markers to her offspring that predispose to a gut-GM-immune-brain interaction favoring HTN development? Is the gut microbiome truly as stable over the lifespan as it appears from the limited studies reported or could HTN modify or be modified by changes over the lifespan? *Therefore, perinatal and early life conditions influence the risk of developing HTN, and this may occur through modulation of the establishment of the gut-immune brain axis.* **In summary, gut-GM-immune interactions are altered during HTN.**

Enteric Nervous System, GM, and Autonomic Nervous System Interactions

The ENS, the nervous system of the gut, is a plexus primarily utilizing the neurotransmitter, serotonin, produced from the precursor tryptophan. It controls gut motility in collaboration with the autonomic nervous system. The GM is an important

component; depleting the GM of mice with antibiotics induced changes in host serotonin biosynthesis and intestinal motility [42]. Constipation, a result of changed gut motility, is linked to HTN in postmenopausal women [43] and in men with chronic kidney disease and end stage renal disease [44, 45]. The ENS communicates with the central nervous system, receiving sympathetic and parasympathetic input to the plexus, as well as neuro-hormonal input. In return, it sends neural and neuro-hormonally coded information to the central nervous system. The GM metabolizes tyrosine with potential to alter sympathetic transmitters (dopamine, norepinephrine, and epinephrine) and contribute to sympathetic dysregulation, another hallmark of HTN. *These findings demonstrate that ENS activity is altered in HTN.*

Autonomic system: Altered sympathetic nervous system activity is associated with HTN in humans and animal models of HTN. Rodent studies describe a change in microbiota secondary to dysfunctional autonomic nervous system activity (ANS) [32•] but it is not clear which occurs first in people. Metabolites of amino acids produced by actions of the GM, such as tryptophan metabolites, and the glutamate metabolite, GABA, can directly impact the central and peripheral nervous system, [46, 47, 48]. Many of these metabolites are freely accessible centrally as well as systemically and have potential to influence blood pressure at multiple sites in the cardiovascular control centers of the brain, as well as peripherally. SCFA also influence ENS activity that in turn signals to the CNS, alterations of this interaction can impact sympathetic activity. *The autonomic nervous system integrates input from the ENS and GM metabolites at peripheral and central sites to change autonomic system activity and blood pressure.* **Dysregulated autonomic nervous system activity is coincident with, or precedes, HTN [33••], and the ENS under the influence of a dysbiotic GM contributes to this dysregulation.**

Gut Pathology Related to HTN

There are few studies of gut pathology related to hypertension in the human, barring a potentially-related finding of decreased blood flow in pro-inflammatory states [49]. This is an area that deserves more study in the wake of recent findings in rodent models of HTN. The SHR, prior to increased blood pressure, reveals both increased sympathetic activity to the gut, and a decrease in tight junction proteins that are essential for the barrier function of gut epithelium. As HTN becomes established, gut pathology becomes more pronounced, with increased permeability, increased stiffness, fibrosis and muscle thickness, and decreased goblet cells and villi length in the small intestine. Similar changes occur in the colon. In the chronic angiotensin II (Ang II)-infusion model of HTN, the pathology is almost the same but with a smaller loss of goblet cells and no changes in the length of villi, perhaps related to the shorter time period of HTN. ACE inhibition with captopril in the SHR reversed the changes including those on sympathetic activity, but not those on goblet cells; however, captopril had no effect on the gut of the WKY, in which there was a relatively smaller decrease in blood pressure [33••]. The implications here are either that the active molecule is Ang II, since captopril prevents the conversion of Ang I to Ang II; or, that high blood pressure is directly related to the gut pathology. Hematopoietic stem cells treated with Ang II had altered differentiation potential and reduced homing capacity [50], suggesting that Ang II rather than high blood pressure is causative at least for the immune dysregulation in Ang II-induced HTN. Ang II has been shown to cause matrix accumulation, inflammation and apoptosis via TGF-ß and its downstream signaling molecules in the kidney [51], but whether this is true in the gut is unknown. If gut pathology precedes HTN in humans or even if it occurs with established HTN remains to be investigated. **In summary, gut pathology precedes HTN in animal models, but has not been investigated in patients with hypertension.**

Gut RAAS and HTN

The gut has a local renin angiotensin aldosterone system that is important for the uptake of sodium and water from the colon and for the control of gut contractility. Key members of both arms of the RAAS are present in the gut. The effector arm includes the angiotensin type I receptor (AT1R), angiotensin converting enzyme (ACE), angiotensin II (Ang II), aldosterone, and the mineralocorticoid receptor (MR), and its counter regulatory system consists of angiotensin converting enzyme 2 (ACE2), the Mas receptor (MAS1R), the angiotensin type 2 receptor (AT2R), and angiotensin 1–7 (Ang1–7). There are high levels of the AT1R and MR in colonic epithelium, but low or non-existent levels of AT2R or MAS1R (the receptor for Ang1–7) [52], although injury increases the expression of the MAS1R [53]. The AT1R is expressed solely in enteroendocrine L-cells that make Glucagon like Peptide 1 (Glp1) and PYY, and affects gut epithelial flux of anions and fluid [52], as does the MR. The AT1R also modulate gut contractility. This arm of the RAS is important for water and salt movement across the gut, but can be pro-hypertensive and pro-inflammatory if in imbalance with the RAAS counter-regulatory arm. Some beneficial actions of antihypertensive drugs that act on the RAAS are caused by altering these effects in the gut. For example, Losartan®, the AT1R blocker, decreases the number of AT1R in the gut and alters gut motility [54].

ACE2 is located primarily in the epithelium of the small intestine [55]; there is little to no RNA expression for ACE2 in the colon and the protein was not detectable there by immunohistochemistry. ACE2 acts as an anti-inflammatory agent by increasing Ang1–7 and decreasing Ang II content of the colon, and has important beneficial effects in some diseases of the gut such as colitis [53].

Interactions between the GM and the gut RAAS that Relate to HTN

Production of RAAS Activators and Inhibitors by the GM

Some symbiotic bacteria produce ACE inhibitors, renin inhibitors, and antioxidant molecules during the digestion of mucin, thus GM dysbiosis could trigger HTN, as reviewed in [56]. Aldosterone (and other steroid metabolites) are synthesized from bile salt-conjugated steroids in the enterohepatic circulation by the microbiota, and elicit HTN [57]. GM-mediated synthesis of steroids that reduce the inactivation of cortisol by acting as inhibitors of 11ß-hydroxysteroid dehydrogenase 2 (11HSD2) have also been described [58]. These two types of steroids produced by the GM have potential to be prohypertensive through local actions in the gut and, being freely diffusible, the brain [59, 60, 61] and kidney. Further, metabolic activity of the GM may explain the pro-hypertensive actions of stress in some of the population if we hypothesize that they have a GM dysbiosis that inhibits the breakdown of cortisol by 11HSD2. Cortisol binds two receptors to have pro-hypertensive actions, the mineralocorticoid receptor, and in the absence of 11HSD2, to the glucocorticoid receptor [62].

The HTN resulting from GM-mediated synthesis of aldosterone was prevented by antibiotic knockdown of the gut microbiota in rodents [57], illustrating the important role of the GM in HTN. This role has been extended to humans by a recent case report showing that HTN was reversed by a course of brain penetrating, anti-inflammatory antibiotics, and returned 6 months after antibiotic therapy ended [28]. This may have been the result of actions on both the GM and the brain, especially since steroid-activated MR can be pro-inflammatory by actions in macrophages and microglia, and the antibiotics have anti-inflammatory as well as antibiotic actions. *From these data, we can conclude that anti-inflammatory antibiotics may be useful to treat HTN by actions in the gut and brain.*

ACE2 Actions on the GM

ACE2 is the most studied component of the gut RAAS that has direct effects on the GM. ACE2 alters antimicrobial peptide secretion in the small intestine leading to altered GM composition in the colon [55]. Polymorphisms of the ACE2 gene have been linked to hypertension [63] and it is likely that this could be, in part, due to altered ACE2 activity in gut and resultant GM alterations. ACE2 is shed as an enzymatically active molecule, sACE2, from lung and kidney proximal tubule epithelia by the actions of ADAM17 [64, 65]. sACE2 interacts with viruses in various ways. sACE2 inhibits the binding of the SARS virus to the epithelium and prevents its uptake. ACE2 is also cleaved by the flu virus neuraminidase and subsequently degraded in the cell [66, 67]. It is unclear whether sACE2 has actions in the gut, or if ACE2 has any interactions with gut viruses, but it would be interesting to have this information and realize its potential in HTN, considering the huge viral load in the gut, estimated at 10^{11} per gram. *Essentially, the gut RAAS and GM interact, and dysbiosis affects HTN by modulating the gut RAAS.*

Some interesting questions arise when considering interactions of GM and RAAS. If antihypertensive drugs, such as ACE inhibitors were prescribed to an individual with GM already producing ACE inhibitors, would these be less effective to treat HTN than in an individual without these GM? Do inter-individual antihypertensive responses vary depending upon the background GM? Can the GM metabolize drugs differently depending upon the composition of the GM [68•]? Is resistance to development of HTN in some individuals due to a particularly beneficial combination of GM and RAAS? Could ACE2's antimicrobial peptide activity be exploited to correct dysbiotic colonic GM populations? Answers to these questions could greatly potentiate the ability to prescribe effective antihypertensive agents.

Genetic Models of ACE2 Expression in Mice

Mice with global overexpression of ACE2 (ACE2 KI) are resistant to anxiety [69]. ACE2 stabilizes the neutral amino acid transporter B^0AT1 in gut epithelia [55]. Overexpression would be expected to increase uptake of tryptophan, the precursor for serotonin, into the host; serotonin decreases anxiety. Central MAS1 receptor antagonism blocks the anxiolytic effect of ACE2 KI, suggesting a central mechanism of anxiolysis via Ang1–7 generation. Dysbiosis of the GM occurs in ACE2 knockout mice (ACE2 KO) where lack of tryptophan uptake in the small intestine changed the secretion of antimicrobial proteins and altered colonic bacterial populations [55]. These studies led to the hypothesis that ACE2 has a major impact on the GM. The ACE2 KI mice were used to test this hypothesis and to discover the contribution of the ACE2 genetically modified mice to our understanding of BP regulation. ACE2 KI mice have a small trend towards a decrease in BP at baseline compared to their littermates, but respond much less to hypertensive stimuli (Ang II infusion), unpublished data. Similarly, ACE2 KO mice have little change in baseline blood pressure. We performed analysis of the fecal microbiota (collected and analyzed as described in [32•]), from ACE2 KI mice and their controls, described in [69] with protocols approved by the Institutional Care and Use Committee at the University of Florida. ACE2 KI mice have increased OTU (or species) abundance, Fig. 1a, and increased alpha diversity of bacteria in the GM in two estimations of alpha diversity (Shannon diversity index, $p < 0.01$ and Fisher's alpha test, $p < 0.05$) compared to their littermate controls, Fig. 1b, and their bacterial populations were distinctly separated in Bray-Curtis PcoA plots, Anosim

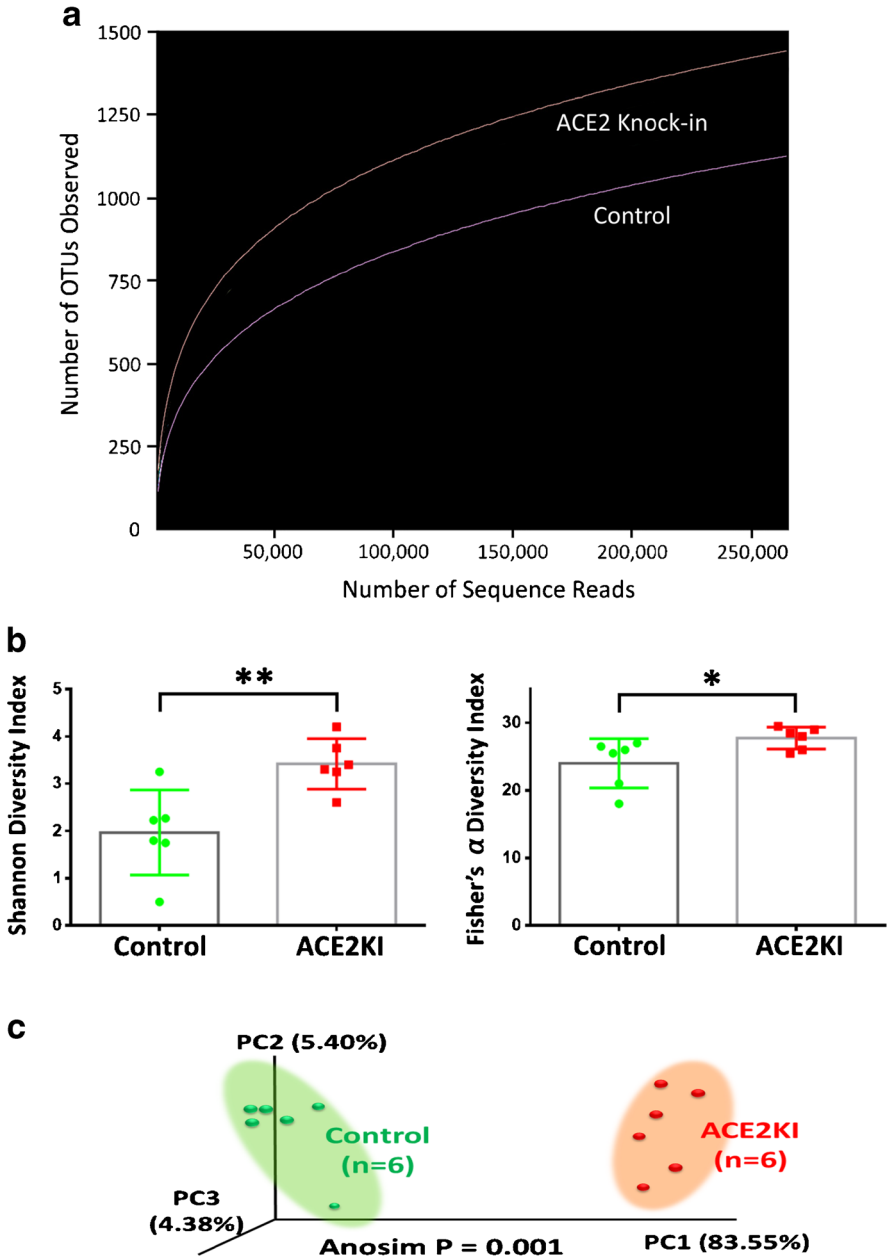

Fig. 1 ACE2 knock-in mice have altered gut microbiota compared to littermate controls. 16S rRNA gene sequence-based identification of bacteria in ACE2 knock-in mice. **a** Number of OTUs (or species) found at multiple rarefaction depths; ACE2 knock-in *red*, littermate controls *purple*. **b** The richness (# of OTUs) and evenness (distribution across OTUs) between ACE2 knock-ins and their littermate controls were significantly different using two tests of alpha diversity, Shannon index (*left*) and Fisher's alpha test (*right*), * = $p \leq 0.05$, ** = $p \leq 0.001$. **c** Principal coordinate analysis (PCoA) plot showing the separation between the bacterial communities found in the feces of ACE2 knock-in and their littermate control mice. The variance explained by each of the first three axes is shown in parentheses (83.55, 4.38, and 5.40%, respectively). **d** Heatmap illustrating the genus-level changes in bacterial abundance in the littermate controls and ACE2 knock-in mice. The relative abundance of a bacterial genus (*row*) in individual animals (*column*) is indicated by the color of the cell (*blue*, low abundance; *red*, high abundance). Bacterial genera altered in expression in both ACE2 knock-out [55] and ACE2 knock-in mice are illustrated by † (*Allobaculum*) and ‡ (*Rikenella*). **e** Bacterial taxa with significantly different abundances between littermate controls and ACE2 knock-in mice identified by linear discriminant analysis coupled with effect size (LEfSe). Bacterial taxa enriched in the ACE2 knock-in mice are shown in *red*, in littermate controls in *green*. Bacterial taxa altered in expression in both ACE2 knock-out [55] and ACE2 knock-in mice are illustrated by † (*Allobaculum*) and ‡ (*Rikenella*). **f** Phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt) analysis showing the significantly different functional capabilities predicted for the bacterial communites in ACE2 knock-in and littermate control mice. The phenylalanine, tyrosine, and tryptophan biosynthetic pathway expected to be altered in the knock-in mice is indicated by §

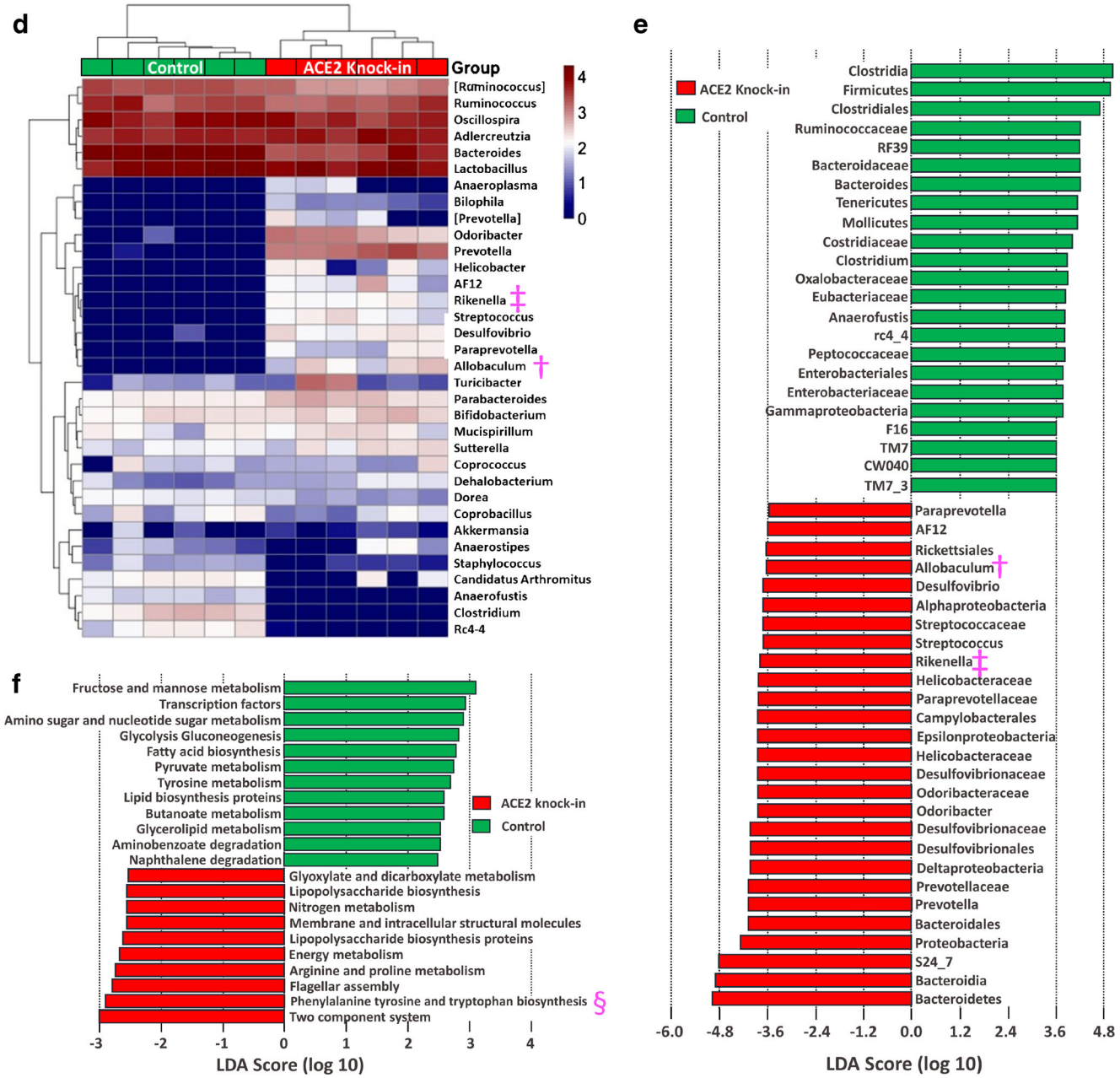

Fig. 1 (continued)

$p = 0.001$, Fig. 1c. One of the pathways suggested to be significantly upregulated in the bacteria present in the GM of ACE2 KI mice compared to littermate controls by PICRUSt analysis [70] is the phenylalanine-tyrosine-tryptophan biosynthetic pathway, (LDA score of 2.8), § in Fig. 1f. This is consistent with the previously described actions of ACE2 on tryptophan uptake in the gut [55]. While tryptophan metabolism was altered in the GM following manipulation of ACE2 gene expression coincident with dysbiosis, the multiple gene pathways and the abundance and diversity of the bacteria modeled as significantly different following ACE2 overexpression suggested that this was not the only cause of the dysbiosis, Fig. 1d, e and f.

ACE2 KI mice, as well as the ACE2 KO mouse, show changes in the abundances of *Allobacullum* and *Rikenella*, Fig. 1d and e; † and ‡ respectively, suggesting that the level of expression of ACE2 can modulate these bacterial populations. The health consequences of changes in abundance of *Rikenella* in the GM are not well researched. Their abundance is decreased by a high-fat diet (HFD) and increased towards the levels seen on a normal diet by the administration of tea with a high-fat diet, coincident with an improvement in health [71]. *Allobaculum* is considered a beneficial bacterium. It is also decreased in mice on a HFD and increased in mice fed prebiotics, that improved health, in association with a HFD

[72]; treatment with berberine prevents obesity and insulin resistance (both associated with metabolic HTN) in rats on a HFD, while also increasing *Allobacullum* abundance [73]. However, *Allobaculum* is a tryptophan auxotrophe, so its increase in ACE2 KI mice GM was unexpected. Further metabolic studies are needed to resolve this conundrum. Nonetheless, these data suggest that gut ACE2 is involved in regulation of the GM in a way that could interact with HTN, as obesity and insulin resistance are risk factors for metabolic HTN. The physiological significance of this regulation remains to be investigated. However, initial evidence suggests the GM-ACE2 interaction to be important; reduced blood pressure responses to hypertensive stimuli (unpublished data) and the anxiolysis in ACE2 KI mice supports this view.

Conclusions

Accumulating evidence suggests the gut and its microbiota are likely important players in control of blood pressure, joining the team that includes autonomic activity, immune activation, and neuroinflammation. The gut and its GM interface with these components in animal models of hypertension, and there are emerging suggestions that similar interfaces occur in human HTN. But what is missing for us to be able to translate this new knowledge into better therapy for HTN? To what extent is gut pathology involved in human hypertension? A better understanding of the state of the gut barrier in HTN would be very helpful, as recently suggested [74]. For example, could bacteria, viruses or fungi escape into the systemic circulation to trigger immune responses to precipitate HTN? Might there be a HTN signature of bacterial, fungal or viral DNA expressed in the serum of hypertensive patients that could be used to predict and treat hypertension? Could the dysbiosis of HTN create a unique profile of metabolites in the blood that could be similarly used? Can GM diversity between individuals result in different metabolism of antihypertensive drugs, such that effective or resistant drug responses are driven by differences in GM composition [68•]. If drug therapy could be tailored individually based on understanding of how the GM will metabolize various classes of antihypertensive agents, significant advances in treating HTN might be achieved. What is the interplay of miRNAs in the communication between the host and the microbiome in HTN [75]. MiRNAs play a role in HTN [76], have recently been shown to be key players in controlling glial activation in diseases such as multiple sclerosis and autoimmune encephalitis [77], and thus may have an important role in mediating HTN. Yet we have no information about miRNAs of the gut and HTN. And finally, would the disparities associated with HTN in African Americans be reflected in these measures, resulting in greater ability to treat the disease and mitigate the damage it does? There are many avenues and treatment opportunities to research in the gut-HTN interaction, it will take guts and intestinal fortitude to fully explore and exploit these potentials.

Compliance with Ethical Standards

Conflict of Interest Drs. Richards, Pepine, Raizada, and Kim declare no conflicts of interest relevant to this manuscript.

Human and Animal Rights and Informed Consent The animal studies reported here were performed with protocols approved by the Institutional Animal Care and Use Committee at the University of Florida that conforms to nationally accepted standards for animal experimentation.

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- • Of importance
- •• Of major importance

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