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The effects of whole grain cereals on tryptophan metabolism and intestinal barrier function: underlying factors of health impact

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This review aims to investigate the relationship between the health impact of whole grains mediated via the interaction with intestinal microbiota and intestinal barrier function with special interest on tryptophan metabolism, focusing on the role of the intestinal microbiota and their impact on barrier function. Consuming various types of whole grains can lead to the growth of different microbiota species, which in turn leads to the production of diverse metabolites, including those derived from tryptophan metabolism, although the impact of whole grains on intestinal microbiota composition results remains inconclusive and vary among different studies. Whole grains can exert an influence on tryptophan metabolism through interactions with the intestinal microbiota, and the presence of fibre in whole grains plays a notable role in establishing this connection. The impact of whole grains on intestinal barrier function is closely related to their effects on the composition and activity of intestinal microbiota, and SCFA and tryptophan metabolites serve as potential links connecting whole grains, intestinal microbiota and the intestinal barrier function. Tryptophan metabolites affect various aspects of the intestinal barrier, such as immune balance, mucus and microbial barrier, tight junction complexes and the differentiation and proliferation of epithelial cells. Despite the encouraging discoveries in this area of research, the evidence regarding the effects of whole grain consumption on intestine-related activity remains limited. Hence, we can conclude that we are just starting to understand the actual complexity of the intestinal factors mediating in part the health impacts of whole grain cereals.

Keywords: Whole grain cereals: Intestinal microbiota: Intestinal barrier: Tryptophan

Cereal grains belong to wide variation of food cultures forming familiar staple foods from the early ages of history until recent years. Cereal grains are major sources of energy, carbohydrates and protein sources globally[1]. However, cereal grains have been neglected as a relevant and culturally familiar source of dietary plant protein. As such they provide feasible plant foods and, from the sustainability viewpoint, assist the need for transition from animal-based diets towards plant-based diets. In their whole grain form, all parts of the cereal grains are retained and as such they are also rich in many vitamins and minerals and bioactive compounds such as phytochemicals and fermentable and non-fermentable fibres delivering many health benefits[2]. Indeed, whole grain consumption is associated with decreased risk of multiple diseases and mortality, such as CVD[3,4], type 2

Abbreviations: AHR, aryl hydrocarbon receptor; IDO1, indoleamine-2, 3-dioxygenase 1; IPA, indole-3-propionic acid; LPS, lipopolysaccharides; PXR, pregnane X receptor.

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diabetes[3–5], hypertension[6–8] and some cancers such as colorectal cancer[4,5,7]. Conversely, a diet low in whole grains has been identified as one of the highest-ranked dietary risk factors for burden of diseases globally[9]. Nevertheless, precise mechanisms behind these health impacts are not well known. Because of the observed health benefits, whole grain consumption is encouraged in dietary guidelines worldwide[10,11]. That been said, the average consumption of whole grains in populations fails to meet the recommendations[12,13]. Recently, Nordic Nutrition recommendations[14] recommended consuming at least 90 g/d of whole grain consumption with the preference of other grain cereals than rice. However, there is no global consensus on the recommended amount or the number of portions of whole grain products to be consumed daily, and the recommendations vary from qualitative to quantitative statements[11,13]. This may make the shift to consume more whole grain foods even more challenging.

Until recently the consensus on the definition for whole grain cereal foods has been missing. This has been a challenge in practical terms in consumer understanding, but also when evaluating and comparing previous scientific reports on the health impact of cereal grains. Other grains, such as pulses, legumes and oilseeds, are not included in any definitions nor in dietary recommendations for whole grains as they differ substantially from cereal grains in their anatomy and composition[10]. Consequently, our focus in this review is exclusively on whole grain cereals. Consensus on global definition of whole grains was published by a working group of Whole Grain Initiative[13]. According to this consensus, 'whole grains shall consist of the intact, ground, cracked, flaked, or otherwise processed kernel after the removal of inedible parts such as the hull and husk. All anatomical components, including the endosperm, germ, and bran must be present in the same relative proportions as in the intact kernel'. Moreover, the whole grain foods have definition of 'A whole-grain food shall contain at least 50% whole-grain ingredients based on dry weight. Foods containing 25–50% whole-grain ingredients based on dry weight, may make a front-of-pack claim on the presence of whole grain but cannot be designated 'whole grain' in the product name'. The definitions have been ratified by the leading international cereal science associations, including the C & A Association, the HealthGrain Forum and the International Association for Cereal Science and Technology[13]. Furthermore, the Nordic Nutrition recommendations[14] have adopted a consensus in defining the whole grains, simplifying the harmonisation process across the Nordic countries. However, there are still challenges in implementing these definitions on a global scale, and their adoption in practical usage requires agreement from regulatory bodies as well.

From the intestine-mediated health impact point of view, whole grains are an interesting combination of various fibres and protein which are bound within the plant cell structures. This combination of fibre and protein in cereals is of great interest, for example, how is it processed and metabolised in the gastrointestinal tract? Fibre composition varies greatly among different whole grains[15]. They are rich in both soluble and insoluble dietary fibres, among which some are fermentable, such as β-glucans, pectins, fructans, arabinoxylan and resistant starch and some non-fermentable such as cellulose and lignans, by the intestinal microbiota[16]. Latter division is partly mixed and overlapping with concepts of fibre being soluble or not. It is currently understood that dietary fibres that are fermentable by the intestinal microbiota induce the increase in the intestinal microbiota diversity and support the growth of bacterial species associated with health beneficial metabolites such as SCFA. Especially the non-fermentable fibre may possess these beneficial impacts on the intestinal microbiota, allowing the adherence of specific bacteria and facilitating the fermentation of the fermentable fibre[17,18]. Most cereal grain proteins are located in the endosperm, but the aleurone and subaleurone layers of the bran have the highest protein content[19]. Fibre and other plant tissue structures have been demonstrated to reduce protein digestibility in both *in vitro* and human trials, but this effect might differ between individuals and also depend on the type of fibre[20]. Due to lower bioavailability of the cereal protein, part of it reaches the colon within the fibre fraction. Processing during the food manufacturing causes changes in grain constituents, which may have an impact on the digestibility and bioavailability of protein[2]. To this end, there is the question of what happens to fibre fraction bound or otherwise non-absorbed cereal protein in the colon?

Our current understanding is mainly based on animal protein fermentation in the colon. Colonic bacteria favour carbohydrate fermentation over proteins, resulting in proteins being fermented mostly in the distal colon[21]. Proteolytic fermentation in the distal colon yields metabolites, such as ammonia, certain phenols and branched-chain amino acids that are usually regarded as harmful for the intestinal barrier function and may activate pro-inflammatory mechanisms in the intestine, while also predisposing the individual to non-communicable diseases through systemic effects[22]. However, it can be assumed that the fermentation activity of dietary fibre in the proximal colon influences the activity of the microbiota, promotes the production of beneficial metabolites and strengthens the intestinal barrier function, thereby promoting beneficial health outcomes that may counterbalance the potential harm of proteolytic activity. In addition to SCFA, cereal fibre fermentation produced many other metabolites[23–25] including derivatives of fibre-embedded phytochemicals that have been associated with health-supporting effects. Moreover, protein content differs between different grains[2]. The metabolically active cereal proteins include mostly enzymes and storage proteins such as albumins and globulins[26]. Some cereal proteins, such as gluten, and peptides, such as prolamins, may trigger immune system and cause symptoms for some individuals[2]. Previous reports, both from the others and our own studies, have shown that the intestinal microbiota-produced metabolites have great importance for the overall health impact of the whole grain cereals. Novel

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research findings link tryptophan, an important amino acid within the cereal protein fraction, with health and risk of disease. This review aims to investigate the relationship between the health impact of whole grains mediated via the interaction with intestinal microbiota and intestinal barrier function with special interest on tryptophan metabolism, focusing on the role of the intestinal microbiota and their impact on barrier function.

Whole grains and intestinal microbes

One potential link between whole grains and their health effects is the impact of whole grains on intestinal microbiota. Consuming various types of whole grains can lead to the growth of different microbiota species, which in turn leads to the production of diverse metabolites, including those derived from tryptophan metabolism[27]. However, despite the efforts to evaluate the impact of whole grains on intestinal microbiota composition, the results remain inconclusive and vary among different studies[28]. This has been pointed in a recent review by Koecher *et al.*, making difficult to draw any conclusions on the effect of whole grains on the intestinal microbiota because there are no consistent effects, even when grouping studies evaluating the same grains and using similar microbial measurement techniques[28]. We agree with Koecher *et al.* that these differences are likely attributed to variations in the understanding of what constitutes whole grains (lack of consistent use of whole grain definition), variations in the quantity of whole grains tested, diverse techniques employed for microbiota determination, lack of control in dietary intake in some of the studies and the interindividual differences in microbiota composition and response, which diminish the statistical power of most of the studies[28,29]. Then there is a need for future studies to clarify the impact of whole grains in microbiota composition. Conversely, whole grains modulate microbiota activity to produce bioactive compounds that may exert a physiological effect. Fibre from whole grains is fermented by intestinal microbiota. This fermentation yields SCFA, including butyrate, acetate and propionate and other metabolites that have been associated with several health impacts[2]. The consumption of diet containing whole grains is associated with higher levels of total SCFA and acetate[30,31]. Notably, the daily consumption of oat and barley β-glucan has been shown to increase the concentration of SCFA in faeces of the subjects[32]. The consumption of rye products has been associated with increased butyrate-producing bacteria and higher levels of butyrate in plasma[33]. Additionally, the postprandial effect on butyrate concentrations, as well as propionate concentrations, in plasma has been observed after the consumption of rye bread[34].

In addition to fibre, whole grains contain other compound that can influence microbiota composition and activity such as polyphenols, sterols, tocols and betaine[35]. The mechanisms of the effects of these other compounds from whole grains are understudied in human subjects. Polyphenols seem to stimulate the production of SCFA and other organic acids[35,36] and studies *in vitro* and in animal models associate betaine with an increase of SCFA-producing bacteria[37,38]. Nonetheless, the specific mechanism of action and contribution to health in human subjects remain unclear.

Intestinal barrier

Intestinal barrier is a dynamic entity that consists of multiple elements. It consists of (1) microbial barrier that is composed of commensal bacteria and chemical barrier composed of gastrointestinal secretions in the lumen, (2) the microclimate including the unstirred water layer, glycocalyx and mucus layer, (3) the epithelium constituted of a single layer of different cell types, such as enterocytes, Paneth cells and goblet cells, that are connected to each other by apical junctional complexes, (4) the immunological barrier including a variety of immune cells and (5) the lamina propria[39–41]. There are numerous ways the intestinal barrier protect host from the harmful substances and pathogens. For example, mucus serves as the first physical defence in the barrier by containing several immune factors and thus protecting the epithelial cells from the direct contact with antigens, toxins and pathogenic bacteria[39–42]. The epithelial cells (enterocytes and Paneth cells) react to noxious stimuli by secretion of antimicrobial peptides and chloride [39,40,43]. In addition, junction complexes regulate intercellular transport, including blocking the entry of pathogens[39,41,43]. The lamina propria provides defence based on innate and acquired immunity cells as well as endocrine and secretomotor mechanisms[39,40].

The dysfunction of the intestinal barrier, also referred as 'leaky gut', is characterised by an increase in intestinal permeability. This condition can be induced by sustained inflammation or infections[40,43]. As a result of increased intestinal permeability, pathogens and lipopolysaccharides (LPS) are able to pass through the intestinal barrier, triggering the production of proinflammatory cytokines. These cytokines can induce systemic proinflammatory immune responses[44] and alter the structure of tight junctions, thereby further disrupting intestinal permeability[45,46]. An example illustrating the impact of altered intestinal homeostasis is the disturbance in the production and secretion of endocrine hormones, that subsequently trigger metabolic diseases[42]. Thus, maintaining the integrity of the intestinal barrier serves as a beneficial target in promoting overall health. Tryptophan and its metabolites may play a role in supporting this essential function of the intestinal barrier by influencing on intestinal permeability, mucus production, immune balance and intestinal microbe composition that will be discussed in following sections.

Tryptophan metabolism in the intestine

Amino acids, peptides and proteins that are attached to cereal structures, such as the fibre fractions, and are not absorbed in the small intestine ultimately reach the large intestine, where some of them undergo

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fermentation by the intestinal microbiota[50]. After being released from the cereal structures, proteins are broken down into smaller peptides and amino acids by proteases and peptidases produced by bacteria in the large intestine. Among the other amino acids, tryptophan can participate in various metabolic pathways, especially those induced by colonic microbes[49,51,52].

The majority of dietary tryptophan, such as other amino acids, is absorbed from the small intestine[47]. In the epithelial cells, tryptophan is released into the peripheral circulation, or it can be degraded to different metabolites by the enzymes of the intestinal cells[48]. Microbial activity could impact tryptophan availability and metabolism in the small intestine, but the current evidence is very limited and mostly focused on the colonic microbiota. Several common groups of intestinal bacteria including *Lactobacillus*, *Streptococcus* and *Escherichia coli* among others have been reported to express tryptophan synthetase[49] and potentially may contribute to tryptophan production. Future studies clarifying the role of the small intestinal microbiota in the tryptophan metabolism may be needed.

Tryptophan has three major metabolic pathways: kynurenine, serotonin and indole pathways (Fig. 1).

Kynurenine pathway

Kynurenine pathway covers a considerable part of the entire tryptophan metabolism in the gastrointestinal tract[52]. Tryptophan is degraded to kynurenine in the intestinal epithelial cells and in the immune cells by the enzyme indoleamine-2,3-dioxygenase 1 (IDO1), whose activity is induced by the inflammatory signalling[53]. Kynurenine can be further metabolised through kynurenic acid or quinolinic acid pathways, which produce multiple derivatives affecting health and disease[53,54].

Indole pathway

The indole pathway is another primary metabolic route for tryptophan, constituting a small percentage of intestinal metabolism[52]. Through the catalytic action of tryptophanase and decarboxylase enzymes, tryptophan can undergo modifications within the indole pathway, resulting in the production of diverse compounds called indole derivatives[55,56]. In contrast to serotonin and kynurenine, indole derivatives are almost exclusively synthesised through the metabolism of the intestinal microbiota[49,55,57], and multiple species have been reported to produce these metabolites[58].

Serotonin pathway

A small portion of the tryptophan obtained from food is metabolised into serotonin within the large intestine. Interestingly, this contribution accounts for a significant proportion, estimated to be some 90 % of the body's overall serotonin production[57]. The synthesis of serotonin in the intestine occurs in enterochromaffin cells, a specific type of enteroendocrine cells, as well as in serotonergic neurons of the enteric nervous system[59]. Within the intestine, the enzyme tryptophan hydroxylase plays a crucial role as a limiting factor in the production of serotonin, catalysing the conversion of tryptophan to 5-hydroxytryptophan[60,61]. Subsequently, 5-hydroxytryptophan is further converted to serotonin through the action of aromatic amino acid decarboxylase.

Whole grains and tryptophan metabolism

The relationship between the consumption of whole grains and the modulation of tryptophan metabolism has been investigated in human studies, although to a limited extent. In healthy adults, it was found that plasma tryptophan concentrations decreased after the consumption of whole grains[62]. However, this effect was not observed in postmenopausal women with dyslipidaemia[63]. Several studies have demonstrated that the consumption of whole grains is associated with the suppressed activity of the kynurenine pathway[62,64,65]. Moreover, there is a direct association between the consumption of whole grains and higher fibre intake with serum indole-3-propionic acid (IPA)[65,66]. The relationship with other indole derivatives varies, with some studies showing an inverse association[65] and others showing a positive association[63,67], depending on the specific indole derivative. Additionally, partial effects on serotonin metabolism have been observed, with some studies reporting an inverse association[62,64,68] and one study showing a positive association[65] between whole grain consumption and serotonin concentrations. Establishing causality in this relationship, however, is challenging, and there are still limited studies available to definitively determine the direction of these associations.

Whole grains can exert an influence on tryptophan metabolism through interactions with the intestinal microbiota, and the presence of fibre in whole grains plays a notable role in establishing this connection. Certain fibre types have been associated with the production of IPA by specific bacterial genera found in faecal samples[65]. Various phyla and genera of intestinal bacteria have been found to employ tryptophan metabolic pathways within the intestine[58,69,70]. Different genera may possess the ability to utilise distinct metabolic pathways for tryptophan[58,65,70], which could exert a significant influence on the tryptophan metabolism in the host. The absence or depletion of the intestinal microbiota in mice has been found to decrease the activity of the kynurenine pathway, resulting in decreased kynurenine levels and increased tryptophan concentrations in the bloodstream[71–73]. In addition, previous studies conducted on germ-free mice have indicated disruptions in serotonin synthesis[71,72,74,75].

The impact of intestinal microbiota on tryptophan metabolism can be attributed to microbial products. Among these products, SCFA are noteworthy. *In vitro* studies have demonstrated that SCFA can enhance intestinal serotonin metabolism by increasing the expression of the tryptophan hydroxylase 1 enzyme[76,77]. However, in mice, propionate has shown an inverse correlation as well[27]. SCFA also have been observed to stimulate serotonin release from enterochromaffin cells in rats[78], but

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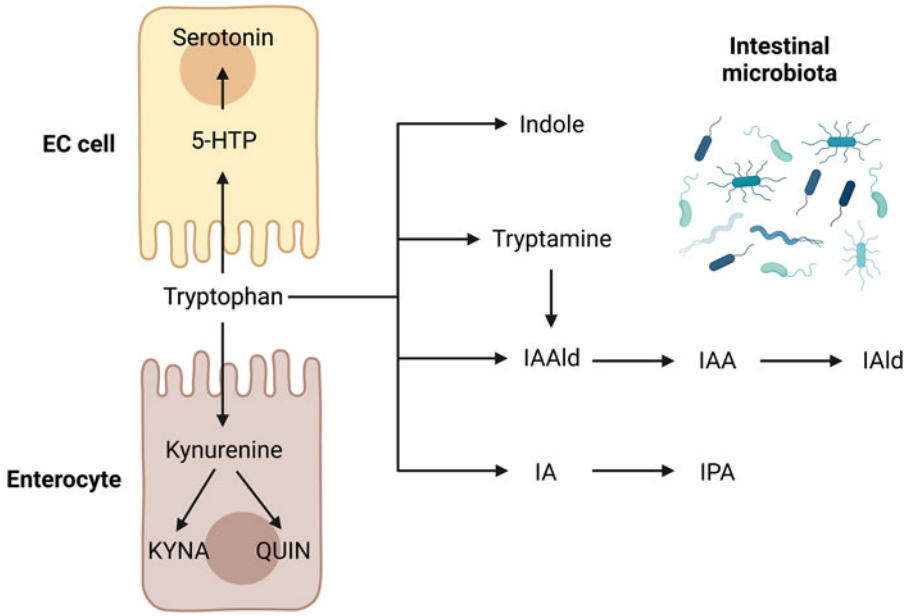

Fig. 1. Simplified illustration depicting tryptophan metabolism in the intestine. The main pathways of tryptophan metabolism are kynurenine pathway, serotonin pathway and indole pathway. Additionally, kynurenine pathway is active in immune cells and serotonin pathway is active in serotonergic neurons (not shown). The production of indole derivatives is attributed to the intestinal microbiota. 5-HTP, 5-hydroxytryptophan; EC, enterochromaffin; IA, indole-3-acrylic acid; IAA, indole-3-acetic acid; IAAld, indole-3-acetaldehyde; IAld, indole-3-aldehyde; IPA, indole-3-propionic acid; KYNA, kynurenic acid; QUIN, quinolinic acid.

this effect has not been replicated *in vitro*[79]. Furthermore, butyrate has been found to reduce the expression of the IDO1 enzyme in the intestine, leading to a decrease in the conversion of tryptophan into kynurenine[80]. In addition to SCFA, another microbial product called deoxycholate, which is a secondary bile acid produced through microbial biotransformation, has been shown to stimulate serotonin release from enterochromaffin cells[77]. Furthermore, alterations in the microbiota can interfere with the functioning of serotonin transporter proteins, resulting in increased concentrations of serotonin[81].

Tryptophan metabolism can be influenced by the intestinal microbiota through immune responses as well. The microbiota plays a role in regulating the immune response through Toll-like receptors[82,83], and previous studies have associated their activation with increased activation of kynurenine pathway in immune cells[84,85]. Conversely, alterations in the intestinal microbiome have been associated with changes in intestinal permeability[86,87]. Consequently, microbes and their metabolites, which would not typically enter the body through normal routes, gain access, triggering immune cell activation and subsequent inflammation[88,89]. This can affect tryptophan metabolism, particularly through the activation of enzymes within the kynurenine pathway.

Conversely, tryptophan has the ability to alter the composition and metabolic activity of the intestinal microbiota. For instance, tryptophan supplementation has been shown to enhance the abundance of SCFA-producing bacteria in LPS-challenged mice[90] and in pigs[91,92]. This has led to higher concentrations of SCFA in the colonic digesta[92]. Furthermore, indoles play a role in interspecies communication, effectively regulating the microbial composition and characteristics within the gastrointestinal tract[93]. They promote the proliferation of beneficial bacteria and inhibit the growth of pathogenic bacteria in the intestinal tract[56]. Meanwhile, IDO1 plays an important role in preserving the intestinal microbial diversity within the intestinal environment[94]. These findings emphasise the bidirectional interaction between tryptophan and the intestinal microbiota.

The composition of the intestinal microbiota and its active metabolic pathways appear to be closely linked to the tryptophan metabolism both in the intestine and within the body. However, it is crucial to exercise caution when generalising the findings from studies conducted on animals or *in vitro* to human populations. Furthermore, the precise mechanisms underlying this interplay are not fully understood, and several unanswered questions remain. Therefore, further investigation is necessary to unravel the cause-and-effect relationships and underlying mechanisms involved in these connections in the context of human biology.

Whole grains, tryptophan metabolism and intestinal barrier

Whole grains and intestinal barrier

The impact of whole grains on intestinal barrier function is closely related to their effects on the intestinal

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microbiota. Firstly, commensal luminal bacteria inhibit the colonisation of pathogens via numerous mechanisms, such as the production of antimicrobial peptides, pH modification of the lumen content and nutrient competition[40,95]. Secondly, the intestinal microbiota has been implicated in modulating the integrity of the intestinal barrier, as it plays a role in regulating epithelium formation[96] and enhancing the mucus layer through its influence on mucus properties and mucosal immunity[97–99]. Similarly, a diet lacking in fibre has been shown to erode the mucus layer and promote mucus penetrability in mice[23,100,101]. This effect could potentially be attributed to the intestinal microbiota utilising mucus glycoproteins as a source of nutrients[23].

The connection between whole grains, intestinal microbiota and intestinal barrier function may be mediated through SCFA[39,43]. SCFA produced in the fermentation of whole grain fibres are responsible for causing a decrease in pH within the intestinal environment, which in turn supports the microbial barrier[102]. Furthermore, SCFA and particularly butyrate plays a crucial role in supporting the intestinal epithelial cells by serving as important energy sources and possessing anti-inflammatory properties[40,41,43,103]. SCFA correlated negatively with the expression of pro-inflammatory cytokine genes in LPS-challenged pigs[92]. In addition, SCFA have been shown to activate transmembrane G protein-coupled receptors, which take part in regulating gastrointestinal homeostasis and intestinal immunity[104]. These qualities make SCFA essential for maintaining homeostasis in the intestinal epithelium.

SCFA play a role in regulating the growth and differentiation of epithelial cells[102,105]. Additionally, they are directly involved in the regulation of intestinal permeability, potentially by accelerating the assembly of tight junctions[27,92,106–108]. Notably, butyrate has been found to promote the aggregation of tight junctions[107] and enhance the expression of tight junction complex proteins *in vitro* studies[109,110]. In mice, the administration of oat and rye bran resulted in increased mRNA expression of tight junction proteins, which correlated with the presence of SCFA, particularly propionate and butyrate[27]. Moreover, a positive association was observed between SCFA and occludin mRNA expression in LPS-challenged pigs[92].

SCFA are thought to provide support to the intestinal barrier through their effects on the mucus layer as well. *In vitro* studies have demonstrated that propionate and acetate can stimulate mucin 2 expression[111], and propionate has also been associated with colonic mucin levels in mice[27]. Butyrate has been involved in supporting the mucus barrier by stimulating the production of mucin both *in vitro*[112–114] and in the colon of mice[27]. However, it is noteworthy that the transcription of mucin was decreased at higher concentrations[113]. Interestingly, Gaudier *et al.*[115] discovered that despite the increased expression of the mucin 2 gene, high concentrations of butyrate led to a reduction in the thickness of the adherent mucus layer in mice. Furthermore, higher concentrations of butyrate have been shown to be involved in the disruption of the intestinal barrier associated with cell apoptosis *in vitro*[116]. These findings emphasise the importance of maintaining an adequate production of SCFA, particularly butyrate, in the intestine. By promoting the growth of SCFA-producing bacteria, including whole grains in the diet has the potential to facilitate this adequate production and support intestinal health.

Tryptophan metabolism and intestinal barrier

Tryptophan in the whole grains and tryptophan metabolism within the colon represent additional potential links connecting whole grains, intestinal microbiota and the intestinal barrier function. Several studies have proposed that specific tryptophan metabolites activate the aryl hydrocarbon receptor (AHR), which plays a role in maintaining the integrity of the intestinal barrier[104,117,118]. Indeed, tryptophan, multiple indole derivatives, 5-hydroxytryptophan and kynurenines have been seen to induce the expression of the AHR genes *in vivo* and *in vitro*[104,114,119–121]. The stimulating effect on AHR activity varies depending on the specific metabolite[122]. Another receptor implicated in the maintenance of intestinal barrier functions is the pregnane X receptor (PXR). It has been proposed as a key regulator of intestinal barrier function[123]. Activation of PXR has been shown to have protective effects on the intestinal barrier during inflammation[124], and PXR is involved in the upregulation of the tight junction complex proteins[125]. It is important to note that the sensitivity of AHR and PXR for tryptophan metabolites differ between human subjects and other mammals[121,123,126]. As a result, the outcomes observed in animal studies may not entirely reflect the actual effects on human subjects.

The absence of tryptophan in the diet can potentially compromise the integrity of the intestinal barrier, as suggested by Régnier *et al.*[42]. Additionally, the depletion of microbial tryptophan metabolism pathways has been found to associate with increased intestinal permeability in a cohort study[127]. Conversely, tryptophan increased goblet cells, antimicrobial peptides and mucins in the ileum of LPS-challenged mice, resulting in a reduction of damage to the intestinal mucosal barrier[90]. Tryptophan has also increased the concentrations and expressions of proteins that contribute to tight junction formation *in vitro* and in pigs[92,128,129], aligning with a decrease in permeability observed *in vitro*[129].

Indole metabolites have been shown to regulate epithelial cell proliferation and the production of antimicrobial peptides by promoting IL-22 production through AHR activation[117,130]. Indole, specifically, has been found to increase the expression of genes involved in fortifying the mucosal barrier and stimulating mucin production, as well as exhibit anti-inflammatory properties *in vitro*[131]. Furthermore, likely mediated by the activation of the PXR, indole has been shown to enhance the expression of proteins involved in the formation of junctional complexes, resulting in increased resistance against epithelial damage both *in vitro*[131] and in the colons of germ-free mice[132].

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IPA has been observed to activate PXR as well, with greater activation occurring in the presence of indole or indole-3-acetic acid[123]. However, this activation has not been consistently observed *in vitro*[120]. IPA has demonstrated the ability to reduce intestinal permeability both *in vitro*[133,134] and in mice studies[123,133]. The changes in permeability observed *in vitro* align with the increased expression of tight junction proteins[134], and the impact on the expression of proteins within the junction complex has been observed in rats as well[135]. In addition, IPA has been shown to elevate the concentrations of mucins and goblet cell secretion products *in vitro*, suggesting its strengthening effects on the mucus barrier[134]. Moreover, IPA has displayed anti-inflammatory properties by reducing proinflammatory cytokines and promoting anti-inflammatory cytokine production after LPS stimulation *in vitro*[120,134].

In both *in vitro* experiments and in mice with ulcerative colitis, indole-3-aldehyde has shown the ability to inhibit intestinal damage by targeting inflammatory pathways[136]. Additionally, indole-3-aldehyde has been found to upregulate the expression of junction proteins *in vitro*, as well as in mice with ulcerative colitis[136] and sclerosing cholangitis[130]. Indole-3-aldehyde has also been observed to restore the expression of the proliferation marker[130]. Moreover, indole-3-aldehyde has demonstrated the ability to restore antifungal resistance in mice with impaired adaptive immunity, suggesting a potential role in supporting microbial symbiosis[117].

Other indole derivatives have also been implicated in the functioning of the intestinal barrier. For example, indoleacrylic acid has demonstrated anti-inflammatory properties by enhancing the production of IL-10, reducing the secretion of proinflammatory cytokines, and promoting antioxidant responses following LPS stimulation *in vitro*[120]. Indoleacrylic acid has also been associated with enhanced goblet cell function and increased mucus production, possibly through the activation of the AHR, as observed *in vitro*[120]. Meanwhile, tryptamine has shown the ability to decrease inflammatory-induced permeability *in vitro*[133]. Conversely, indoxyl sulphate, a uremic toxin, has been found to inhibit genes related to tight junctions *in vitro*[132,137].

The primary function of kynurenine pathway in the intestine is to maintain immune balance[138]. Treatment with kynurenine can alleviate intestinal inflammation induced by LPS[90]. Kynurenic acid demonstrates potential anti-inflammatory properties in the gastrointestinal tract by inhibiting inflammatory enzymes[139] and acting as a ligand for G protein-coupled receptor[104]. Similarly, quinolinic acid plays a role in immunoregulatory processes within the intestine[54]. Furthermore, the expression of IDO1 in the intestine is involved in preserving the integrity of the intestinal barrier and mediating anti-inflammatory effects on the intestinal mucosa through various mechanisms[40,104]. For instance, IDO1 has promoted the differentiation of secretory cells of the intestinal epithelial in mice and its increased expression correlated with increased levels of mucin 2 and AHR in both healthy individuals and Crohn's disease patients[140]. However, limited evidence exists regarding other roles of the kynurenine pathway in the intestine, emphasising the need for further investigation in this area.

Regarding intestinal barrier functions, serotonin has displayed both protective and detrimental effects on the intestinal system, with outcomes varying based on the methodology employed in different studies. The administration of serotonin has been shown to alleviate LPS-induced intestinal inflammation *in vitro*[90]. Neuronal, rather than mucosal, serotonin has promoted intestinal mucosal growth in mice[141]. Contrary, increased levels of mucosal serotonin have been associated with aggravated inflammation[142] and inhibiting the production of mucosal serotonin has shown potential in attenuating inflammation in mice with intestinal inflammation[143]. Moreover, while serotonin administration led to decreased intestinal permeability in healthy controls, it resulted in decreased expression of the tight junction protein occludin in patients with irritable bowel syndrome[144]. Conversely, melatonin, derived from serotonin and known for its anti-inflammatory properties, has demonstrated the ability to reduce intestinal permeability[145].

Clinical implications

To the best of our knowledge, the relationship between whole grains as a hybrid source of dietary fibre and protein, tryptophan metabolism, intestinal microbiota and intestinal barrier function has not been previously addressed simultaneously. The interplay between these factors is intricate and complex, and disruptions in the delicate balance in the gastrointestinal tract can lead to increased intestinal permeability and the development of associated diseases. Despite the encouraging discoveries in this area of research, the evidence regarding the effects of whole grain consumption on intestine-related activity remains limited. Hence, it is essential to investigate the mechanisms underlying these interactions to enhance both individual and public health outcomes.

It is important to acknowledge the vast individual variability. Defining the precise portion of whole grains and the ideal composition of intestinal microbiota for optimal responses on the intestinal barrier functions is exceptionally challenging due to these inherent individual variations. Moreover, certain gastrointestinal conditions, such as irritable bowel syndrome, can influence how one reacts to certain metabolites or stimuli compared to healthy comparisons. Thus, it is essential that personalised dietary strategies are implemented based on physiological assessments. Despite this complexity, the positive effects of whole grain consumption are inevitable, and even moderate consumption can lead to numerous improvements in both the intestinal environment and overall health.

Intestinal microbiota and intestinal barrier function are crucial factors in mediating the health effects associated with the whole grain consumption. Notably, the effects of the intestinal microbiota and intestinal barrier function extend beyond the colon and can impact other

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parts of the body. For instance, intestinal microbiota can influence the gut–brain axis. This modulation occurs through the production of various metabolites, particularly tryptophan metabolites, as well as the modulation of the intestinal barrier[44,49,146]. Furthermore, the intestinal microbiota and its metabolic activity are involved in the bidirectional connection between the intestine and the liver, referred to as the gut–liver axis, and are associated with liver diseases through this axis[147,148]. These pathways present promising targets for investigating the health impacts of whole grain consumption in the future.

Studies have shed light on the critical role of tryptophan metabolites, particularly indoles, in maintaining the integrity of the intestinal barrier. Further investigations are needed to fully understand the broader physiological significance of these metabolites in the gastrointestinal tract and uncover their potential as a novel approach for supporting both intestinal and overall health. This may require novel methods for determination of concentrations of microbial tryptophan catabolites as well. Moreover, while there is evidence regarding how whole grain consumption can influence growth and metabolic activity of certain bacteria connected with tryptophan metabolism, further investigation is needed to fully comprehend these interactions accurately and potentially uncover previously unknown microorganisms involved in the equation.

Besides the indirect impact of whole grains on tryptophan metabolism through the intestinal microbiota, tryptophan present in whole grains can directly participate in the modulation of metabolism within the colon. Nevertheless, the bioavailability of proteins and other nutrients in the whole grains is restricted due to the presence of fibre and other anti-nutrients. As a result, the potential health benefits derived from these nutrients and whole grains in general are diminished. In addition, despite improvements in assessment techniques, both *in vitro* and *in vivo*, numerous challenges persist. *In vivo* studies are typically expensive and involve invasive procedures, which raise ethical concerns. Because of these ethical limitations, direct access to the human intestinal tract is only allowed under certain circumstances. Furthermore, it is more challenging to control external factors that may introduce confounding effects as well as establishing cause-and-effect conclusions in the habitual environment. Animal studies, in addition to the ethical implications, may produce results that are not extrapolated to human subjects because of the differences in physiology and diet among species. Conversely, *in vitro* models still possess some limitations in replicating the complex processes of human gastrointestinal environment. In the future, we need more food technological approaches to enhance nutrient bioavailability while concurrently refining accurate methods for assessing bioavailability. However, we need to be aware of the potential impact of increasing the bioavailability of both beneficial and unbeneficial elements found in whole grains.

Discrepancy in defining and assessing whole grain intake further complicates drawing unbiased conclusions about associations. Omics technologies are high-throughput techniques that provide high amounts of data about a specific type of molecules including human and bacterial DNA, RNA, proteins and metabolites[149]. While the use of omics technologies may help to identify many potential biomarkers or biomarker profiles related with whole grain consumption and their impact on health, the application of this information in clinical practice may have some challenges. The challenges relate to sensitivity and specificity of the biomarkers as well as to the vast complexity of the metabolic pathways involved and technical issues such as reproducibility and high false-positive rate of omics technologies[150]. Identification of metabolites such as alkylresorcinol, avenacosides and benzoxazinoid-derived phenylacetamide sulphates, and their metabolites, that have been proposed as biomarkers of whole grain consumption[151,152], may be linked in the future with health outcomes in human subjects.

To this end, we can conclude that we are just starting to understand the actual complexity of the intestinal factors mediating in part the health impacts of whole grain cereals, whether beneficial or unbeneficial. The investigation of these underlying factors, such as intestinal microbiota and intestinal barrier function, will also produce overall knowledge on diet–microbiota interactions. We need to study how microbial metabolites impact epithelial cells' metabolism as well as related immune functions that contribute to promoting optimal intestinal barrier integrity. In addition, much more research is needed on the faith of plant protein in the intestine and the factors that have an impact on it, both biological and food processing technologies. A highly interesting future is ahead for nutrition and food sciences and related research on intestinal activities, with much needed to understand benefits of whole grains and underlying mechanisms. This will in turn provide knowledge and means to direct the nutritional advice and related development of healthier plant-based foods towards more personalised approaches.

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Conflict of Interest

None.

Authorship

V. L. drafted the manuscript and had primary responsibility for final content. C. G.-G. and M. K. participated in and supervised writing and editing the manuscript.

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