Indole and Indole-Related Compounds by the Intestinal Microbiota

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The intestinal microbiota metabolic activity towards the available substrates generates myriad bacterial metabolites that may accumulate in the luminal fluid. Indole and indole-related compounds are, first, involved in intestinal microbial community communication, regulating important aspects of bacterial physiology. These molecules are also known to be active on the intestinal mucosa, exerting overall beneficial effects in different experimental situations, notably in inflammatory situations. After absorption, indole is partly metabolized in the liver into the co-metabolite indoxyl sulfate.

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1. The Amino Acid L-tryptophan Is Used by the Intestinal Microbiota to Produce Indole and Indole-Related Compounds That Are Recovered in the Large Intestine Fluid

L-tryptophan is one of the nine indispensable amino acids that must be provided in the diet to meet the body's requirements [1]. L-tryptophan, in addition to its role as a precursor for protein synthesis in host tissues, is also a precursor for numerous compounds with biological activities, including the neurotransmitters serotonin and tryptamine, the hormone melatonin, and the vitamin-like compounds niacin and nicotinic acid [2]. In humans, the tryptophan requirement is 4.0 mg/kg body weight/day. The mean amount of tryptophan provided from all sources is 0.91 g/day, thus representing a usual consumption equal to 13.0 mg/kg body weight/day; thus, this value is well above the requirement [3]. In addition to being used by the different organs and tissues of the host body, a minor part of available tryptophan, approximately 5%, is metabolized by the gut microbes [4]. Exogenous tryptophan used by the gut microbes mainly originates from the undigested, or not fully digested, proteins, which are transferred from the small to the large intestine [5]. In fact, based on a regular Western diet, 6–12 g of proteins and peptides from both dietary and endogenous origin escape digestion in the small intestine, thus reaching the colonic lumen [6][7][8][9]. This quantity of nitrogenous materials can be usefully compared to the dietary protein consumption in Western countries, which averages 85 g per day [10][11].

Bacterial proteases and peptidases release amino acids, including tryptophan, from available dietary and endogenous luminal protein, and since amino acids are little absorbed by the colonic epithelial cells [12], they are mainly used by the large intestine microbes in the course of their metabolic activity.

Intestinal bacteria convert tryptophan mainly into indole through the action of the bacterial enzyme tryptophanase; the latter is induced by tryptophan itself [13]. Of note, in mammals, indole originates exclusively from bacterial metabolic activity since host cells do not have the metabolic capacity for the production of this compound [14]. Indole is synthesized from various Gram-positive and Gram-negative bacterial species, including *Escherichia coli*, *Proteus Vulgaris*, *Clostridium* spp., and *Bacteroides* spp. [14][15][16]. Tryptophan can also be converted directly or indirectly by the intestinal microbiota into several indole-related compounds, including indole-3-pyruvate, indole-3-lactate, indole-3-propionate, indole-3-acetamide, indole-3-acrylate, indole acetaldehyde, indole-3-acetate, indole-3-aldehyde, 3-methyl-indole (skatole), and indole-3-acetaldehyde [4][13][17][18][19][20]. However, the precise metabolic pathways involved in the stepwise conversion of tryptophan into these minor indolic compounds by the intestinal microbiota in the large intestine remain unclear. Regarding the bacterial species involved in their production, indole-3-propionate has been shown to be produced by *Clostridium sporogenes* [21]. Skatole is known to be produced by *Clostridium* spp. and *Bacteroides* spp. [15][22][23]. Other indolic compounds are produced by *Bacteroides* species, *Peptostreptococcus* spp. *Lactobacillus* spp., and *Bifidobacterium* spp. [15][24].

The fecal indole content has been measured in volunteers, but huge inter-individual differences exist between volunteers, ranging from 0.30 to 6.64 millimolar concentrations [25]. Such a large range is presumably due to differences in intestinal microbiota composition between volunteers and to different levels of dietary protein consumption [26]. Incidentally, since indole appears to be absorbed through the colonic epithelium [27], the concentration of indole in the colon is likely higher than that recorded in the feces, with concentrations higher in the distal large intestine than in the more proximal parts [28].

Fecal skatole concentration in healthy individuals averages 0.04 mM [29], but the concentration in the colonic content is again presumably higher since skatole is absorbed through the gut epithelium [30], and then released in the circulation [31]. In the pig model, skatole colonic concentration averages 0.23 mM [32]. Skatole concentration increases in the large intestine after a high-meat diet, or when luminal fermentation increases as a result of longer intestinal stasis [29]. In rodents, indole-3-propionate is found only in blood recovered from animals with intestinal microbiota, but not in blood originating from germ-free animals [33], thus reinforcing the view that this metabolite originates from the intestinal microbiota's metabolic activity.

2. Indole and Indolic Compounds Are Involved in Intestinal Microbial Community Communication

Among amino acid-derived bacterial metabolites, indole has attracted growing interest since this compound is involved in bacterial physiology and metabolism in relationship with antibiotic resistance, virulence factors, sporulation, and biofilm formation [14][34][35][36]. Indeed, indole is a bacterial signal involved in the communication between bacteria within the same species and between bacteria of different species. It acts as an inter-and intra-cellular signal in bacterial ecosystems. Indole diminishes the related virulence of *L. monocytogenes* by reducing cell motility and aggregation [37]. Indole influences host cell invasion by non-indole-producing species, such as *Salmonella enterica* and *P. aeruginosa*, and the fungal species *Candida albicans* [38] In addition, indole reduces *E. coli* motility [39]. In another study, indole was shown to display bacteriostatic effects on lactic acid bacteria, while

affecting their survival [40]. Of note, indole mitigates cytotoxicity by *Klebsellia spp*. by suppressing toxin production [41].

3. Indole and Indolic Compounds Exert Beneficial Effects on the Intestinal Mucosa

Several indolic compounds, including indole-3-acetate, indole-3-propionate, indole-3-aldehyde, indole-3-acetaldehyde, and indole acrylate, bind to the aryl hydro carbon receptor [42], which is present in different cell types of the host, including cells present in the intestinal mucosa, notably intestinal epithelial cells and immune cells [43]. The binding of indolic compounds to AhR participates in the maintenance of the intestinal mucosa homeostasis by acting on the control of the intestinal epithelium renewal, its barrier function, and the activity of several intestinal immune cell types [44]. AhR can also be activated by dietary compounds [45], but bacterial metabolites appear to play a preponderant role in the AhR activation [46]. Only a few commensal bacteria have been identified as able to produce AhR ligands, such as *Peptostreptococcus russellii* [24] and *Lactobacillus* spp. [47].

Among the indolic compounds, indole-3-propionate, when given by the oral route, exerts beneficial effects on the intestinal barrier function when the latter is experimentally altered in rodent models, such as via radiation injury [48], or rodents fed a high-fat diet [49]. In addition, indole-3-propionate reduces intestinal permeability and inflammation in a rodent model [50]. In such an experimental context, it is of interest to note that circulating indole-3-propionate acid is reduced in patients suffering from ulcerative colitis when compared with their healthy counterparts, whereas an increased level of this bacterial metabolite is associated with remission [51].

In a model of experimental colitis induced in mice, other indolic compounds given by the oral route, namely indole-3-pyruvate, indole-3-aldehyde, and indole-3-ethanol, protect against increased intestinal permeability observed in this model [52]. Indole itself, when given by the oral route, decreases mucosal inflammation and injury in a model of enteropathy in rodents [53]. In mice, microbiota-derived indole-3-aldehyde contributes to aryl hydrocarbon receptor-dependent IL-22 gene transcription, allowing the survival of mixed microbial communities, while providing colonization resistance to the fungal species *C. albicans*, and protecting the mucosa from inflammation [47]. Indole-3-acrylate diminishes in mice intestinal inflammation and upregulates Mucin 2 gene expression [24].

Protective effects of microbiota-derived aryl hydrocarbon receptor agonists on the intestinal mucosa have been suggested by both experimental and clinical data, with presumed consequences for the risk of metabolic syndrome [54]. In this latter study, metabolic syndrome was found to be associated with an impaired capacity of the gut microbiota to produce from tryptophan aryl hydrocarbon agonist receptors; this impaired capacity is paralleled by increased gut permeability and decreased secretion of GLP-1 from enteroendocrine cells [54].

Regarding the effects of indole on intestinal epithelial cells, exposure of human enterocytes to indole, but not to other indole-like compounds, increases the expression of genes involved in the intestinal epithelial barrier function and mucin production [55]. Interestingly, these effects were paralleled by an effect of indole on the expression of different cytokines, with decreased expression of the pro-inflammatory IL-8, and increased expression of the

regulatory cytokine IL-10. However, in this study, the expression of several genes linked to inflammation was also found to be increased [55], making the indole effect on the intestinal epithelial cells more complicated than it appears at first sight.

Moreover, oral administration of an indole-containing capsule to rodents results in an increased expression in colonocytes of genes coding for tight junction proteins between epithelial cells [56]. Following these results, indole was found to increase transepithelial resistance in in vitro experiments using colonocyte monolayers [57], thus reinforcing the view that indole ameliorates the basal barrier function. Thus, indole and several indolic compounds exert beneficial effects on the intestinal mucosa in different situations. Overall, the protective effects of bacterial metabolites derived from tryptophan on intestinal mucosa may contribute to the beneficial effects of tryptophan supplementation observed in animal models of colitis [58][59].

However, surprisingly, indole used at a 2.5 millimolar concentration affects the respiration of colonocytes by diminishing mitochondrial oxygen consumption [57], and thus mitochondrial ATP production. This last effect was accompanied by transient oxidative stress in colonocytes, which was followed by an increase in the expression of antioxidant enzymes, presumably as an adaptive process against the deleterious effect of excessive indole exposure.

Little is known about the effects of skatole on intestinal cells, but high concentrations of skatole induce cell death in human colonocytes [60].

In in vitro experiments performed with immortalized and primary mouse colonic enteroendocrine L cells, indole modulates the secretion of glucagon-like peptide-1 (GLP-1) [61]. Since GLP-1 slows down gastric emptying, stimulates insulin secretion by pancreatic beta cells, and diminishes appetite [62], it would be of major interest to learn in vivo the effects of indole on these physiological parameters.

4. After Absorption, Indole Is Partly Metabolized into Indoxyl Sulfate in the Liver

Indole, after absorption in the portal vein, has been shown to exert some anti-inflammatory effects on liver cells. In a rodent model, indole reduces the production of pro-inflammatory mediators by the liver [63]. In a model of obese mice, indole reduces hepatic damage and the associated inflammatory response [64]. The indolic compound indole-3-acetic acid alleviates, in mice, the high-fat diet-induced hepatotoxicity [65]. This latter bacterial metabolite reduces the expression of fatty acid synthase in hepatocytes [66].

The indole that reaches the liver cells is partly metabolized by several cytochrome (CYP) family enzymes, including, notably, CYP2E1 [67]. Several molecules are produced from indole, with indoxyl sulfate being the main co-metabolite produced [68]. As can be anticipated, subjects who consumed a high-protein diet showed overall greater indoxyl sulfate urinary excretion than those who consumed a low-protein diet [69]. In addition, in a randomized, parallel, double-blind trial in overweight volunteers, protein supplementation provoked an increased

concentration of indoxyl sulfate in urine [70]. CYP enzymes are presumably involved in the conversion of indole to indoxyl, an intermediate in the synthesis of indoxyl sulfate. CYP2E1 represents the major enzymatic isoform responsible for the oxidation of indole to indoxyl [71]. CYP2E1 is detected in the colonic epithelium [72][73]. However, data obtained with human colonocytes of the HT-29 Glc-/+ line, chosen because these cells have retained metabolic features that are characteristic of healthy colonocytes (such as the capacity to oxidize butyrate and acetate [74][75], show that the capacity of these cells to convert indole to indoxyl sulfate is modest but measurable [57], and the vast majority of indoxyl sulfate is presumably produced within the liver.

5. Indoxyl Sulfate Is a Recognized Uremic Toxin That Exerts Deleterious Effects on Tubular Kidney Cells and Accelerates Chronic Kidney Disease

Indoxyl sulfate after production in the liver is released in the peripheral blood and then excreted in urine [76]. Indoxyl sulfate concentrations in the blood can be increased from micromolar concentrations in healthy individuals, up to 1.1 millimolar in severe chronic kidney disease [77]. Indoxyl sulfate belongs to the family of uremic toxins that may accumulate in body fluids leading to the so-called uremic syndrome [78]. Indeed, indoxyl sulfate at excessive concentrations aggravates chronic kidney disease in patients [79]. In healthy individuals, indoxyl sulfate is almost entirely bound to proteins in the blood (approximately 93% of this co-metabolite is in bound form [80]). The main binding protein in the blood is albumin, with two binding sites for indoxyl sulfate [81]. Circulating IS in free form is then efficiently excreted in the urine by proximal tubular cells through basolateral organic anion transporters [82].

However, in patients with chronic kidney disease, only 85% of this co-metabolite is protein-bound [76], and thus a higher part of indoxyl sulfate is in free form. As kidney function declines, indoxyl sulfate total concentration increases in the blood and this elevation contributes to further progression of chronic kidney disease [83]. In a cohort study, it was found that blood indoxyl sulfate concentrations are higher in patients with chronic kidney failure progression than in stabilized patients [84]. Then, indoxyl sulfate concentration in blood has been proposed as an indicator of chronic kidney disease progression in dialyzed patients [85].

In experimental in vitro and in vivo studies, indoxyl sulfate has been shown to have deleterious effects on kidney cells when present in excess. This co-metabolite increases the expression of inflammation-associated genes in cultured proximal renal tubular cells [86]. This effect coincides with a capacity of indoxyl sulfate to increase the net production of reactive oxygen species in the proximal tubular cells [87][88], and oxidative stress in these cells [89]. In addition, this compound reduces the glutathione concentration in renal tubular cells [90]. Taking into account the central role of reduced glutathione in the process of excessive reactive oxygen species disposal [91], it is plausible that a reduced intracellular concentration of glutathione will render renal tubular cells even more vulnerable to oxidative stress. The fact that indoxyl sulfate also reduces the superoxide scavenging activity in the kidneys of normal and uremic rodents [92] contributes to the sensitivity of the kidney cells to oxidative stress. Indeed, superoxide is one of the reactive oxygen species that is deleterious to cells when its intracellular concentration exceeds a threshold value [93]. These effects are of major importance when considering that reactive oxygen species are elevated in renal tubular cells in the process of chronic kidney disease progression [94].

Indoxyl sulfate in excess is involved in renal fibrosis. Briefly, renal fibrosis results notably from excessive accumulation of extracellular matrix after renal insult [95]. Administration of indoxyl sulfate in experimental models of chronic kidney disease leads to glomerular sclerosis and interstitial fibrosis [96]. These effects of indoxyl sulfate can be explained by its capacity to promote the transformation of kidney fibroblasts into matrix-producing phenotype, thus increasing collagen deposition, a process that is linked to interstitial fibrosis [97]. This proposition is reinforced by the fact that indoxyl sulfate increases the expression of genes implicated in kidney fibrosis [98].

References

1. Gietzen, D.W.; Rogers, Q.R. Nutritional homeostasis and indispensable amino acid sensing: A new solution to an old puzzle. Trends Neurosci. 2006, 29, 91–99.
2. Kaluzna-Czaplinska, J.; Gatarek, P.; Chirumbolo, S.; Chartrand, M.S.; Bjorklund, G. How important is tryptophan in human health? Crit. Rev. Food Sci. Nutr. 2019, 59, 72–88.
3. Blachier, F.; Blais, A.; Elango, R.; Saito, K.; Shimomura, Y.; Kadowaki, M.; Matsumoto, H. Tolerable amounts of amino acids for human supplementation: Summary and lessons from published peer-reviewed studies. Amino Acids 2021, 53, 1313–1328.
4. Hyland, N.P.; Cavanaugh, C.R.; Hornby, P.J. Emerging effects of tryptophan pathway metabolites and intestinal microbiota on metabolism and intestinal function. Amino Acids 2022, 54, 57–70.
5. Blachier, F.; Beaumont, M.; Portune, K.J.; Steuer, N.; Lan, A.; Audebert, M.; Khodorova, N.; Andriamihaja, M.; Airinei, G.; Benamouzig, R.; et al. High-protein diets for weight management: Interactions with the intestinal microbiota and consequences for gut health. A position paper by the my new gut study group. Clin. Nutr. 2019, 38, 1012–1022.
6. Gibson, J.A.; Sladen, G.E.; Dawson, A.M. Protein absorption and ammonia production: The effects of dietary protein and removal of the colon. Br. J. Nutr. 1976, 35, 61–65.
7. Smiddy, F.G.; Gregory, S.D.; Smith, I.B.; Goligher, J.C. Faecal loss of fluid, electrolytes, and nitrogen in colitis before and after ileostomy. Lancet 1960, 1, 14–19.
8. Kramer, P. The effect of varying sodium loads on the ileal excreta of human ileostomized subjects. J. Clin. Investig. 1966, 45, 1710–1718.
9. Gaudichon, C.; Bos, C.; Morens, C.; Petzke, K.J.; Mariotti, F.; Everwand, J.; Benamouzig, R.; Dare, S.; Tome, D.; Metges, C.C. Ileal losses of nitrogen and amino acids in humans and their importance to the assessment of amino acid requirements. Gastroenterology 2002, 123, 50–59.
10. Dubuisson, C.; Lioret, S.; Touvier, M.; Dufour, A.; Calamassi-Tran, G.; Volatier, J.L.; Lafay, L. Trends in food and nutritional intakes of French adults from 1999 to 2007: Results from the INCA surveys. Br. J. Nutr. 2010, 103, 1035–1048.

11. Pasiakos, S.M.; Agarwal, S.; Lieberman, H.R.; Fulgoni, V.L., III. Sources and Amounts of Animal, Dairy, and Plant Protein Intake of US Adults in 2007-2010. Nutrients 2015, 7, 7058–7069.
12. van der Wielen, N.; Moughan, P.J.; Mensink, M. Amino Acid Absorption in the Large Intestine of Humans and Porcine Models. J. Nutr. 2017, 147, 1493–1498.
13. Gao, J.; Xu, K.; Liu, H.; Liu, G.; Bai, M.; Peng, C.; Li, T.; Yin, Y. Impact of the Gut Microbiota on Intestinal Immunity Mediated by Tryptophan Metabolism. Front. Cell. Infect. Microbiol. 2018, 8, 13.
14. Lee, J.H.; Wood, T.K.; Lee, J. Roles of indole as an interspecies and interkingdom signaling molecule. Trends Microbiol. 2015, 23, 707–718.
15. Roager, H.M.; Licht, T.R. Microbial tryptophan catabolites in health and disease. Nat. Commun. 2018, 9, 3294.
16. Keszthelyi, D.; Troost, F.J.; Masclee, A.A. Understanding the role of tryptophan and serotonin metabolism in gastrointestinal function. Neurogastroenterol. Motil. Off. J. Eur. Gastrointest. Motil. Soc. 2009, 21, 1239–1249.
17. Dong, F.; Perdew, G.H. The aryl hydrocarbon receptor as a mediator of host-microbiota interplay. Gut Microbes 2020, 12, 1859812.
18. Oliphant, K.; Allen-Vercoe, E. Macronutrient metabolism by the human gut microbiome: Major fermentation by-products and their impact on host health. Microbiome 2019, 7, 91.
19. Taleb, S. Tryptophan Dietary Impacts Gut Barrier and Metabolic Diseases. Front. Immunol. 2019, 10, 2113.
20. Jensen, M.T.; Cox, R.P.; Jensen, B.B. 3-Methylindole (skatole) and indole production by mixed populations of pig fecal bacteria. Appl. Environ. Microbiol. 1995, 61, 3180–3184.
21. Dodd, D.; Spitzer, M.H.; Van Treuren, W.; Merrill, B.D.; Hryckowian, A.J.; Higginbottom, S.K.; Le, A.; Cowan, T.M.; Nolan, G.P.; Fischbach, M.A.; et al. A gut bacterial pathway metabolizes aromatic amino acids into nine circulating metabolites. Nature 2017, 551, 648–652.
22. Cook, K.L.; Rothrock, M.J., III; Loughrin, J.H.; Doerner, K.C. Characterization of skatole-producing microbial populations in enriched swine lagoon slurry. FEMS Microbiol. Ecol. 2007, 60, 329–340.
23. Whitehead, T.R.; Price, N.P.; Drake, H.L.; Cotta, M.A. Catabolic pathway for the production of skatole and indoleacetic acid by the acetogen Clostridium drakei, Clostridium scatologenes, and swine manure. Appl. Environ. Microbiol. 2008, 74, 1950–1953.
24. Wlodarska, M.; Luo, C.; Kolde, R.; d'Hennezel, E.; Annand, J.W.; Heim, C.E.; Krastel, P.; Schmitt, E.K.; Omar, A.S.; Creasey, E.A.; et al. Indoleacrylic Acid Produced by Commensal Peptostreptococcus Species Suppresses Inflammation. Cell Host Microbe 2017, 22, 25–37.e26.

25. Darkoh, C.; Chappell, C.; Gonzales, C.; Okhuysen, P. A rapid and specific method for the detection of indole in complex biological samples. Appl. Environ. Microbiol. 2015, 81, 8093–8097.
26. Leong, S.C.; Sirich, T.L. Indoxyl Sulfate-Review of Toxicity and Therapeutic Strategies. Toxins 2016, 8, 358.
27. Huc, T.; Konop, M.; Onyszkiewicz, M.; Podsadni, P.; Szczepańska, A.; Turło, J.; Ufnal, M. Colonic indole, gut bacteria metabolite of tryptophan, increases portal blood pressure in rats. Am. J. Physiol. Regul. Integr. Comp. Physiol. 2018, 315, R646–R655.
28. Whitt, D.D.; Demoss, R.D. Effect of microflora on the free amino acid distribution in various regions of the mouse gastrointestinal tract. Appl. Microbiol. 1975, 30, 609–615.
29. Yokoyama, M.T.; Carlson, J.R. Microbial metabolites of tryptophan in the intestinal tract with special reference to skatole. Am. J. Clin. Nutr. 1979, 32, 173–178.
30. Witte, F.; Pajic, A.; Menger, F.; Tomasevic, I.; Schubert, D.C.; Visscher, C.; Terjung, N. Preliminary Test of the Reduction Capacity for the Intestinal Adsorption of Skatole and Indole in Weaning Piglets by Pure and Coated Charcoal. Anim. Open Access J. MDPI 2021, 11, 2720.
31. Claus, R.; Raab, S. Influences on skatole formation from tryptophan in the pig colon. Adv. Exp. Med. Biol. 1999, 467, 679–684.
32. Yoshihara, I.; Maruta, K. Gas Chromatographic Microdetermination of Indole and Skatole in Gastrointestinal Contents of Domestic Animals. Agric. Biol. Chem. 1977, 41, 2083–2085.
33. Wikoff, W.R.; Anfora, A.T.; Liu, J.; Schultz, P.G.; Lesley, S.A.; Peters, E.C.; Siuzdak, G. Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. Proc. Natl. Acad. Sci. USA 2009, 106, 3698–3703.
34. Kim, J.; Park, W. Indole: A signaling molecule or a mere metabolic byproduct that alters bacterial physiology at a high concentration? J. Microbiol. 2015, 53, 421–428.
35. Lee, J.-H.; Lee, J. Indole as an intercellular signal in microbial communities. FEMS Microbiol. Rev. 2010, 34, 426–444.
36. Vega, N.M.; Allison, K.R.; Samuels, A.N.; Klempner, M.S.; Collins, J.J. Salmonella typhimurium intercepts Escherichia coli signaling to enhance antibiotic tolerance. Proc. Natl. Acad. Sci. USA 2013, 110, 14420–14425.
37. Rattanaphan, P.; Mittraparp-Arthorn, P.; Srinoun, K.; Vuddhakul, V.; Tansila, N. Indole signaling decreases biofilm formation and related virulence of Listeria monocytogenes. FEMS Microbiol. Lett. 2020, 367, fnaa116.
38. Li, G.; Young, K.D. Indole production by the tryptophanase TnaA in Escherichia coli is determined by the amount of exogenous tryptophan. Microbiology 2013, 159, 402–410.

39. Bansal, T.; Englert, D.; Lee, J.; Hegde, M.; Wood, T.K.; Jayaraman, A. Differential effects of epinephrine, norepinephrine, and indole on Escherichia coli O157:H7 chemotaxis, colonization, and gene expression. Infect. Immun. 2007, 75, 4597–4607.
40. Nowak, A.; Libudzisz, Z. Influence of phenol, p-cresol and indole on growth and survival of intestinal lactic acid bacteria. Anaerobe 2006, 12, 80–84.
41. Ledala, N.; Malik, M.; Rezaul, K.; Paveglio, S.; Provatas, A.; Kiel, A.; Caimano, M.; Zhou, Y.; Lindgren, J.; Krasulova, K.; et al. Bacterial Indole as a Multifunctional Regulator of Klebsiella oxytoca Complex Enterotoxicity. mBio 2022, 13, e03752-21.
42. Agus, A.; Planchais, J.; Sokol, H. Gut Microbiota Regulation of Tryptophan Metabolism in Health and Disease. Cell Host Microbe 2018, 23, 716–724.
43. Pernomian, L.; Duarte-Silva, M.; de Barros Cardoso, C.R. The Aryl Hydrocarbon Receptor (AHR) as a Potential Target for the Control of Intestinal Inflammation: Insights from an Immune and Bacteria Sensor Receptor. Clin. Rev. Allergy Immunol. 2020, 59, 382–390.
44. Hubbard, T.D.; Murray, I.A.; Bisson, W.H.; Lahoti, T.S.; Gowda, K.; Amin, S.G.; Patterson, A.D.; Perdew, G.H. Adaptation of the human aryl hydrocarbon receptor to sense microbiota-derived indoles. Sci. Rep. 2015, 5, 12689.
45. Hubbard, T.D.; Murray, I.A.; Perdew, G.H. Indole and Tryptophan Metabolism: Endogenous and Dietary Routes to Ah Receptor Activation. Drug Metab. Dispos. Biol. Fate Chem. 2015, 43, 1522–1535.
46. Lamas, B.; Richard, M.L.; Leducq, V.; Pham, H.P.; Michel, M.L.; Da Costa, G.; Bridonneau, C.; Jegou, S.; Hoffmann, T.W.; Natividad, J.M.; et al. CARD9 impacts colitis by altering gut microbiota metabolism of tryptophan into aryl hydrocarbon receptor ligands. Nat. Med. 2016, 22, 598–605.
47. Zelante, T.; Iannitti, R.G.; Cunha, C.; De Luca, A.; Giovannini, G.; Pieraccini, G.; Zecchi, R.; D'Angelo, C.; Massi-Benedetti, C.; Fallarino, F.; et al. Tryptophan catabolites from microbiota engage aryl hydrocarbon receptor and balance mucosal reactivity via interleukin-22. Immunity 2013, 39, 372–385.
48. Xiao, H.W.; Cui, M.; Li, Y.; Dong, J.L.; Zhang, S.Q.; Zhu, C.C.; Jiang, M.; Zhu, T.; Wang, B.; Wang, H.C.; et al. Gut microbiota-derived indole 3-propionic acid protects against radiation toxicity via retaining acyl-CoA-binding protein. Microbiome 2020, 8, 69.
49. Jennis, M.; Cavanaugh, C.R.; Leo, G.C.; Mabus, J.R.; Lenhard, J.; Hornby, P.J. Microbiota-derived tryptophan indoles increase after gastric bypass surgery and reduce intestinal permeability in vitro and in vivo. Neurogastroenterol. Motil. Off. J. Eur. Gastrointest. Motil. Soc. 2018, 30, e13178.
50. Venkatesh, M.; Mukherjee, S.; Wang, H.; Li, H.; Sun, K.; Benechet, A.P.; Qiu, Z.; Maher, L.; Redinbo, M.R.; Phillips, R.S.; et al. Symbiotic bacterial metabolites regulate gastrointestinal

barrier function via the xenobiotic sensor PXR and Toll-like receptor 4. Immunity 2014, 41, 296–310.

51. Alexeev, E.E.; Lanis, J.M.; Kao, D.J.; Campbell, E.L.; Kelly, C.J.; Battista, K.D.; Gerich, M.E.; Jenkins, B.R.; Walk, S.T.; Kominsky, D.J.; et al. Microbiota-Derived Indole Metabolites Promote Human and Murine Intestinal Homeostasis through Regulation of Interleukin-10 Receptor. Am. J. Pathol. 2018, 188, 1183–1194.
52. Scott, S.A.; Fu, J.; Chang, P.V. Microbial tryptophan metabolites regulate gut barrier function via the aryl hydrocarbon receptor. Proc. Natl. Acad. Sci. USA 2020, 117, 19376–19387.
53. Whitfield-Cargile, C.M.; Cohen, N.D.; Chapkin, R.S.; Weeks, B.R.; Davidson, L.A.; Goldsby, J.S.; Hunt, C.L.; Steinmeyer, S.H.; Menon, R.; Suchodolski, J.S.; et al. The microbiota-derived metabolite indole decreases mucosal inflammation and injury in a murine model of NSAID enteropathy. Gut Microbes 2016, 7, 246–261.
54. Natividad, J.M.; Agus, A.; Planchais, J.; Lamas, B.; Jarry, A.C.; Martin, R.; Michel, M.L.; Chong-Nguyen, C.; Roussel, R.; Straube, M.; et al. Impaired Aryl Hydrocarbon Receptor Ligand Production by the Gut Microbiota Is a Key Factor in Metabolic Syndrome. Cell Metab. 2018, 28, 737–749.e734.
55. Bansal, T.; Alaniz, R.C.; Wood, T.K.; Jayaraman, A. The bacterial signal indole increases epithelial-cell tight-junction resistance and attenuates indicators of inflammation. Proc. Natl. Acad. Sci. USA 2010, 107, 228–233.
56. Shimada, Y.; Kinoshita, M.; Harada, K.; Mizutani, M.; Masahata, K.; Kayama, H.; Takeda, K. Commensal bacteria-dependent indole production enhances epithelial barrier function in the colon. PLoS ONE 2013, 8, e80604.
57. Armand, L.; Fofana, M.; Couturier-Becavin, K.; Andriamihaja, M.; Blachier, F. Dual effects of the tryptophan-derived bacterial metabolite indole on colonic epithelial cell metabolism and physiology: Comparison with its co-metabolite indoxyl sulfate. Amino Acids 2022.
58. Hashimoto, T.; Perlot, T.; Rehman, A.; Trichereau, J.; Ishiguro, H.; Paolino, M.; Sigl, V.; Hanada, T.; Hanada, R.; Lipinski, S.; et al. ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. Nature 2012, 487, 477–481.
59. Kim, C.J.; Kovacs-Nolan, J.A.; Yang, C.; Archbold, T.; Fan, M.Z.; Mine, Y. l-Tryptophan exhibits therapeutic function in a porcine model of dextran sodium sulfate (DSS)-induced colitis. J. Nutr. Biochem. 2010, 21, 468–475.
60. Kurata, K.; Kawahara, H.; Nishimura, K.; Jisaka, M.; Yokota, K.; Shimizu, H. Skatole regulates intestinal epithelial cellular functions through activating aryl hydrocarbon receptors and p38. Biochem. Biophys. Res. Commun. 2019, 510, 649–655.

61. Chimerel, C.; Emery, E.; Summers, D.K.; Keyser, U.; Gribble, F.M.; Reimann, F. Bacterial metabolite indole modulates incretin secretion from intestinal enteroendocrine L cells. Cell Rep. 2014, 9, 1202–1208.
62. Holst, J.J. The physiology of glucagon-like peptide 1. Physiol. Rev. 2007, 87, 1409–1439.
63. Beaumont, M.; Neyrinck, A.M.; Olivares, M.; Rodriguez, J.; de Rocca Serra, A.; Roumain, M.; Bindels, L.B.; Cani, P.D.; Evenepoel, P.; Muccioli, G.G.; et al. The gut microbiota metabolite indole alleviates liver inflammation in mice. FASEB J. Off. Publ. Fed. Am. Soc. Exp. Biol. 2018, 32, lb359.
64. Knudsen, C.; Neyrinck, A.M.; Leyrolle, Q.; Baldin, P.; Leclercq, S.; Rodriguez, J.; Beaumont, M.; Cani, P.D.; Bindels, L.B.; Lanthier, N.; et al. Hepatoprotective Effects of Indole, a Gut Microbial Metabolite, in Leptin-Deficient Obese Mice. J. Nutr. 2021, 151, 1507–1516.
65. Ji, Y.; Gao, Y.; Chen, H.; Yin, Y.; Zhang, W. Indole-3-Acetic Acid Alleviates Nonalcoholic Fatty Liver Disease in Mice via Attenuation of Hepatic Lipogenesis, and Oxidative and Inflammatory Stress. Nutrients 2019, 11, 2062.
66. Krishnan, S.; Ding, Y.; Saedi, N.; Choi, M.; Sridharan, G.V.; Sherr, D.H.; Yarmush, M.L.; Alaniz, R.C.; Jayaraman, A.; Lee, K. Gut Microbiota-Derived Tryptophan Metabolites Modulate Inflammatory Response in Hepatocytes and Macrophages. Cell Rep. 2018, 23, 1099–1111.
67. Gillam, E.M.; Notley, L.M.; Cai, H.; De Voss, J.J.; Guengerich, F.P. Oxidation of indole by cytochrome P450 enzymes. Biochemistry 2000, 39, 13817–13824.
68. King, L.J.; Parke, D.V.; Williams, R.T. The metabolism of indole in the rat. Biochem. J. 1966, 98, 266–277.
69. Poesen, R.; Mutsaers, H.A.; Windey, K.; van den Broek, P.H.; Verweij, V.; Augustijns, P.; Kuypers, D.; Jansen, J.; Evenepoel, P.; Verbeke, K.; et al. The Influence of Dietary Protein Intake on Mammalian Tryptophan and Phenolic Metabolites. PLoS ONE 2015, 10, e0140820.
70. Beaumont, M.; Portune, K.J.; Steuer, N.; Lan, A.; Cerrudo, V.; Audebert, M.; Dumont, F.; Mancano, G.; Khodorova, N.; Andriamihaja, M.; et al. Quantity and source of dietary protein influence metabolite production by gut microbiota and rectal mucosa gene expression: A randomized, parallel, double-blind trial in overweight humans. Am. J. Clin. Nutr. 2017, 106, 1005–1019.
71. Banoglu, E.; Jha, G.G.; King, R.S. Hepatic microsomal metabolism of indole to indoxyl, a precursor of indoxyl sulfate. Eur. J. Drug Metab. Pharmacokinet. 2001, 26, 235–240.
72. Bulus, H.; Oguztuzun, S.; Guler Simsek, G.; Kilic, M.; Ada, A.O.; Gol, S.; Kocdogan, A.K.; Kaygin, P.; Bozer, B.; Iscan, M. Expression of CYP and GST in human normal and colon tumor tissues. Biotech. Histochem. Off. Publ. Biol. Stain Comm. 2019, 94, 1–9.

73. Rosenberg, D.W.; Mankowski, D.C. Induction of cyp2e-1 protein in mouse colon. Carcinogenesis 1994, 15, 73–78.
74. Andriamihaja, M.; Chaumontet, C.; Tome, D.; Blachier, F. Butyrate metabolism in human colon carcinoma cells: Implications concerning its growth-inhibitory effect. J. Cell. Physiol. 2009, 218, 58–65.
75. Leschelle, X.; Delpal, S.; Goubern, M.; Blottiere, H.M.; Blachier, F. Butyrate metabolism upstream and downstream acetyl-CoA synthesis and growth control of human colon carcinoma cells. Eur. J. Biochem. 2000, 267, 6435–6442.
76. Hobby, G.P.; Karaduta, O.; Dusio, G.F.; Singh, M.; Zybailov, B.L.; Arthur, J.M. Chronic kidney disease and the gut microbiome. Am. J. Physiol.. Ren. Physiol. 2019, 316, F1211–F1217.
77. Vanholder, R.; Schepers, E.; Pletinck, A.; Nagler, E.V.; Glorieux, G. The uremic toxicity of indoxyl sulfate and p-cresyl sulfate: A systematic review. J. Am. Soc. Nephrol. JASN 2014, 25, 1897–1907.
78. Vanholder, R.; Baurmeister, U.; Brunet, P.; Cohen, G.; Glorieux, G.; Jankowski, J.; European Uremic Toxin Work, G. A bench to bedside view of uremic toxins. J. Am. Soc. Nephrol. JASN 2008, 19, 863–870.
79. Cheng, T.H.; Ma, M.C.; Liao, M.T.; Zheng, C.M.; Lu, K.C.; Liao, C.H.; Hou, Y.C.; Liu, W.C.; Lu, C.L. Indoxyl Sulfate, a Tubular Toxin, Contributes to the Development of Chronic Kidney Disease. Toxins 2020, 12, 684.
80. Devine, E.; Krieter, D.H.; Ruth, M.; Jankovski, J.; Lemke, H.D. Binding affinity and capacity for the uremic toxin indoxyl sulfate. Toxins 2014, 6, 416–429.
81. Viaene, L.; Annaert, P.; de Loor, H.; Poesen, R.; Evenepoel, P.; Meijers, B. Albumin is the main plasma binding protein for indoxyl sulfate and p-cresyl sulfate. Biopharm. Drug Dispos. 2013, 34, 165–175.
82. Enomoto, A.; Takeda, M.; Tojo, A.; Sekine, T.; Cha, S.H.; Khamdang, S.; Takayama, F.; Aoyama, I.; Nakamura, S.; Endou, H.; et al. Role of organic anion transporters in the tubular transport of indoxyl sulfate and the induction of its nephrotoxicity. J. Am. Soc. Nephrol. JASN 2002, 13, 1711–1720.
83. Fujii, H.; Goto, S.; Fukagawa, M. Role of Uremic Toxins for Kidney, Cardiovascular, and Bone Dysfunction. Toxins 2018, 10, 202.
84. Wang, W.; Hao, G.; Pan, Y.; Ma, S.; Yang, T.; Shi, P.; Zhu, Q.; Xie, Y.; Ma, S.; Zhang, Q.; et al. Serum indoxyl sulfate is associated with mortality in hospital-acquired acute kidney injury: A prospective cohort study. BMC Nephrol. 2019, 20, 57.

85. Tan, X.; Cao, X.; Zou, J.; Shen, B.; Zhang, X.; Liu, Z.; Lv, W.; Teng, J.; Ding, X. Indoxyl sulfate, a valuable biomarker in chronic kidney disease and dialysis. Hemodial. Int. Int. Symp. Home Hemodial. 2017, 21, 161–167.

86. Sun, C.Y.; Hsu, H.H.; Wu, M.S. p-Cresol sulfate and indoxyl sulfate induce similar cellular inflammatory gene expressions in cultured proximal renal tubular cells. Nephrol. Dial. Transplant. Off. Publ. Eur. Dial. Transpl. Assoc.-Eur. Ren. Assoc. 2013, 28, 70–78.

87. Bolati, D.; Shimizu, H.; Yisireyili, M.; Nishijima, F.; Niwa, T. Indoxyl sulfate, a uremic toxin, downregulates renal expression of Nrf2 through activation of NF-kappaB. BMC Nephrol. 2013, 14, 56.

88. Shimizu, H.; Yisireyili, M.; Higashiyama, Y.; Nishijima, F.; Niwa, T. Indoxyl sulfate upregulates renal expression of ICAM-1 via production of ROS and activation of NF-kappaB and p53 in proximal tubular cells. Life Sci. 2013, 92, 143–148.

89. Motojima, M.; Hosokawa, A.; Yamato, H.; Muraki, T.; Yoshioka, T. Uremic toxins of organic anions up-regulate PAI-1 expression by induction of NF-kappaB and free radical in proximal tubular cells. Kidney Int. 2003, 63, 1671–1680.

90. Edamatsu, T.; Fujieda, A.; Itoh, Y. Phenyl sulfate, indoxyl sulfate and p-cresyl sulfate decrease glutathione level to render cells vulnerable to oxidative stress in renal tubular cells. PLoS ONE 2018, 13, e0193342.

91. D’Autréaux, B.; Toledano, M.B. ROS as signalling molecules: Mechanisms that generate specificity in ROS homeostasis. Nat. Rev. Mol. Cell Biol. 2007, 8, 813–824.

92. Owada, S.; Goto, S.; Bannai, K.; Hayashi, H.; Nishijima, F.; Niwa, T. Indoxyl sulfate reduces superoxide scavenging activity in the kidneys of normal and uremic rats. Am. J. Nephrol. 2008, 28, 446–454.

93. Peskin, A.V. Cu,Zn-superoxide dismutase gene dosage and cell resistance to oxidative stress: A review. Biosci. Rep. 1997, 17, 85–89.

94. Ratliff, B.B.; Abdulmahdi, W.; Pawar, R.; Wolin, M.S. Oxidant Mechanisms in Renal Injury and Disease. Antioxid. Redox Signal. 2016, 25, 119–146.

95. Humphreys, B.D. Mechanisms of Renal Fibrosis. Annu. Rev. Physiol. 2018, 80, 309–326.

96. Miyazaki, T.; Ise, M.; Hirata, M.; Endo, K.; Ito, Y.; Seo, H.; Niwa, T. Indoxyl sulfate stimulates renal synthesis of transforming growth factor-beta 1 and progression of renal failure. Kidney Int. Suppl. 1997, 63, S211–S214.

97. Milanesi, S.; Garibaldi, S.; Saio, M.; Ghigliotti, G.; Picciotto, D.; Ameri, P.; Garibotto, G.; Barisione, C.; Verzola, D. Indoxyl Sulfate Induces Renal Fibroblast Activation through a Targetable Heat Shock Protein 90-Dependent Pathway. Oxidative Med. Cell. Longev. 2019, 2019, 2050183.

98. Shimizu, H.; Yisireyili, M.; Nishijima, F.; Niwa, T. Stat3 contributes to indoxyl sulfate-induced inflammatory and fibrotic gene expression and cellular senescence. Am. J. Nephrol. 2012, 36, 184–189.