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Tumor necrosis factor alpha and the gastrointestinal epithelium: Implications for the gut-brain axis and hypertension

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Abstract

The colonic epithelium is the site of production and transport of many vasoactive metabolites and neurotransmitters that can modulate the immune system, affect cellular metabolism, and subsequently regulate blood pressure. As an important interface between the microbiome and its host, the colon can contribute to the development of hypertension. In this critical review, we highlight the role of colonic inflammation and microbial metabolites on the gut brain axis in the pathology of hypertension, with special emphasis on the interaction between tumor necrosis factor α (TNFα) and short chain fatty acid (SCFA) metabolites. Here, we review the current literature and identify novel pathways in the colonic epithelium related to hypertension. A network analysis on transcriptome data previously generated in spontaneously hypertensive rats (SHR) and Wistar-Kyoto (WKY) rats reveals differences in several pathways associated with inflammation involving TNFα (TNF −> NF-κB Expression Targets; TNF −> STAT Expression Targets) as well as oxidative stress. We also identify down-regulation of networks associated with gastrointestinal function, cardiovascular function, enteric nervous system function, and cholinergic and adrenergic transmission. In addition, the analysis uncovered transcriptome responses related to glycolysis, butyrate oxidation, and mitochondrial function, in addition to gut neuropeptides that serve as modulators of blood pressure and metabolic function. We present a model for the role of TNFα in regulating bacterial metabolite transport and neuropeptide signaling in the gastrointestinal system, highlighting the complexity of host-microbiota interactions in hypertension.

Keywords

butyrate; blood pressure; microbiome; gene networks; Inflammation; hypertension

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Declarations

The authors have no conflict of interest to disclose. We declare that this work is original and has not been considered for publication elsewhere. The computational analysis on the published dataset by Yang et al. is a new contribution and has not been published previously. CLS, JZ, and CJM all contributed to writing and editing of the article.

1. Hypertension: A continuing health concern

Hypertension remains one of the most concerning preventable conditions for the global human population. Based upon the updated 2017 definition of hypertension, the American Heart Association estimated that (1) 46% of the total adult population above the age of 20 (2013–2016) had some form of hypertension, (2) that estimates for the cost of treating high blood pressure was $55.9 billion dollars (2014–2015); and (3) that the total mortality attributed to high blood pressure alone was 90,098 (859,125 for cardiovascular disease) in 2017 (Virani et al. 2020). Most concerning is the realization that mortality rates for hypertension have been rising since 2013 (Nambiar et al. 2020), and account for about 9.4 million deaths globally (Vallianou et al. 2020). Moreover, between 2017–2018, estimates revealed that 22.4% of adults aged 18–39, 54.5% of adults 40–59, and 74.5% of adults over 60 presented with hypertension (Ostchega et al. 2020). This earlier onset of hypertension is especially troubling since it has been associated with higher risk of cardiovascular disease (CVD) and premature mortality (Wang et al. 2020).

The underlying causes of hypertension are many and include both genetic and environmental factors such as diet, lack of activity, pollution, drug abuse, smoking, in addition to other factors (Virani et al. 2020). In terms of underlying mechanism, hypertension is often associated with inflammation, oxidative stress, increased sympathetic activity, sodium retention, renin-angiotensin-aldosterone system (RAAS) over-activation, and endothelial cell dysfunction (Manrique et al. 2009). Due to these pro-hypertensive factors, many comorbidities such as metabolic syndrome, diabetes mellitus, dyslipidemia, stroke, congestive heart failure, chronic kidney disease, dementia, coronary heart disease, as well as auto-immune dysfunctions and gut dysbiosis occur in those patients with severe hypertension (Yang et al. 2015; Ventura andand Lavie, 2016; Wolf andand Ryan 2019; Virani et al. 2020). It is clear that hypertension exhibits complex etiology and is influenced by multiple factors, highlighting the need for continued efforts to develop new therapeutic interventions and treatment strategies. Here we conduct a comprehensive review to determine the role of TNF alpha in the gastrointestinal system and its relation to hypertension. To do this, our Google Scholar search terms included TNF-α, Colon, Colonocyte, Differentiation, Inflammation, Blood pressure, Hypertension, Vasodilation, Vasoconstriction, Gut permeability, Dysbiosis, Microbiome, Fermentation, Metabolism, Short Chain Fatty Acid, Branched SCFA, Transporter, Formate, Acetate, Propionate, Butyrate, Valerate, Isovalerate, Isobutyrate, 2-methylbutyrate, Concentration in Serum, and HDAC inhibition. The search was conducted between August, 2019 and July, 2020.

2. Inflammation is a hallmark of hypertension: A role for TNFα signaling

Hypertension is closely associated with a low grade chronic inflammatory response both in the periphery and within the central nervous system (CNS). Systemically, activation of innate immune system has been demonstrated in human hypertension and rodent models of hypertension (Barbaro andand Harrison 2019; Guzik et al. 2007; Kim et al. 2015), while activation of CNS glial cells and infiltration of peripheral immune cells in cardio-regulatory brain regions has been described (Santisteban et al. 2015; Shi et al. 2010; Stern et al. 2016). In addition, elevated serum and tissue levels of pro-inflammatory cytokines such

as interleukins 1 and 6 (IL1 and IL6), and TNFα are reported in human and rodent hypertension studies (Segiet et al. 2019; Sriramula et al. 2008; Tanase et al. 2019; Mehaffey and Majid 2017; Navarro-Gonzalez et al. 2010). TNFα is a proinflammatory cytokine involved in acute phase response to tissue damage or infection (Gruys et al. 2005). The role of TNFα signaling is complex and cell type- and dose-dependent, regulating apoptosis, proliferation, cell survival, or differentiation (Aggarwal 2003), in addition to regulating the secretion of many secondary inflammatory cytokines. In humans, severity of resistant hypertension and an increased 6-year mortality odds ratio have both been linked to significantly higher levels of TNFα among other cytokines (Barbaro andand Harrison 2019), while TNFα inhibitors have been shown to lower blood pressure in both hypertensive humans and experimental models (Guzik et al. 2007; Yoshida et al. 2014). In salt-sensitive rodents, TNFα has also been shown to precede changes in blood pressure and was accompanied by reduced soluble tumor necrosis factor receptor 1 (TNFR1), which functions as a TNFα inhibitor, as well as elevated intracellular TNFR1 in hypertension-prone compared to hypertension-resistant rodents (Mazor et al. 2010). TNFα is produced by many different cells in the body; however, the main producers are monocyte/macrophages and other immune cells such as Natural Killer, T and B cells (Aggarwal, 2003). Once released from macrophages, TNFα can activate other immune cells (Gamble et al. 1985; Maini et al. 1995), as well as directly act on its receptors present in various tissues and cell types including those localized to the gastrointestinal (GI) system (Watson and Hughes, 2012). Considering the prominent role of monocytes and T and B cells in immune responses in hypertension including that observed in the GI tract (Kim et al. 2015; Santisteban et al. 2017), the evidence that elevated levels of TNFα are present in human and rodent hypertension (Navarro-Gonzalez et al. 2010; Yoshida et al. 2014; Yang et al. 2020; Silveira-Nunes et al. 2020), and data showing hypertension and gut microbiota can be modified with dietary interventions (Mazor et al. 2010), it becomes apparent there is a compelling rationale for further research into the role of TNFα in hypertension, with particular emphasis on the GI tract.

3. Inflammation, TNFα and the gastrointestinal microbiome in hypertension

The intersection between inflammation, diet, and microbiome in the onset of hypertension has received significant attention in recent years, with numerous reports linking gut dysbiosis to hypertension (e.g. Jose and Raj, 2015; Vallianou et al. 2020; Yang et al. 2015; Mell et al. 2015; Silveira-Nunes et al. 2020). Gut barrier function, microbial composition, and immune cell function are all important determinants of cardiovascular health. Animal models of hypertension are associated with gut inflammation, dysbiosis, and increased gut permeability (Richards et al. 2017, Santisteban et al. 2017), and 16S bacterial sequencing of human hypertensive microbiota and metabolomics analysis of human hypertensive plasma suggest gut dysbiosis and elevated circulating markers of gut permeability (Kim et al. 2018; Cheema and Pluznick, 2019; Karbach et al. 2016; Calderón-Pérez et al. 2020). Pathological levels of TNFα can increase cell shedding in the gut, leading to micro-erosions of multiple gut cells that negatively impact the epithelial barrier (Watson andand Hughes 2012), thus suggesting involvement of TNFα in gut permeability. Indeed, TNFα is critical for

maintenance of intestinal homeostasis, and a fine balance between epithelial cell death and survival depends on the levels of TNFα and crosstalk between the TNFα receptors 1 and 2 (Ruder et al. 2019). Traditionally, microbiota is considered a crucial modulator of intestinal immune responses throughout one's lifetime, and it involves TNFα signaling (Liebisch et al. 2019; Segain et al. 2000). Conversely, modulation of TNFα signaling can affect the gut microbiota composition (Busquets et al. 2015; Rooks et al. 2014), suggesting that reciprocal host-microbiota interactions can be modulated via TNFα signaling in the gut. Recently, our group demonstrated that reduced systemic and GI inflammation and decreased expression of TNFα signaling molecules such as membrane-spanning 4-domains subfamily A member 4B (Ms4a4b) was associated with beneficial shifts in the gut microbiota composition and reduced blood pressure (Yang et al. 2017; Ahmari et al. 2016). On the other hand, increased GI TNFα in rodent models of hypertension is associated with gut dysbiosis (Yang et al. 2020) and hypertensive individuals show increased TNFα to interferon γ (IFNγ) ratio and reduced fecal microbial diversity (Silveira-Nunes et al. 2020). Thus, TNFα may be an important mediator between host and microbiota in hypertension and related disorders characterized by inflammation and gut dysbiosis.

4. Microbial short chain fatty acids in regulation of inflammation and blood pressure in hypertension

Microbial enzymes in the gastrointestinal tract are critical moderators of human disease via the production of many vasoactive and immunomodulatory metabolites, including SCFAs. These microbial metabolites can modulate inflammation and gene expression (Venegas et al. 2019; Vinolo et al. 2011). For example, consumption of dietary fiber and supplementation with SCFAs such as acetate, butyrate, propionate, and valerate, or ample availability of their microbial producers in the GI tract have been shown to negatively associate with a variety of inflammatory conditions, including ulcerative colitis (Machiels et al. 2014), asthma (Arrieta et al. 2015), colorectal cancer (Chen et al. 2019; Hibberd et al. 2017; Yoon and Kim, 2018), and hypertension (Brown and Hazen, 2018; Chambers et al. 2018; Yang et al. 2019; Chang et al. 2020). On the other hand, elevated formate has been linked to gut dysbiosis and inflammation (Hughes et al. 2017; Jakobsdottir et al. 2013) while the branched chain SCFAS, isobutyrate and isovalerate, have been implicated in hypercholesterolemia (Granado-Serrano et al. 2019). Thus, the relative concentrations and ratios of the different SCFA metabolites may be important factors in the development and progression of many conditions characterized by inflammation, which have also been shown to be responsive targets of prebiotic and probiotic therapies (Ganesh et al. 2018; Robles-Vera et al. 2018; Robles-Vera et al. 2020a; Robles-Vera et al. 2020b; O'Connor et al. 2020). For instance, in an obstructive sleep apnea model of hypertension in which cecal acetate levels were reduced by 48%, hypertension was prevented by the probiotic *Clostridium butyricum* and prebiotic Hylon VII (both of which increase acetate and butyrate production) or by cecal acetate infusion (Ganesh et al. 2018). Another group has shown that acetate and butyrate supplementation, or some of their probiotic producers (*Bifidobacterium breve* CECT7263 or *Lactobacillus fermentum* CECT5716), can lower blood pressure in a variety of hypertensive models, including the SHR and Angiotensin II (Ang II) infusion, but not a chronic nitric oxide synthase inhibition model of hypertension despite improvements of many endpoints

including reduced dysbiosis, inflammation, and oxidative stress (Robles-Vera et al. 2018; Robles-Vera et al. 2020a; Robles-Vera et al. 2020b).

SCFAs also exert direct effects on blood pressure (for review see Poll et al. 2020) either as vasodilators or vasoconstrictors. The effects of the smallest SCFA, formate (1C), on blood pressure is complicated by its role in chloride exchange in the kidneys. Administration of sodium formate at millimolar concentrations has produced vasoconstriction in canine basilar arteries (DeFelice et al. 1976). On the other hand, acetate (2C), propionate (3C), and butyrate (4C) are physiologically the most abundant SCFAs and exert well described anti-hypertensive effects (Bartolomaeus et al. 2019; Natarajan et al. 2016; Yang et al. 2019). Acetate and butyrate also have direct CNS effects (Soliman et al. 2012; Yang et al. 2019). Valerate (5C) is present in lower concentrations in the colon and has been less studied, however it appears to be at equivalent levels in the serum as propionate and butyrate (Jakobsdottir et al. 2013), and may favorably influence gut health and prevent certain types of hypertension (Chang et al. 2020; Onyszkiewicz et al. 2020; Li et al. 2020). For instance, intracolonic infusion of sodium valerate has been recently shown to lower blood pressure and heart rate in rats via a GPR41/43 dependent mechanism (Onyszkiewicz et al. 2020). However, increased plasma valerate has also been associated with preexisting cardiovascular disease in patients with chronic kidney disease (Jadoon et al. 2018). Due to these somewhat conflicting results, further study of the mechanisms of SCFA effects in hypertension is warranted.

5. Interaction between TNFα and SCFAs in the gut

Under physiological conditions, SCFAs can affect TNFα secretion and signaling, and conversely, TNFα has been shown to modulate the SCFA uptake in the gut. For example, a study in cardiomyocytes showed that histone deacetylase (HDAC) 3, a target for some SCFAs, mediates secretion of TNFα following lipopolysaccharide stimulation (Zhu et al. 2010), while mice treated with the HDAC6 specific inhibitor, tubastatin A, had lower TNFα levels than those without the inhibitor (Magupalli et al. 2020).

The extent to which TNFα secretion relies on HDAC-dependent mechanisms is not fully elucidated. However, the role of HDAC in TNFα signaling directly links inflammation to gut health, as many SCFAs, and particularly butyrate, are known HDAC inhibitors. Indeed, butyrate and other HDAC inhibitors have been shown by multiple groups to reduce inflammation in many organ systems. For a comprehensive review into the roles of SCFAs in inflammation, the reader is directed to Vinolo et. al. (2011) and Venegas et al. (2019). There are many examples of reciprocal regulation between butyrate and TNFα. Butyrate, for example, has been demonstrated to lower macrophage secretion of TNFα and other pro-inflammatory cytokines, while also increasing anti-inflammatory IL-10 secretion (Vinolo et al. 2011). Butyrate has also been shown to decrease TNFα production in intestinal biopsies from Crohn's Disease patients through inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) signaling (Segain et al. 2000). In an experimental colitis model, mice with a knockout to solute carrier family 5 member 8 (SLC5a8) (the high affinity transporter of butyrate and other SCFAs), which were fed low fiber diets, presented with decreased epithelial repair and increased inflammation compared

to mice with functioning SLC5a8 (Sivaprakasam et al. 2019). We have recently shown a link between reduced colonic expression of Slc5a8 and reduced butyrate colonic transport in the SHR (Yang et al. 2019), a model also presenting with high colonic TNFα (Yang et al. 2020). In line with this, TNFα has been shown to influence SCFA uptake in the colon. For example, TNFα treatment decreased both the SLC5a8 mRNA expression levels and butyrate uptake transport in intestinal epithelial cell (IEC) 6 cells, while pretreatment with *Lactobacilli plantarum* exerted a positive effect on butyrate uptake and SLC5a8 expression (Borthakur et al. 2010). Thus, an important interaction between the SCFAs, TNFα and the gut epithelium may exist in hypertension. Figure 1a summarizes probable molecular interactions between gut epithelial SCFAs transporters and inflammatory cytokines (notably TNFα) as well as various cellular proteins. These were generated using Pathway Studio as previously described (Cao et al. 2019) and are based on gene and protein expression and their close relationships within the interactome developed from an extensive review of the current literature. As illustrated in Figure 1b, TNFα can regulate the expression of solute carrier family 16 member 1 (SLC16a1), a low affinity SCFA transporter, which is the predominant conveyor of butyrate and several other SCFAs under physiological conditions (Sivaprakasam et al. 2018) as well as SLC5a8, the high affinity SCFA transporter, which increases SCFA transport when concentrations are low. During pathological processes, such as lower SCFA production, the transport of butyrate and other SCFAs falls on Slc5a8 (Sivaprakasam et al. 2019), thus highlighting its importance in conditions such as hypertension where its expression is reduced while TNFα is high.

In addition to reducing TNFα secretion, SCFAs may also modulate downstream TNFα signaling and conversely, TNFα can subsequently influence the metabolism of SCFAs (i.e. butyrate, propionate) (Figure 1). We present in the figure those downstream proteins most likely involved in TNFα-mediated regulation of SCFAs, such as hypoxia-inducible factor 1 (HIF1) and peroxisome proliferator-activated receptors (PPARs). These are but a few examples of proteins that are putative mediators of such a pathway. Butyrate cotreatment can modulate pathways downstream of TNFα through transcription factors, reducing NF-κB signaling and secretion of factor B and IL-8, while increasing Complement component 3 (C3) secretion (Andoh et al. 1999). As indicated in Figure 1a,b, this may occur through specificity protein 1 (SP1), PPARs, or HIF1a signaling. However, these interactions and regulatory relationships remain to be rigorously tested in the gut epithelium.

It is important to note that the effect of butyrate on complex cellular responses is dependent upon the metabolic state of the cell (Ryu et al. 2018) as well as the gut luminal pH (Gálfi and Neogrády, 2001). As such, certain pathological conditions may exacerbate, rather than alleviate, the GI inflammatory responses. For instance, while the effects of butyrate are generally reported as beneficial to barrier function and inflammation, butyrate reportedly increases transepithelial electrical resistance in cells obtained from ulcerative colitis patients (a measure of membrane integrity and permeability) when applied alone, but can lead to a synergistic loss of membrane integrity when in the presence of TNFα and IFNγ, when TNFα and IFNγ applied alone did not significantly increase permeability (Vancamelbeke et al. 2019). Thus, although butyrate and TNFα may exert opposing effects under certain physiological conditions, during pathology they have been shown to determine gut permeability and the inflammatory state. The precise role of butyrate and other

SCFAs and their interaction with TNFα and other inflammatory cytokines in regulation of homeostasis and barrier function of gut epithelial cells remains largely unanswered, especially in the pathophysiology of hypertension.

6. Microbial short chain fatty acids in regulation of epithelial cell homeostasis and cellular metabolism

SCFAs can be produced via multiple pathways, such as the catabolic fermentation of either dietary fiber or amino acids, the anabolic formation from smaller molecules such as pyruvate, lactate, carbon dioxide, or other SCFAs (Gonzalez-Garcia et al. 2017; Morrison and Preston, 2016; Oliphant and Allen-Vercoe, 2019), or during the metabolism of certain xenobiotic compounds such as ethanol (Pardo et al. 2013). Total SCFA concentrations vary by diet and microbial composition, and in physiological conditions, they are the highest in the proximal colon where they range between 70–140 mM, dropping to 20–70 mM in the distal colon (Topping and Clifton, 2001). Acetate, propionate, and butyrate absorption has been assessed in humans undergoing abdominal surgery and per hour account for roughly 34.9 μmol/kg body weight (Bloemen et al. 2009). In physiological conditions, the main function of many SCFAs is as an energy source/fuel for the host epithelial cells, with butyrate generally being the most potent and predominant energy source (Waldecker et al. 2008). SCFAs are metabolized in mitochondria through a β oxidation pathway to Acetyl-CoA (and methylmalonyl-CoA in the case of the odd carbon SCFAs), which then enter the tricarboxylic acid (TCA) cycle which functions to reduce NAD^+ to NADH, culminating in ATP production via oxidative phosphorylation (Donohoe et al. 2011; Halarnkar and Blomquist, 1989). Far from being just an energy source, and as mentioned before, SCFAs are also HDAC inhibitors of varying potency (Waldecker et al. 2008). In line with this, Acetyl-CoA also supplies the acetyl group for histone acetylases (HATs) (Roth et al. 2001). Intriguingly, class III HDAC enzymes have an absolute requirement for NAD^+ to remove the acetyl groups (Licciardi et al. 2011), suggesting that downstream TCA cycle activity might lower this class of HDAC activity in the presence of high SCFA levels. On the other hand, when butyrate and other SCFAs are low in abundance, such as the case in microbiome depleted or germ-free organisms (Hughes et al. 2017; Zarrinpar et al. 2018), the colonocytes exist in an energy-deprived condition, with a decrease in genes involved in the oxidation of butyrate, a lower NADH to NAD^+ ratio, lower ATP levels, and increased AMP-activated protein kinase (AMPK) activation, cyclin-dependent kinase inhibitor 1B (P27KIP1) phosphorylation, and autophagy, which importantly, are rescued with butyrate supplementation (Donohoe et al. 2011). Thus, the presence or absence of specific SCFAs such as butyrate may lead to a switch in the metabolic functions of the colonic epithelial cells.

A shift in colonic butyrate metabolism has been shown in a number of inflammatory conditions of the gut such as colon cancer and ulcerative colitis (Chen et al. 2019; Sivaprakasam et al. 2018). Indeed, colon cancer cells are thought to maintain higher HDAC activity due to a shift from butyrate metabolism to glycolysis (Chen et al. 2019). Several butyrate transporters have been shown to be tumor-suppressor genes and have decreased expression levels in inflamed tissues in ulcerative colitis (Sivaprakasam et al. 2018). In both

these conditions, decreased butyrate transport functionally leads to higher HDAC levels, lower acetylated histones and altered transcription patterns (Sivaprakasam et al. 2018; Chen et al. 2019). Thus, butyrate and other SCFAs represent major fuel source that maintain homeostasis of the gut epithelium, and absence of this fuel may lead to metabolic shifts in the GI tract. As these SCFAs are lower in hypertension, the metabolic function of colonic epithelia in hypertension may be affected.

Butyrate metabolism by enterocytes is also thought to protect intestinal stem cells in the crypt base (Rath et al. 2018). The intestinal epithelium has staggering regenerative abilities, and each crypt villus can shed almost the entire epithelial cell total daily, and a very small proportion of these are intestinal stem cells from which the villi regenerate (Nooteboom et al. 2010; Rath et al. 2018). These stem cells in the crypt base grow and differentiate into either secretory or absorptive lineages as they migrate upward, eventually undergoing senescence and a type of programmed cell death, anoikis, involving shedding into the lumen via tight junction reformation at the villus tip (Rath et al. 2018; Watson and Hughes, 2012). Mitochondrial function is a key determinant of IEC fate during differentiation (for review see Rath et al. 2018) and differentiated cells are also capable of dedifferentiation in response to damage and loss of columnar polarity (Weichselbaum and Klein, 2018).

IECs maintain a highly regulated process of cell division that may be dysregulated via chronic inflammation, including by TNFα (Thoo et al. 2019). Thus, in hypertension, elevated gut TNFα may result in decreased gut renewal and shifts in epithelial cell metabolism, and it is unclear whether butyrate mitigates or compounds this process. Increased luminal butyrate but lower circulating levels have been documented in the SHR (Yang et al. 2019) and lower circulating butyrate has also been shown in hypertensive patients (Calderón-Pérez et al. 2020), suggesting that altered butyrate transport and utilization is a feature of hypertension. However, whether there exists a shift in butyrate metabolism in the hypertensive colon, and what the consequence of that may be to hypertension, remains unknown.

7. Short chain fatty acids: effects on blood pressure

The relative abundance of SCFAs in circulation has been shown to vary considerably depending on diet, genetics, environment, and health condition, as well as the SCFA in question. Formate is physiologically in the range of 10 to 100 μM in the serum of healthy adults but has been reported to reach 300–400 μM in response to various vitamin deficiencies (Brosnan and Brosnan, 2016; Pietzke et al. 2020). Lower millimolar concentrations have been reported clinically in blood as a consequence of methanol poisoning, and at this concentration formate is mitotoxic, directly interfering with mitochondrial respiration (Zakharov et al. 2015). Branched chain amino acids can be catabolized in host tissues via cytosolic or mitochondrial Branched-chain amino acid aminotransferase (BCAT) enzymes (Zhang et al. 2018), though whether this contributes to circulating branched SCFA levels is poorly understood. Of the branched SCFAs isovalerate appears to be the highest in circulation, reported in the 30–40μM range, whereas isobutyrate is lower around 12 μM (Jakobsdottir et al. 2013). Less is known about 2-methylbutyric acid concentrations in circulation, although it has been reported around 220 nM in patients

undergoing hemodialysis (Wu et al. 2019). Acetate is generally viewed as the most abundant SCFA produced in the colon, with human serum levels normally within the 25–200 μM range in healthy adults, with indication that higher serum concentrations are possible (Hosios and Vander Heiden, 2014; Zordoky et al. 2015; Verbeke, 2017) and acetate uptake to the brain appears to be saturated at 2–3 mM in rats (Deelchand et al. 2009). Propionate and butyrate undergo substantial metabolism in the liver and therefore are typically present in the higher nanomolar and lower μM range in the serum of healthy adults (Verbeke, 2017; Jakobsdottir et al. 2013). These concentrations may also be elevated in some pathological conditions such as propionic acidemia, where serum propionate concentrations have been reported in the high μM and low mM range (Thompson et al. 1990; Wolf et al. 1981) and are known to exert toxic effects (Wolf et al. 1978; Wolf et al. 1981). Thus, circulating quantities of SCFAs are quite specific to the SCFA in question but generally span the nM to μM range. Furthermore, they are sensitive to many environmental factors such as the health status and diet of the individual.

Reduced circulating levels of acetate, propionate, butyrate, and valerate in particular have been associated with human and rodent hypertension (Yang et al. 2019; Brown and Hazen, 2018; Huart et al. 2019; Chang et al. 2020). The reduced presence of beneficial SCFAs in circulation may partly be due to reduced absorption of these SCFAs in the proximal colon (Poll et al. 2020; Yang et al. 2019). Currently, it is unclear whether other factors such as increased liver metabolism, excretion by monocarboxylate efflux pumps, or decreased blood flow to the colon could also contribute to lower absorption into circulation. It is likely that the latter is involved to some degree as prolonged inflammation can lead to reduced blood supply (Rath et al. 2018) and low GI perfusion has been reported in the SHR (Santisteban et al. 2017). Butyrate and acetate can also directly affect the CNS cardio-regulatory regions and thereby modulate cardiovascular responses (Soliman et al. 2012; Yang et al. 2019). In addition, many SCFAs have a direct vasodilatory effect (Poll et al. 2020), while also having the ability to modify expression of genes such as Ang II receptors and other inflammatory markers (Yang et al. 2019). Thus, reduction of SCFAs in circulation partly due to reduced intestinal absorption can potentially exacerbate the pro-hypertensive responses.

8. Molecular markers for inflammatory, metabolic, and neuronally active gut peptides in SHR colonic epithelium: implications for hypertension

In previous sections, we outlined some published evidence in support of the TNFα -SCFA interaction in pathophysiology of the gut. To identify additional mechanisms in the gut that might be related to this interaction in hypertension, we utilized a computational method previously published by us (Zubcevic et al. 2017) and reanalyzed a recently published transcriptome data set from gut epithelial cells obtained from SHR and WKY rodents (Yang et al. 2020). In the study, epithelial cells were mechanically removed from adult male WKY and SHR, and differential gene expression analysis was conducted using RNA-seq. We downloaded the available published NCBI GEO dataset (GSE130658) and conducted the gene set enrichment and sub network enrichment analyses, two powerful approaches used to identify pathways and gene networks (Zubcevic et al. 2017). The analysis leverages literature information to build networks and to identify key pathways altered based on

transcriptome data. Our reanalysis of these data identified intriguing new pathways not previously identified which supports our hypothesis of TNFα -SCFA nexus in hypertension. It is important to note that while WKY is considered to be a genetic control for SHR, there are limitations to this model as it has been linked to irritable bowel syndrome (O'Mahony et al. 2013), and thus may differ from other common normotensive control strains such as Wistar-Han or Sprague Dawley rats. Thus, this is one consideration when interpreting the present RNA-seq analysis. Nevertheless, such gene expression comparisons are relative and differences between WKY and SHR are expected to shed light into mechanisms and pathology of hypertension.

Indeed, several metabolic pathways are differentially expressed in the WKY and SHR, including those involved with xenobiotics, pterins, omega-3 fatty acids, estrogens, and pyrimidines (Supplemental Table 1). Within the Pterin family, folate metabolism has been linked to oxidative stress, elevated blood pressure, and insulin resistance in SHR (Pravenec et al. 2013). Folate also has a role in one carbon addition in nucleic and amino acid synthesis (Jamerson et al. 2013). Many genes in the folate cycle are differentially expressed in SHR colonic epithelium, including aminomethyltransferase (*AMT),* dihydrofolate reductase *(DHFR),* methylenetetrahydrofolate dehydrogenase 1 and 1-like *(MTHFD1 and MTHFD1L),* methylenetetrahydrofolate reductase *(MTHFR)*, serine hydroxymethyltransferases 1 and 2 *(SHMT1 and SHMT 2)*, and solute carrier family 19 member 1 *(SLC19a1).* Of note, *MTHFR,* the rate limiting enzyme of folate production, is expressed at higher levels in the SHR colonic epithelium. The vast majority of differentially expressed pyrimidine metabolic pathway genes have lower expression levels in the SHR, including carbamoyl-phosphate synthetase 2 (*CAD),* cytidine/uridine monophosphate kinase 1 *(CMPK1),* dihydroorotate dehydrogenase *(DHODH),* deoxythymidylate kinase *(DTYMK)*, DUTP pyrophosphatase (*DUT),* ectonucleoside triphosphate diphosphohydrolase 1 and 4 *(ENTPD1 andENTPD4),* nucleoside diphosphate kinase A and B *(NME1 and NME2)*, cytosolic 5'-nucleotidase 3 *(NT5C3A),* DNA polymerase alpha catalytic subunit *(POLA1),* DNA polymerase delta catalytic subunit and subunit 2 *(POLD1 and POLD2),* DNA polymerase epsilon catalytic subunit, subunit 2, and accessory subunit *(POLE, POLE2, and POLE4*), DNA-directed RNA polymerase III subunits RPC6, RPC8, and RPC10 *(POLR3F, POLR3H, and POLR3K),* DNA primase small subunit *(PRIM1),* Ribonucleoside-diphosphate reductase large and small subunits *(RRM1 and RRM2),* thymidine kinase 1 *(TK1),* and thymidylate synthase *(TYMS),* whereas guanine deaminase (*GDA)* and DNA polymerase delta subunit 4 *(POLD4)* are seemingly the only differentially expressed genes with higher expression. This is significant as nucleotide metabolism is an important component of both cell proliferation and differentiation in intestinal epithelial cells (Sanderson and He, 1994). Altered nucleic acid metabolism, the process by which DNA and RNA are synthesized and degraded, is a concern in metabolic disturbances, genetic mutation, and cancer.

Overall, there was overwhelming data to support a dysregulated immune system in the gut of SHR, specifically various downstream effectors of the TNFR1 signaling pathway (Table 1). In the hypertensive rats, this pathway was overexpressed, and gene expression of pro-apoptotic, pro-necrotic, and pro-inflammatory mediators appears to be higher in SHR rodents, suggesting that TNFα -related inflammation and epithelial damage may

contribute to hypertension in this model. Caspase expression patterns favored the SHR, with Caspases 3, 4, 8, and 9 observed with higher expression levels in SHR, further demonstrating an association between hypertension and TNFα -related inflammation in the gut. These transcriptional responses in the gut epithelium also correspond to oxidative stress and dysfunction, such as apoptosis and oxidative stress (Table 2). Studies have pointed out that oxidative stress and reactive oxygen species (ROS) production underlie the intersection between TNFα, butyrate, and hypertension. For instance, in the Sabra salt-sensitive and resistant models of hypertension, the NADPH oxidase inhibitor, apocynin, exerts a protective effect on blood pressure in salt fed-rodents, while also significantly reducing the TNFα levels (Mazor et al. 2010). Several SCFAs have been shown to affect the production of ROS in rat endothelial cells. For example, when Ang II was co-incubated with acetate and butyrate, but not propionate, ROS levels were reduced in epithelial colonic cells (Robles-Vera et al. 2020b). Furthermore, for butyrate but not acetate, this effect was shown to be GPR41/43 dependent (Robles-Vera et al. 2020b), though, as β-hydroxybutyrate is a substrate of SCL5a8 and SLC16a1, this effect could also be mediated intracellularly.

Evidence from literature implicates that a shift in how the cells utilize SCFAs and particularly butyrate may be a marker of several inflammatory GI disorders. Here, lower butyrate utilization by the SHR gut epithelium may involve key transcription factors. For example, STAT3 and NF-κB are known to be downregulated by butyrate (Chen et al. 2019); however, our analysis shows increased STAT and NF-κB pathway expression in the SHR (Table 1, Figure 2). This may indicate a shift in metabolic capacity within the gut epithelium as a compensatory response to impaired oxidative phosphorylation. Genes involved in SCFA metabolism, acyl-CoA synthetase short-chain family members 1 and 3 *(ACSS1* and *ACSS3)*, are also differentially expressed in SHR colon, underscoring alterations to SCFA utilization. Additionally, several genes involved in glycolysis are differentially expressed, with aldehyde dehydrogenase 3 family member B1 (*ALDH3B1),* enolase 1 *(ENO1),* 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4 *(PFKFB4),* Phosphofructokinase, platelet *(PFKP)* being expressed at higher levels in SHR and Phosphoenolpyruvate carboxykinase 2 (*PCK2),* aldehyde dehydrogenase 1 family member A7 *(ALDH1A7),* lactate dehydrogenase H subunit *(LDHB),* hexokinase-1 *(HK1),* enolase 3 *(ENO3),* aldo-keto reductase family 1 member A1 *(AKR1A1)* being expressed at lower levels. *PFKP a*nd *PFKFB4* code isozymes of Phosphofructokinase 1 and 2 respectively, which are known to be key regulatory steps in pH-sensitive glycolysis (Ui, 1966).

Lastly, our pathway analysis uncovered differentially expressed gene sets in the SHR epithelium that are associated with cardiovascular, GI and nervous system physiology and function, as well as with adrenergic, cholinergic, serotonergic and dopaminergic transmission (Table 3). Of particular importance may be those genes in the SHR epithelium that are related to neuro- and cardio-modulators such as serotonin (5HT), histamine, nesfatin-1, cholecystokinin (CCK), and glucagon-like peptides (GLP) 1 and 2 (Supplemental Table 2), with the last four also having a prominent role in metabolic functions. With regards to 5HT, the transcriptome data shows that the SHR express higher levels of the enteroendocrine cell differentiation marker, regenerating islet-derived protein 4 (*REG4*), aligning with previous reports that enteroendocrine cell numbers in the GI are elevated in hypertension (Niezgoda and Kasacka, 2017), during inflammation and in presence of

high TNFα (Xiao et al. 2019). Enteroendocrine cells are responsible for 90% of total 5HT synthesis from tryptophan in the body, and their function can be modulated by both the host inflammatory responses as well as by microbiota and particularly butyrate (Reigstad et al. 2015). Indeed, we found that several genes involved in tryptophan metabolism were differentially expressed in the SHR epithelium, including a decrease in expression of tryptophan hydroxylase 1 (*TPH1)* and aromatic l-amino acid decarboxylase (*DDC)*, enzymes responsible for 5HT synthesis. This may be significant for hypertension, as 5HT has been implicated regulation of blood pressure and in hypertension (Watts et al. 2012) as well as having an effect on the immune system, partially via its ability to block the inflammatory effects of TNFα through 5HT2A receptors (Pelletier and Siegel, 2009). Indeed, analysis of tryptophan metabolites in circulation of hypertensive patients suggests that these pathways may be important in hypertension (Qi et al. 2017). 5HT can also stimulate its 5HT3a receptors present on the vagal afferents (Li et al. 2000; Browning, 2015), and acute stimulation of 5HT3a receptors by selective agonists reportedly leads to decreased blood pressure (Ferreira et al. 2004; Urzedo-Rodrigues et al. 2011).

In addition, both nesfatin-1 and histamine can regulate blood pressure via systemic and central action (Osaki and Shimizu, 2014; de Almeida et al. 2015; Yamanaka et al. 2017; Güneş et al. 2020). Nucleobindin-2 (*NUCB2)*, the gene coding for nesfatin-1 has been shown to be primarily produced in the gastric mucosa and may be responsible for the secretion of incretins, such as GLP-1 and 2 and glucose dependent insulinotropic polypeptide (GIP), which further control meal-responsive insulin secretion (Ramesh et al. 2015). While the *GIP* gene does not appear in this dataset, pre-proglucagon (*GCG)*,, which in L cells can be proteolytically cleaved to produce GLP-1 and 2, also appears to be downregulated in SHR gut epithelium, underscoring decreased insulin signaling in this model (Suzuki et al. 2018).

TNFα is also known to downregulate Gcg expression via G-protein coupled receptor 120 (Worthington et al. 2018). CCK is additionally expressed at lower levels in SHR, and apart from its known metabolic effects on protein and lipid metabolism, it may play a role in inflammation as it has been shown to promote a T helper 2 and regulatory T cell phenotype *in vitro* (Worthington et al. 2018). CCK and GLP-1 can also modulate the gut-brain neural axis involved in metabolic, digestive and behavioral functions by stimulating specific receptors on the vagal afferents in the gut (Krieger et al. 2016; Davis et al. 2020). Thus, a decrease in production of several gut peptides by the SHR epithelium may be important in pathophysiology of hypertension. Others have shown a potent anti-hypertensive effect of central injections of GLP-1 receptor agonist, which was reportedly mediated via activation of specific catecholaminergic neurons present in the nucleus of the solitary tract (NTS) (Katsurada et al. 2019). The NTS is the primary site of projection of gut vagal afferents and a major cardio-regulatory brain region, and the catecholaminergic neurons in the NTS can both suppress feeding via vagal activation in the gut (Chen et al. 2020) as well as restrain the development of hypertension (Duale et al. 2007). Moreover, while an antihypertensive effect of CCK has previously been reported in the SHR (Koyama et al. 2020), and reduced CCK levels were found in several SHR brain regions including the pituitary (Shulkes et al. 1989), the potential mechanistic link between hypertension, diabetes and obesity that lies within the gut needs to be further explored.

Indeed, diets high in sugars and fats (Areas et al. 1990; Reaven et al. 1990; Cao et al. 2011) can change the gut microbiota and exacerbate the SHR hypertension via several mechanisms of action. Conversely, inflammation can shift the colonic epithelial metabolism which in turn can reportedly modulate the composition of the gut bacteria towards dysbiosis (Litvak et al. 2018). Knowing what we know now regarding the role of microbiome in hypertension and the intricacies of the host-microbiota interactions, studies employing sugar and fat modifications in investigation of hypertension should be revisited. For example, our transcriptome pathway analysis identified differences in expression levels of genetic components of Omega-6-Fatty Acid Metabolism between WKY and SHR (Table 1), and this pathway could be explored as a therapeutic dietary intervention. To date, the role of Omega-6 fatty acids in hypertension is inconclusive. Several studies note that Omega-6 fatty acids do not appear to exert direct effects on blood pressure, but may lead to dyslipidemia and inflammation (Khandelwal et al. 2013). Thus, metabolic shifts in the gut epithelium that may arise due to inflammation and exacerbated by gut dysbiosis may impact production and release of several gut-associated neuropeptides that could contribute to the gut-brain axis dysfunction in hypertension.

9. Conclusions

A significant relationship exists between gut dysbiosis, GI inflammatory processes and hypertension. However, specific molecular mechanisms of these interactions in health and disease remain unknown. There are clearly defined changes in microbial metabolites throughout development of different inflammatory conditions, and a decrease in beneficial microbial metabolites such as SCFAs is a hallmark of hypertension. Here, we present the case for a potential TNFα-SCFA interaction in the hypertensive gut characterized by shifts in the gut epithelial cell metabolism, which may affect the production of metabolically active and neuroactive gut peptides within the gut-brain axis (Figure 3). We uncover specific transcriptome differences underlying hypertensive rodent models, which includes genes also involved in gastric inflammation and cancer (Supplemental Figure 1), and these proteins are proposed to contribute to hypertensive conditions within the gut. The comprehensive review of the literature and extensive pathway analysis reveals novel mechanisms and potential therapeutic targets in gut dysbiosis-associated hypertension.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

1C	1 Carbon
2C	2 Carbon

3C	3 Carbon
4C	4 Carbon
5C	5 Carbon
PFKFB4	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 4
ACSS1	Acyl-CoA synthetase short-chain family member 1
ACSS3	Acyl-CoA synthetase short-chain family member 3
ALDH1A7	Aldehyde dehydrogenase 1 family member A7
ALDH3B1	Aldehyde dehydrogenase 3 family member B1
AKR1A1	Aldo-keto reductase family 1 member A1
AMT	Aminomethyltransferase
AMPK	AMP-activated protein kinase
DDC	Aromatic l-amino acid decarboxylase
BCAT	Branched-chain amino acid aminotransferase
CAD	Carbamoyl-phosphate synthetase 2
CVD	Cardiovascular disease
CNS	Central nervous system
CCK	Cholecystokinin
C3	Complement component 3
P27KIP1	Cyclin-dependent kinase inhibitor 1B
CMPK1	Cytidine/Uridine Monophosphate Kinase 1
NT5C3A	Cytosolic 5’-nucleotidase 3
DTYMK	Deoxythymidylate Kinase
DHFR	Dihydrofolate reductase
DHODH	Dihydroorotate dehydrogenase
POLA1	DNA polymerase alpha catalytic subunit
POLD1	DNA polymerase delta catalytic subunit
POLD2	DNA polymerase delta subunit 2
POLD4	DNA polymerase delta subunit 4
POLE	DNA polymerase epsilon catalytic subunit

POLE2	DNA polymerase epsilon subunit 2
PRIM1	DNA primase small subunit
POLR3K	DNA-directed RNA polymerase III subunit RPC10
POLR3F	DNA-directed RNA polymerase III subunit RPC6
POLR3H	DNA-directed RNA polymerase III subunit RPC8
DUT	DUTP pyrophosphatase
ENTPD1	Ectonucleoside triphosphate diphosphohydrolase 1
ENTPD4	Ectonucleoside triphosphate diphosphohydrolase 4
ENO1	Enolase 1
ENO3	Enolase 3
GI	Gastrointestinal
GLP	Glucagon-like peptide
GIP	Glucose dependent insulinotropic polypeptide
GDA	Guanine deaminase
HK1	Hexokinase-1
HAT	Histone acetylase
HDAC	Histone deacetylase
HIF1	Hypoxia-inducible factor 1
IFNγ	Interferon gamma
IL	Interleukin
IEC	Intestinal epithelial cell
LDHB	Lactate dehydrogenase H subunit
Ms4a4b	Membrane-spanning 4-domains, subfamily A, member 4B
MTHFD1	Methylenetetrahydrofolate dehydrogenase 1
MTHFD1L	Methylenetetrahydrofolate dehydrogenase 1-like
MTHFR	Methylenetetrahydrofolate reductase
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NUCB2	Nucleobindin-2
NME1	Nucleoside diphosphate kinase A

NME2	Nucleoside diphosphate kinase B
NTS	Nucleus of the solitary tract
PPARs	Peroxisome proliferator-activated receptors
PCK2	Phosphoenolpyruvate carboxykinase 2
PFKP	Phosphofructokinase, platelet
POLE4	Polymerase (DNA-directed), epsilon 4, accessory subunit
GCG	Pre-proglucagon
ROS	Reactive oxygen species
REG4	Regenerating islet-derived protein 4
RAAS	Renin-angiotensin-aldosterone system
RRM1	Ribonucleoside-diphosphate reductase large subunit
RRM2	Ribonucleotide reductase small subunit
SHMT1	Serine hydroxymethyltransferase 1
SHMT2	Serine Hydroxymethyltransferase 2
5HT	Serotonin
SCFA	Short chain fatty acid
SLC16A1	Solute Carrier Family 16 Member 1
SLC19A1	Solute Carrier Family 19 Member 1
SLC5A8	Solute Carrier Family 5 Member 8
SP1	specificity protein 1
SHR	Spontaneously hypertensive rats
TK1	Thymidine kinase 1
TYMS	Thymidylate synthase
TCA	Tricarboxylic acid
TPH1	Tryptophan hydroxylase 1
TNFR1	Tumor necrosis factor receptor 1
TNFα	Tumor necrosis factor α
WKY	Wistar-Kyoto

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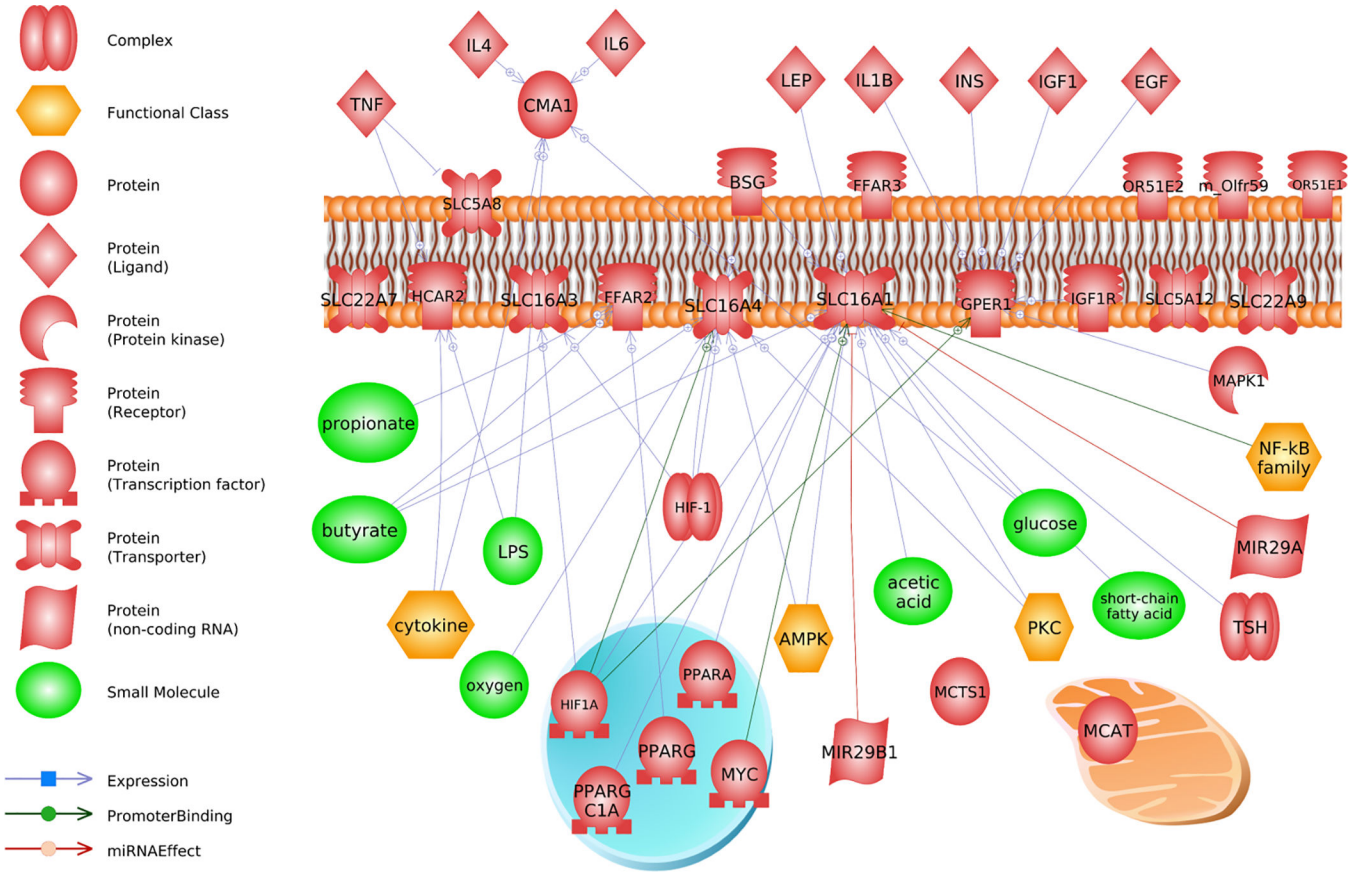

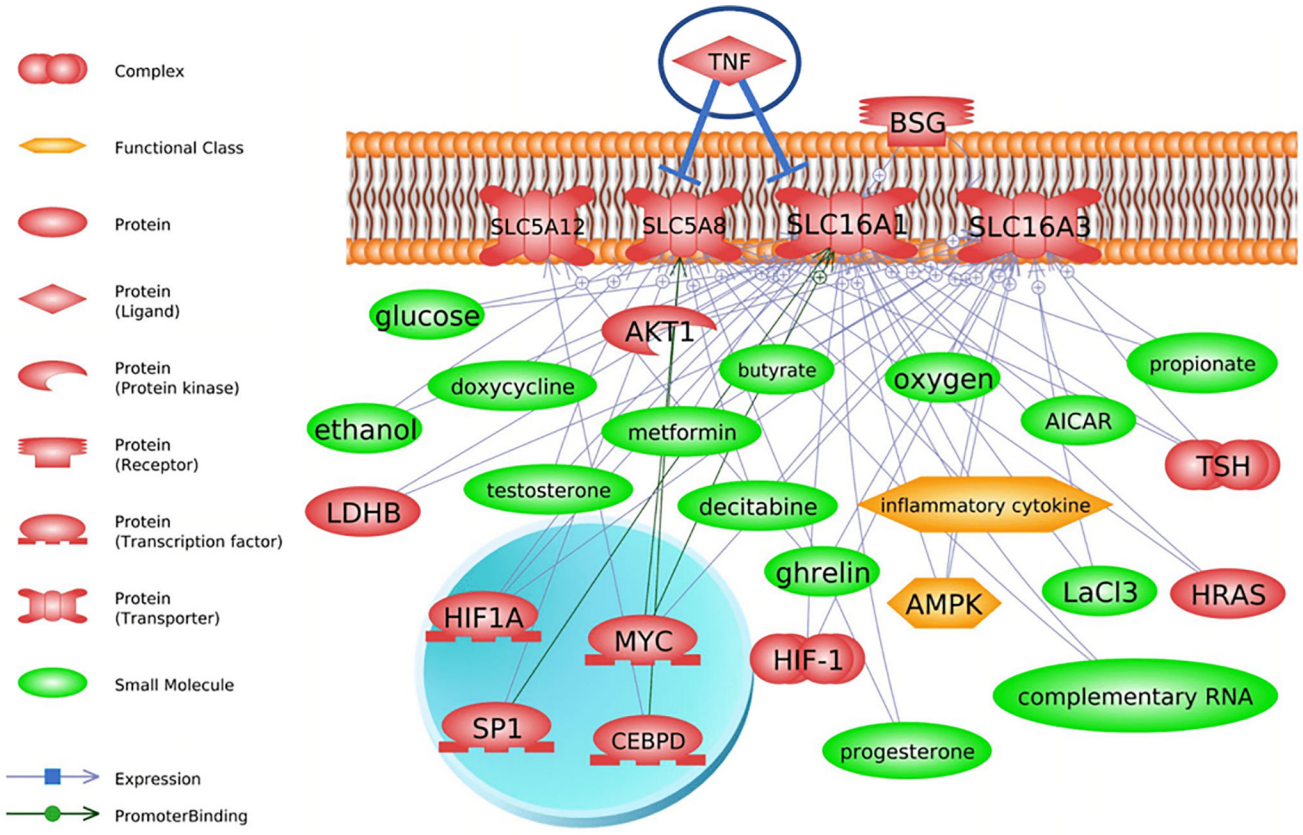

Fig. 1.

Literature connections established in Pathway Studio (Elsevier) between TNF-, SLC transporter transcriptional regulators, and downstream metabolites and hormones. The top graph (a) shows that TNF interacts at the level of the gene or protein with solute carriers on the plasma membrane. Cytokines also regulate these transporters which can affect production and synthesis of various short chain fatty acids (i.e. butyrate and propionate). There is direct evidence that TNF exerts regulatory control over SLC5A8 (bottom graph, b). This in turn can affect several small molecules, such as sex steroids and short chain fatty acids. Abbreviations are found is Supplemental Data 1

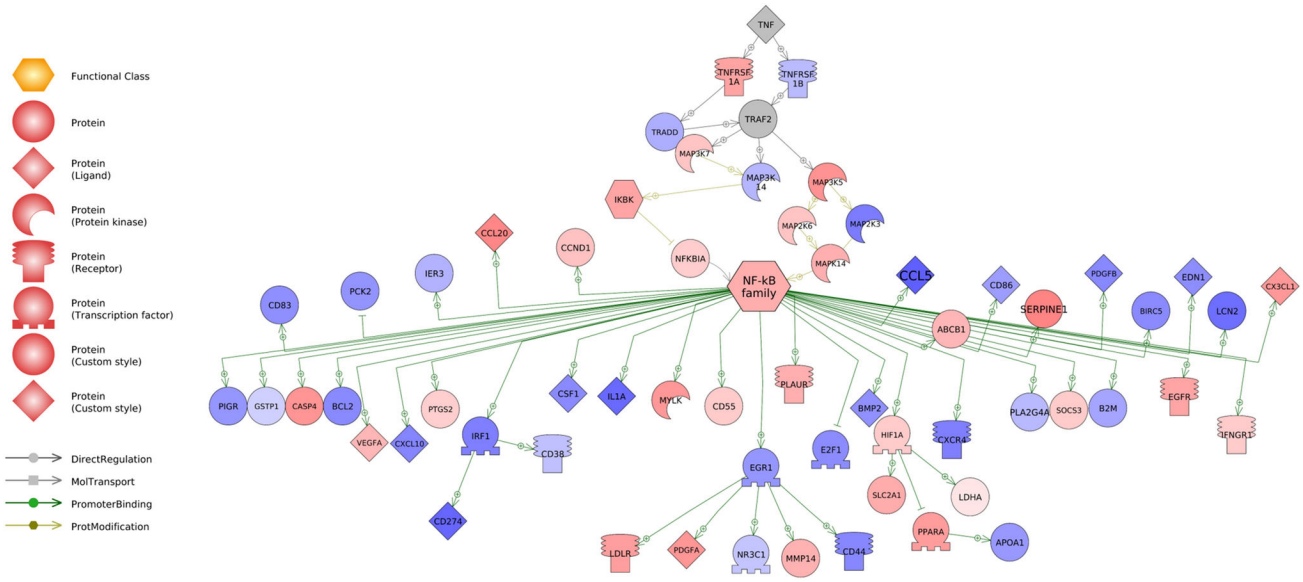

Fig. 2.

Relative expression levels of downstream targets of TNFα and NF-κB signaling molecules in the colonic epithelium of WKY and SHR rodents. Red corresponds to genes that are upregulated in SHR, while blue corresponds to genes with higher expression in WKY. Shade denotes degree the transcript is differentially expressed, with darker shades corresponding to higher level of differential expression. Grey denotes genes whose transcription levels were not assessed in this dataset. Transcriptome data for gut epithelial cells were obtained from Yang et al. 2020. Abbreviations are found is Supplemental Data 1

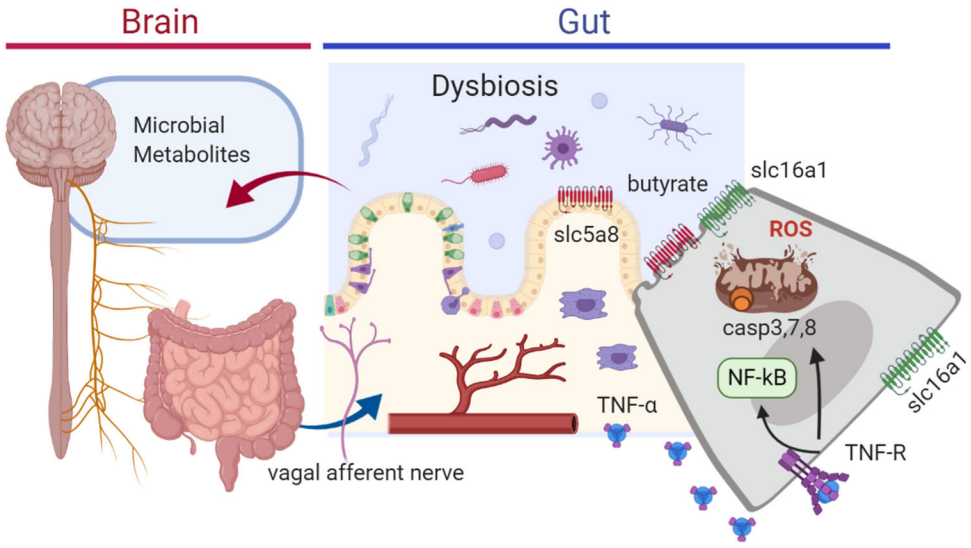

Fig. 3.

Conceptual model for the role of TNF-α in gastrointestinal dysbiosis and impacts on the gut-brain axis. Inflammation in the gut results in mitochondrial dysfunction and altered oxidative respiration, leading to oxidative stress and caspase-induced apoptosis. This in turn can impair butyrate uptake from the gastrointestinal tract, altering the expression of solute transporters. Gut dysbiosis can result in altered short chain fatty acids (e.g. butyrate, propionate) and neurotransmitter synthesis (serotonin, ghrelin, cholecystokinin and somatostatin) in gut epithelium. These mechanisms are proproposed to contribute to a hypertensive state

Table 1.

Subnetwork enrichment analysis for metabolic processes that differ significantly between WKY and SHR. These pathways are those that include TNF or TNF receptor signaling. Provided in the table is the name of the metabolic pathway, a description of the pathway, the number of entities measured in the pathway, the median fold change, normalized enrichment score, and the P value. Transcriptome data for gut epithelial cells were obtained from Yang et al. 2020

Name	Pathway Description	# of Entities	Expanded # of Entities	# of Measured Entities	Median change	NormalizedScore	p-value
IFNAl/lFNR Expression Targets	Interferons Expression Biomarkers; Interferons Expression Biomarkers (Cytokines Expression Biomarkers)	40	49	22	1.05	1.5	0.04
IL2 Expression Targets	Interleukins Expression Biomarkers; Interleukins Expression Biomarkers (Cytokines Expression Biomarkers)	97	138	54	−1.04	1.72	0
IL13 Expression Targets	Interleukins Expression Biomarkers; Interleukins Expression Biomarkers (Cytokines Expression Biomarkers)	66	73	30	−1.03	1.65	0.01
IL15 Expression Targets	Interleukins Expression Biomarkers; Interleukins Expression Biomarkers (Cytokines Expression Biomarkers)	69	85	35	1.02	1.49	0.05
Interleukin Receptors -> Expression Targets in Lymphoid System and Blood	Lymphoid System and Blood; Lymphoid System and Blood (Receptor Signaling with Elevated Expression in Anatomy); blood; hematological System; lymphatic System	60	88	25	−1.04	1.79	0
CD40 -> Expression Targets in Lymph Node	Lymphoid System and Blood; Lymphoid System and Blood (Receptor Signaling with Elevated Expression in Anatomy); lymphatic System	97	109	37	−1.12	1.69	0.01
TCR->NF-kB Expression Targets	T-cell Receptors Expression Biomarkers; T-cell Receptors Expression Biomarkers (CD Molecules Expression Biomarkers)	62	73	31	−1.25	1.73	0
TCR -> STAT Expression Targets	T-cell Receptors Expression Biomarkers; T-cell Receptors Expression Biomarkers (CD Molecules Expression Biomarkers)	41	49	24	−1.03	1.54	0.03
CD40LG -> STAT Expression Targets	TNF Receptor Superfamily Expression Biomarkers; TNF Receptor Superfamily Expression Biomarkers (Expression Biomarkers: Receptor-specific)	45	61	24	−1.11	1.78	0
CD40LG -> NF-kB /ELK/SRF -> CREB/NFATC	TNF Receptor Superfamily Expression Biomarkers; TNF Receptor Superfamily Expression Biomarkers	88	101	52	1.03	1.64	0.01

Name	Pathway Description	# of Entities	Expanded # of Entities	# of Measured Entities	Median change	NormalizedScore	p-value
Expression Targets	(Expression Biomarkers: Receptor-specific)						
CD72 -> AP-1 Expression Targets	TNF Receptor Superfamily Expression Biomarkers; TNF Receptor Superfamily Expression Biomarkers (Expression Biomarkers: Receptor-specific)	64	77	39	−1.11	1.67	0.01
TNF -> NF-kB Expression Targets	TNF Receptor Superfamily Expression Biomarkers; TNF Receptor Superfamily Expression Biomarkers (Expression Biomarkers: Receptor-specific)	127	132	61	−1.06	1.54	0.02
TNF->STAT Expression Targets	TNF Receptor Superfamily Expression Biomarkers; TNF Receptor Superfamily Expression Biomarkers (Expression Biomarkers: Receptor-specific)	83	98	46	1.03	1.47	0.03
TLR1 -> 2/6 Expression Targets	Toll-like Receptors Expression Biomarkers; Toll-like Receptors Expression Biomarkers (CD Molecules Expression Biomarkers)	77	88	39	−1.1	1.55	0.02
TLR4 -> AP- 1/ EGR1/HIF1A Expression Targets	Toll-like Receptors Expression Biomarkers; Toll-like Receptors Expression Biomarkers (CD Molecules Expression Biomarkers)	84	116	47	−1.06	1.48	0.04

Table 2.

Sub-networks related to mitochondria and apoptosis. Provided in the table is the gene set seed, number of entities measured in the pathway, the median fold change, the P-value, and the activation score for the network. Transcriptome data for gut epithelial cells were obtained from Yang et al. 2020

Gene Set Seed	Total # of Neighbors	# of Measured Neighbors	Median change	p-value	Activation Score
apoptosis	9457	4780	−1.04	0.00063	7.07
apoptotic cell clearance	310	157	1.02	0.028	−2.36
cell stress	1226	722	−1.03	0.00085	1.34
extrinsic pathway of apoptosis	221	137	1.01	0.002	−0.24
glucose import	1304	679	−1.02	0.00033	0.19
intestinal D-glucose absorption	74	36	1.05	0.0088	0.38
intrinsic pathway of apoptosis	386	232	−1.01	0.014	1
oxidative stress	2889	1579	−1.04	0.0012	1.06
response to oxidative stress	514	323	−1.02	0.033	0
ROS generation	2188	1170	−1.03	0.00019	0.82
Superoxide anión generation	707	349	−1.03	0.019	0.26

Table 3.

Sub-networks related to the nervous system, cardiovascular function, and the gastrointestinal system. Provided in the table is the gene set seed, number of entities measured in the pathway, the median fold change, the P-value, and the activation score for the network. Transcriptome data for gut epithelial cells were obtained from Yang et al. 2020

Gene Set Seed	Total # of Neighbors	# of Measured Neighbors	Median change	p-value	Activation Score
adrenergic transmission	58	13	−1.05	0.0045	−1.00
cardiovascular deconditioning	200	66	1.05	0.01	−0.45
cardiovascular function	325	123	1.04	0.00038	−0.45
cardiovascular physiology	61	22	1.04	0.036	1.00
central nervous System function	276	104	1.01	0.015	0.00
cholinergic synaptic transmission	125	40	−1.01	0.00066	1.13
dopaminergic activity	99	29	−1.02	0.036	0.58
dopaminergic transmission	140	51	1.04	0.0059	−1.34
enteric nervous System	188	82	−1.07	0.0001	−1.00
gastrointestinal function	120	39	−1.01	0.0078	−1.00
long-term synaptic depression	439	206	1.02	0.02	−1.28
nerve regeneration	498	216	−1.03	2.30E-07	0.17
nervous System physiology	529	218	−1.07	5.90E-09	−1.00
neuromodulation	105	31	−1.09	0.023	unknown
neuron development	370	143	−1.03	0.00034	−1.26
neuron differentiation	316	122	−1.04	0.0043	0.00
neuron sensitivity	90	30	1.05	0.015	0.45
neuronal activity	699	275	1.02	1.40E-06	−1.40
neuronal death	1535	828	−1.03	0.0056	0.00
renin-angiotensin System	257	104	1.05	0.017	−1.51
synaptic plasticity	1226	603	1.02	0.0006	0.17