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MINI-REVIEW

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Beneficial actions of microbiota-derived tryptophan metabolites

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Abstract

Tryptophan is an important dietary amino acid and it is the precursor for 5-hydroxytryptamine synthesis in the nervous system and by enterochromaffin cells in the gut mucosa. Tryptophan is also metabolized by enzymes in the gut mucosa and also by enzymes produced by the gut microbiome. Diet and the microbiome can contribute to metabolic disease in part by causing intestinal inflammation and increased permeability. In this issue of Neurogastroenterology and Motility, Jennis et al. test the hypothesis that indole tryptophan metabolites produced by gut bacteria might be responsible for the anti-inflammatory and beneficial metabolic effects of the gut microbiome and Roux-en-Y gastric bypass surgery for weight loss by obese patients. The authors identified indole-3-propionic acid as the beneficial metabolite. A review of the literature also revealed the beneficial effects of tryptophan metabolites on diabetes and metabolic disease and on inflammatory bowel disease. Taken together, these data highlight another health benefit of the intestinal microbiome, which produces beneficial products from dietary amino acids especially tryptophan.

KEYWORDS
gut microbiome, indole-3-proprionic acid, obesity, tryptophan metabolism, Type 2 diabetes

1 | OBESITY, TYPE 2 DIABETES AND THE GUT MICROBIOME

Obesity is a global public health problem as there are 2.1 billion obese people worldwide and 35% of the US population is obese.[1] Obesity causes many adverse health problems especially cardiovascular disease, insulin resistance and Type 2 diabetes (T2D).[2] The gut microbiome is also affected by obesity which can cause alterations in bacterial populations, intestinal and systemic inflammation, increased intestinal permeability and endotoxemia.[3,4]

The dietary amino acid tryptophan is the precursor for 5-hydroxytryptamine (5-HT, serotonin) synthesis in the nervous system and in the gut mucosa. Dietary tryptophan is taken up by mucosal enterochromaffin (EC) cells, where tryptophan is enzymatically converted to 5-HT. 5-HT is released by the EC cells as a local signaling molecule. Locally released 5-HT acts on the nerve endings of intrinsic enteric sensory nerve endings and extrinsic sensory nerve endings to initiate motor and secretomotor reflexes and gut sensation.[5-7] The gut microbiome produces a number of important signaling molecules that can affect gut motility, secretion and sensation. These molecules include H_2S, CH_4, and short chain fatty acids (SCFA) such acetate, propionate, and butyrate. In addition, gut bacteria can produce tryptamine from dietary tryptophan and tryptamine stimulates intestinal motility.[8]

Abbreviations: 5-HT, 5-hydroxytrypamine; EC cells, enterochromaffin cells; IDO, indoleamine 2,3-dioxygenase.IPA, Indole-3-propionic acid; RYGB, Roux-en-Y gastric bypass; SCFA, short chain fatty acids T2D, type 2 diabetes; TPH, tryptophan hydroxylase.

2 | OBESITY, THE GUT MICROBIOME, AND TRYPTOPHAN METABOLISM

Jennis et al.[9], in this issue tested the hypothesis that tryptophan metabolites produced by gut bacteria could reduce intestinal inflammation in high fat diet (HFD) fed mice, in lean human and in obese T2D human patients undergoing Roux-en-Y gastric by-pass surgery for weight loss. Tryptophan is the precursor for 5-HT synthesis by EC cells in the gut (via tryptophan hydroxylase 1, TPH1) and neurons in the enteric and central nervous systems (via TPH2). Only about 2% of gut

tryptophan is converted to 5-HT.[10] Tryptophan is also metabolized in the host gut lumen to kynurenine and kynurenic acid by indoleamine 2,3-dioxygenase (IDO) and kynurenine-oxoglutarate transaminase, respectively. Kynurenine is also metabolized to 3-hydroxy-L- kynurenine and then quinolinic acid by kyenurenine monooxygenase and kynurenase, respectively. 3-hydroxy-L- kynurenine is also converted to xanthurenic acid by kynurenine-oxoglutarate transaminase. Up to 95% of intestinal tryptophan is metabolized by the host kynurenine pathway.[11] Tryptophan can also be metabolized to tryptamine by the host enzyme L-aromatic acid decarboxylase. An additional tryptophan metabolic pathway is carried out by gut bacteria (microbiome). In this pathway (see figure 1 in the paper by Jennis et al.), tryptophan is metabolized to indole by tryptophanse (TnaA) and indole is subsequently metabolized to indole-3-propionic acid (IPA) by tryptophan amino transferase (Tam1).[11] Bacterial production of IPA is important for gut homeostasis as it has been shown previously to improve intestinal barrier function at least in germ free mice.[12] Jennis et al. showed that mice fed a HFD for up to 22 weeks had increased intestinal permeability (measured as increased blood levels of FITC-dextran administered by gavage). IPA treatment of HFD fed mice reduced intestinal permeability (lower circulating FITC-dextran) and also reduced circulating lipopolysaccharide (LPS) levels. This is consistent with previous work showing gut IPA enhances gut barrier function.[12] These data were backed up by in vitro studies using T84 cells grown as monolayers and incubated with the pro-inflammatory cytokines interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α). FITC dextran permeability was measured. The investigators screened a number metabolites using this assay and found that only IPA and tryptamine decreased monolayer permeability. They also used a gene array to assess the effects of IPA on expression of 41 selected genes activated by IFN-γ. The genes selected encoded proteins involved in tryptophan metabolism, epithelial tight junction formation, pro-inflammatory genes, and transcription factors. IFN-γ upregulated most of these genes but only the gene encoding GLUT5, the fructose transporter (SLC2A5) was downregulated by IPA treatment. The last set of studies was done in human subjects. Jennis et al. studied circulating tryptophan metabolites in lean subjects and in obese T2D subjects before and 1 week or 3 months after RYGB surgery. It was found that the bacterially derived indoles, IPA, and indole sulfuric acid (ISA) were lower in blood samples from T2D subjects compared to lean control subjects. At 3 months post-RYGB surgery, blood levels of IPA and ISA had increased significantly compared to the 1 week samples.

3 | HOST GENETICS, OBESITY, MICROBIOME, AND GUT HEALTH

A seminal study done using genetically determined obese mice (*ob/ob*) with a mutation in the gene encoding leptin was the first to show that obesity can alter the gut microbiome.[13] In this study, non-obese C57Bl/6J *ob/+* and *ob/ob* mice were fed the same high polysaccharide diet but only the *ob/ob* mice became obese. Analysis of cecal bacteria revealed that the *ob/ob* mice had a significant increase in the number of bacteria from the phylum *Firmicutes* and a significant reduction in bacteria from the phylum *Bacteroidetes* compared to lean mice. Microbiota transplant from ob/ob mice to lean C57Bl/6J *ob/+* caused the lean mice to become obese.[13]

Key Points

- The beneficial effects of tryptophan metabolism by gut bacteria in obesity and type 2 diabetes are not well understood.
- This review provides a summary of existing knowledge and therapeutics benefits of tryptophan metabolites produced by the gut microbiome.
- Identification of active and beneficial bacteria derived tryptophan metabolites might be used as treatments for diabetes, obesity, metabolic syndrome and inflammatory bowel disease.

The study summarized above was done using mice with a genetic defect that affected appetite regulation. However, the type of diet consumed by animal subjects or humans has a major impact on the gut microbiota. It is well-established that a HFD changes the bacterial composition contributing to obesity, metabolic disease, and diabetes.[14-17] Host genetics is also an important factor in determining diet-induced weight gain and the composition of the gut microbiome and metabolic disease. Ussar et al.[14] studied three inbred strains of mice and their metabolic and microbiome responses when fed a HFD. C57Bl/6J mice had significant weight gain and they developed insulin resistance and glucose intolerance when placed on a HFD. 129S1/SvImJ mice did not gain as much weight nor did they develop insulin resistance and glucose intolerance. Both strains were purchased from Jackson Laboratories. 129S6/SvEvTac mice are obesity prone but diabetes resistant when fed a HFD. These mice were obtained from a different supplier (Taconic) and came from a different environment. C57Bl/6J mice gained the most weight and had the highest glucose intolerance compared to the two 129S mouse strains. C57Bl/6J mice also had the highest levels of inflammatory mediators compared to the 129 mouse strains. When the three strains of mice were housed in the same facility for 3 generations so that the gut microbiome was similar across groups, the129S6/SvEvTac mice became obesity resistant. These data indicate that mouse strain differences in microbiome can influence the metabolic responses to a HFD despite genetic differences in each strain.[14]

The obese/T2D subjects in the study by Jennis et al. underwent RYGB surgery for weight loss and this reduced intestinal permeability and increased IPA levels suggesting changes occurred in intestinal microbiota. Physical exercise is a non-surgical approach to weight loss and this could increase the population of beneficial bacteria in the gut. For example, mice fed a high fat (60 kcal% fat) for 12 weeks were heavier with reduced glucose tolerance compared to mice fed a low fat (10% kcal fat).[17] However, exercise (running wheel) reduced body weight gain and blood glucose in high fat fed mice. Exercise increased the Bacteroides and decreased the Firmicutes bacterial populations in

the colon of mice regardless of diet. These data highlight the important interaction between diet and exercise in promoting good health.[17]

While host metabolism is the main pathway for tryptophan metabolism, the microbiota also produces benefical metabolites. IDO1 is a host enzyme that metabolizes tryptophan to kynurenine.[9] The role of IDO1 and tryptophan metabolism by the host and the microbiota in maintaining gut mucosal homeostasis was studied in wild type and in IDO$^{-/-}$ mice.[18] As IDO is a host enzyme, it would be expected that tryptophan metabolism by this enzyme would not occur in the in the IDO$^{-/-}$ mice. However, it was found that in IDO1$^{-/-}$ mice, the host Lactobacilli population expands and that these bacteria metabolize dietary tryptophan via indole-3-lactic acid dehydrogenase to indole-3 lactic acid (ILA) and indole-3 aldehyde (IAld). IAld is an arylhydrocarbon receptor (AhR) agonist and the AhR links to IL-22 transcription. IL-22 is a cytokine that stimulates immune responses in epithelial cells including the gut epithelium. While IL-22 activates the immune system, it also activates pathways which promote survival of host bacteria while also having an antifungal effect (*Candida albicans*).[18] So microbiota based tryptophan metabolism produces signaling molelcules that promote survival of beneficial bacteria while also suppressing other infectious organisms such as fungi.

Tryptophan metabolism by the microbiota may also play a role in inflammatory bowel disease (IBD). Caspase recruitment domain family member 9 (*CARD9*) is a colitis susceptibility gene and the CARD9 protein is part of the pattern recognition receptor signaling pathway mediating responses to fungal and bacterial infections. *CARD9* also stimulates IL-22 production which suppresses colitis while *Card9*$^{-/-}$ mice develop colitis.[19] The microbiome is also altered in the *Card9*$^{-/-}$ mice as their gut bacteria do not metabolize tryptophan to form AhR agonists. However, when *Card9*$^{-/-}$ mice were inoculated with Lactobacilli capable of producing AhR agonists from dietary tryptophan, intestinal inflammation was reduced.[19] These investigators also studied tryptophan metabolism in IBD patients and control subjects. It was found that fecal samples from control but not IBD patients activated the AhR while tryptophan levels were lower and kynurenine levels were higher in fecal samples of IBD patients. These data indicate that tryptophan metabolism was altered in the colon of IBD patients. Finally, IBD patients in this study were genotyped for an IBD associated single nucleotide polymorphism in the *CARD9* gene (rs 10781499). This SNP is associated with impaired activation of AhR by microbiota produced AhR agonists and disruption of the host immune response.[19]

As discussed by Jennis et al. the host kynurenine pathway is the major route of tryptophan metabolism in the gut with 90% of dietary tryptophan metabolized by this pathway.[20,21] Kynureine is the immediate precursor for kynurenic acid synthesis by the enzyme IDO and kynurenic acid can act as antagonist of NMDA glutamate receptors[22] and as an antagonist at nicotinic acetylcholine receptors.[23] Kynurenic acid inhibits inflammation in the 2,4,6-trinitrobenzenesulfonic acid (TNBS) model of colitis in rats by blocking enteric NMDA receptors.[22,24] Gut derived kynurenic acid may also have effects on the central nervous system by affecting mood and cognition.[18,25,26]

4 | SUMMARY AND CONCLUSIONS

Jennis et al.[9] have identified an important role for gut microbiome-derived tryptophan metabolites, especially IPA, for gut health. Obesity and T2D are both associated with increased intestinal permeability (endotoxemia) and disturbances in tryptophan metabolism resulting in lower levels of bacterial-derived and beneficial metabolites. Formerly obese patients who had undergone RYGB surgery for weight loss had an improved metabolic profile and also had increased plasma IPA levels and reduced intestinal permeability. The microbiome has an important role in regulating nutrient metabolism and certain microbiome profiles make the host susceptible to development of metabolic disease and diabetes. Alternatively, the microbiome profile can provide protection against metabolic disease and diabetes. The microbiome profile is also an important determinant of gut tryptophan metabolism and some tryptophan metabolites can provide protection against gastrointestinal infections and IBD.

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DISCLOSURES

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