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Abstract

Tight junction proteins are important for intestinal homeostasis. They prevent paracellular transport of large molecules and maintain cell polarity. Impaired tight junction function leads to a more permeable intestinal epithelial barrier and therefore potentially increases disease risk. Limited information is available concerning the effects of food components on the intestinal barrier, particularly paracellular permeability and tight junction proteins. In vitro studies with intestinal epithelial cells and in vivo studies using animal models have demonstrated that dietary n-3 and n-6 polyunsaturated fatty acids (PUFAs), particularly n-3, can reduce intestinal inflammation and permeability. PUFAs can induce transcriptional regulators which may act in combination with their target molecules in defense against oxidative stress, thereby maintaining the intestinal barrier function. More studies that take into account the type and/or amount of individual fatty acids are needed in order to elucidate the molecular mechanisms of PUFAs on intestinal epithelial barrier function.

Keywords

during, function, barrier, intestinal, acids, fatty, colitis, polyunsaturated, influence

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Influence of polyunsaturated fatty acids on intestinal barrier function during colitis



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KEYWORDS: polyunsaturated fatty acids, intestinal barrier function, intestinal inflammation, oxidative stress.

ABSTRACT: Tight junction proteins are important for intestinal homeostasis. They prevent paracellular transport of large molecules and maintain cell polarity. Impaired tight junction function leads to a more permeable intestinal epithelial barrier and therefore potentially increases disease risk. Limited information is available concerning the effects of food components on the intestinal barrier, particularly paracellular permeability and tight junction proteins. *In vitro* studies with intestinal epithelial cells and *in vivo* studies using animal models have demonstrated that dietary n-3 and n-6 polyunsaturated fatty acids (PUFAs), particularly n-3, can reduce intestinal inflammation and permeability. PUFAs can induce transcriptional regulators which may act in combination with their target molecules in defense against oxidative stress, thereby maintaining the intestinal barrier function. More studies that take into account the type and/or amount of individual fatty acids are needed in order to elucidate the molecular mechanisms of PUFAs on intestinal epithelial barrier function.

INTRODUCTION

The luminal environment of the gastrointestinal tract contains nutrients and beneficial microbes but is also laden with potentially hostile microbes and toxins. Therefore, the intestinal tract has two primary functions; to digest and absorb nutrients, and to act as a protective barrier (microbiological, chemical, epithelial, immunological and neural) between the body and the intestinal lumen.

The epithelial cell layer of the gastrointestinal barrier is an important gatekeeper that determines what will enter the bloodstream. In addition to other forms of intestinal transport (e.g. transcellular carrier-mediated, passive diffusion, facilitated) the paracellular transport pathway is important for the transport of small molecules and large hydrophilic compounds and preventing movement of large molecules.

Tight junctions are a mesh-like network of lipoproteins and filamentous strands traversing the lateral plasma membrane that interact with the adjacent cell's proteins (1, 2, Figure 1). These form a dynamic multi-protein complex that is connected to the actin cytoskeleton (3-8) and by their apical location, they determine paracellular permeability (9). They also restrict transport of membrane lipids and proteins from the basolateral to the apical compartment and vice versa. This ensures the maintenance of cell polarity and the specialized properties of the different compartments such as differential transporter expression (3, 10). Moreover, tight junctions play a crucial role in intestinal cell differentiation, proliferation and polarization, and are part of many intracellular signaling cascades (11). They also act as a docking site for endosome vesicle/cytoskeleton polarized transport pathways (12, 13). Tight junction function is regulated

by a multitude of developmental (14, 15), physiological (16), and pathological stimuli (17). During normal food absorption, the tight junctions loosen to allow nutrient absorption, a process which involves a number of factors including luminal glucose levels and the sodium/glucose-co-transporter-mediated myosin light chain phosphorylation (18-20). Cytokines (5, 21, 22) or hormones (23) also influence tight junction-mediated paracellular permeability. Alteration of the barrier function of the intestine is accompanied by an inflammatory response. When the intestinal barrier is disturbed, antigen uptake is increased and may intensify or even initiate inflammation. Water from the bloodstream is also able to reach the intestinal lumen to a much greater extent, resulting in leak flux diarrhea (24). Impaired tight junction function leads to a more permeable intestinal epithelial barrier, which is thought to be a contributing factor in the occurrence of a number of conditions, including autoimmune and/or allergic diseases such as inflammatory bowel disease (IBD, 25-30), multiple sclerosis (31), type I diabetes (32), asthma (33) and atopic eczema (34).

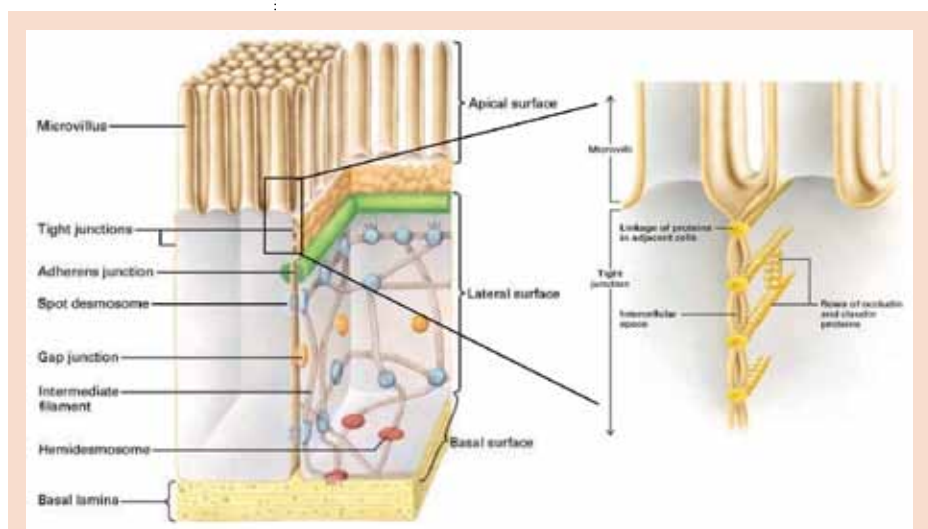


Figure 1. Schematic representation of enterocytes showing the main types of cell junctions (e.g. tight junctions). Tight junctions are a mesh-like network of lipoproteins and filamentous strands traversing the lateral plasma membrane that interact with the cell architecture. (Adapted from HF Lodish, 2000, Molecular Cell Biology, 4th edition, WH Freeman, New York).

There is increasing interest in reducing disease risk based on dietary supplementation with food components which have potential immunomodulatory and barrier protective effects. Limited information is available concerning the effect of food components on intestinal barrier function, paracellular permeability and tight junction proteins. Immunomodulatory and barrier-protective effects have previously been described for glutamine, polyunsaturated fatty acids (PUFAs), zinc, and polyphenols. This short review focuses on the effects of dietary PUFAs on intestinal barrier function in the context of intestinal inflammation.

IMPACT OF PUFAs ON INTESTINAL HOMEOSTASIS

Several immunomodulatory effects on PUFAs in intestinal inflammation have recently been investigated, e.g. modulation of pro- and anti-inflammatory eicosanoids, membrane fluidity, signal transduction, antigen presentation, gene expression and gastrointestinal flora (35). Eicosanoids act directly on immune cells and are modulated themselves by PUFAs (35). PUFAs play an important role in membrane structure where their incorporation into cellular membrane phospholipids increases membrane fluidity, affects the conformation of membrane protein complexes and serves as a precursor to eicosanoid synthesis (36). Moreover, PUFAs can stimulate cell differentiation, support intestinal maturation, reduce transcellular permeability and may improve tight junction formation (37, 38) and have been shown to stabilize the intestinal barrier by increased expression of tight junction proteins (39).

INTESTINAL PERMEABILITY

There are few reports of *in vitro* studies into the effects of n-3 and n-6 PUFAs on intestinal permeability. It has been reported that n-3 eicosapentaenoic acid (EPA), n-6 γ -linolenic acid (GLA), and n-3 docosahexaenoic acid (DHA) affect tight junction permeability in human colonic adenocarcinoma (Caco-2) monolayer cells. This occurs both specifically and concentration dependently, by enhancing permeability and lowering transepithelial electrical resistance (TER) (40, 41). In contrast, EPA and DHA support epithelial barrier integrity in T84 cell monolayers by improving TER and reducing IL4-mediated permeability (42). A role for n-6 arachadonic acid (AA) in supporting barrier integrity has also been suggested (42). Other studies using Caco-2 cells showed that EPA and n-6 linolenic acid (LA) do not affect TER (43), while prolonged LA incubation enhances TER (44). Improved barrier function observed with GLA and EPA appears to be linked to increased expression of the tight junction protein occludin in endothelial cells (38, 45). In another *in vitro* study, DHA and EPA prevented the disruption of epithelial barrier function and redistribution of key tight junction proteins (occludin and ZO1) induced by pro-inflammatory cytokines (46). Understanding the mechanisms by which n-3 PUFAs prevent disruption of intestinal barrier will contribute to the development of therapeutic strategies to intestinal barrier defects.

There is also *in vivo* evidence of PUFAs affecting intestinal barrier function. A diet enriched in n-6 PUFA (safflower oil) reduced the permeability and mucosal neutrophil infiltration in rats with neutrophil-mediated ileal inflammation; this effect was not observed in rats fed an n-3 PUFA-enriched (fish oil) diet (47). AA and DHA have been shown to decrease the development of necrotizing enterocolitis in neonatal rats, most likely by improving barrier function (48). On the other hand, a study showed that dietary n-3 PUFAs reduce clinical colitis and colonic immunopathology possibly by enhancing epithelial barrier function and mucosal wound healing processes in a colitis mouse model (49). n-3 PUFAs also improved the histological signs

of inflammation and prevented redistribution of tight junction proteins in a rat model of experimental colitis (50).

EFFECT ON IMMUNE CELLS

The effects of PUFAs on immune cells and other tissues are partly due to changes in the lipid composition of the cell membrane and alterations to the binding of ligands or signaling molecules. Fish oil contains n-3 PUFAs, such as EPA and DHA, which have been shown to improve the immune response in experimental models of IBD (51) and to have beneficial effects on inflammatory bowel disorders through modulation of immune responses (52). Some membrane-bound enzymes and receptors are sensitive to the fatty acids that surround them. Genome-wide analysis of dietary EPA compared to oleic acid (OA, fatty acid control diet) in the colon of the interleukin-10 gene-deficient (*Il10^{-/-}*) mouse model of IBD identified typical fatty acid responsive genes involved in fatty acid binding, transport and oxidation. This included known peroxisome proliferator-activated receptor (PPAR) target genes and other genes that respond to fatty acids via a mechanism that is likely to be PPAR-independent (53). Dietary EPA increased the expression levels of the *Ppara* gene and some of the PPAR-dependently regulated genes involved in fatty acid catabolism in the colon of *Il10^{-/-}* mice (53). The *Ppara* gene most likely suppresses inflammatory responses via inhibition of NF κ B.

While n-3 and n-6 PUFAs are both natural ligands for PPAR α , n-3 PUFAs are considered as more potent activators of PPAR α than n-6 PUFAs (54). The type of individual fatty acid has to be taken into account in order to elucidate the molecular mechanisms of PUFAs on the intestinal epithelial barrier function. It has been reported that intestinal absorption of the n-9 monounsaturated fatty acid OA, but not its ethyl ester, induces mucosal injury and increases intestinal permeability in the developing piglet intestine (55). This OA-induced mucosal injury is abolished when the carboxylic group of the fatty acid is esterified with a nonpolar ethyl group. These authors also suggest that fatty acids supplied in an ethyl ester form could provide fatty acids to the intestine without causing mucosal damage. Although fatty acid ethyl esters are absorbed and metabolized similarly to free fatty acids, triglycerides containing fatty acid ethyl esters represent a more natural way of delivery.

PUFAs, OXIDATIVE STRESS AND INTESTINAL BARRIER FUNCTION

There is evidence that IBD is accompanied by increased levels of reactive oxygen species and decreased levels of antioxidants (56). Oxidative stress results from diminished cellular antioxidant protection mechanisms and can lead to increased cell damage and apoptosis (57). Oxidative stress has been suggested as a potential trigger for IBD (56). Increased oxidative stress during intestinal inflammation can also lead to increased endogenous oxidation of dietary PUFAs (58), with n-3 PUFAs being more prone to peroxidation than n-6 PUFAs. Sethi et al. (58) showed that oxidized, but not native unoxidized, EPA inhibits lipopolysaccharide-induced endothelial-leukocyte interactions through PPAR α activation during inflammation *in vitro* and *in vivo*. They also suggested that activation of PPAR α by oxidized EPA is responsible for the anti-inflammatory properties of fish oil.

Many genes implicated in oxidative stress, such as cytochrome P450 isoenzymes (CYP450s), glutathione S-transferases (GSTs) and solute carrier (SLC) transporters, are involved in intestinal barrier function (59). The effect of dietary lipids (in the form of synthetic triglycerides consisting of one single unsaturated fatty acid such as EPA, DHA or OA) on small intestinal barrier gene expression in *Ppara^{-/-}* and wildtype mice has been investigated (59). This study found that many intestinal barrier genes were regulated by PPAR α , which seems to be an important receptor

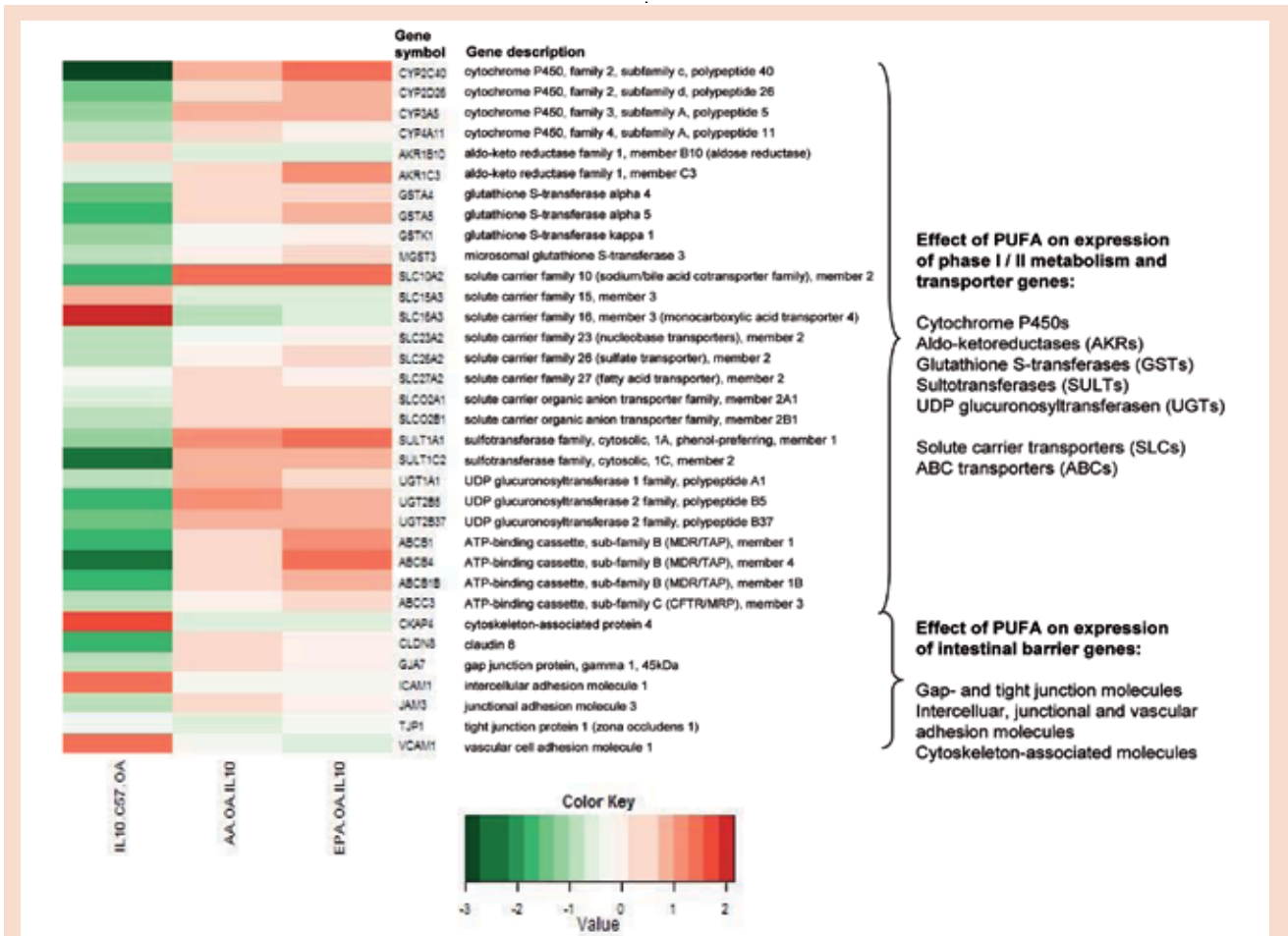


Figure 2. Heat map (supervised hierarchical clustering) showing the expression levels of some of the genes associated with the regulation of the intestinal barrier function that are differentially expressed in the colon of: (a) oleic acid (OA)-fed interleukin-10 gene-deficient (*Il10*^{-/-}) compared to C57 (wild-type) mice on the same diet; (b) arachidonic acid (AA)- compared to OA-fed *Il10*^{-/-} mice, and (c) eicosapentaenoic acid (EPA)- compared to OA-fed *Il10*^{-/-} mice. Up-regulated genes are represented in red tones and down-regulated genes in green tones.

mediating the impact of dietary lipids on the intestinal barrier. Similarly, the expression levels of several genes coding for CYP450s and GSTs were increased in the colon of EPA- and AA-fed *Il10*^{-/-} mice compared to OA-fed *Il10*^{-/-} mice (53, 60, Figure 2). CYP450s are phase I metabolic enzymes that induce oxidative stress by oxidative, peroxidative and reductive metabolism of endogenous substrates such as fatty acids (61), whereas GSTs are phase II metabolic enzyme genes involved in oxidative stress defense (62). The expression levels of other phase II enzyme genes such as sulfotransferases (SULTs) and UDP-glucuronosyltransferases (UGTs) were also increased in the colon of *Il10*^{-/-} mice after feeding EPA- and AA-enriched diets (Figure 2). These represent target genes of the hepatocyte nuclear factor 4a (HNF4a) which, itself, was not modulated under inflammatory conditions (Figure 3). HNF4a plays a crucial role in the promotion of intestinal epithelial barrier function (63). It increases the abundance of cell junction proteins

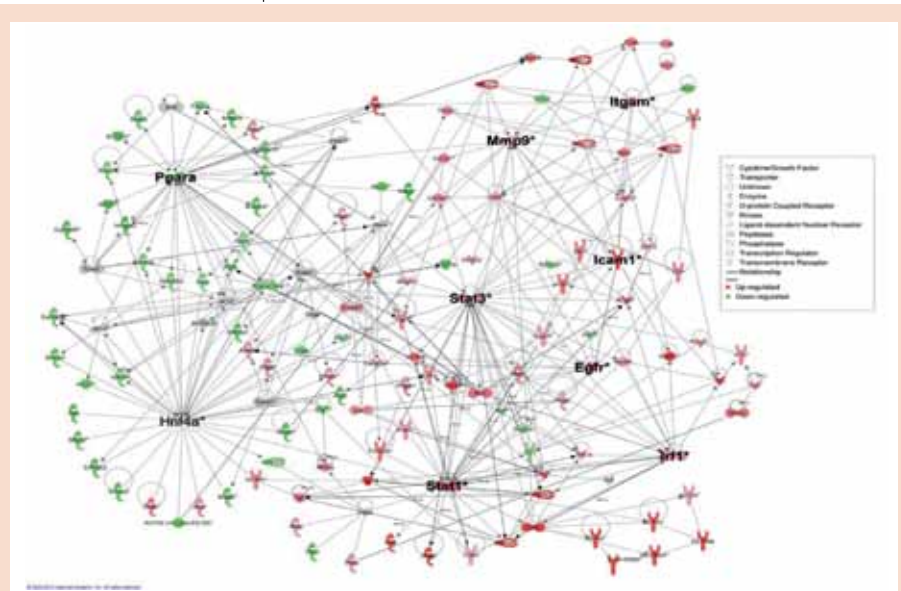


Figure 3. Biological interaction network of genes associated with the top-regulated pathways in the colon of interleukin-10 gene-deficient (*Il10*^{-/-}) mice compared to C57 (wild-type) mice fed an oleic acid (OA) diet, generated using Ingenuity Pathway Analysis software. The "Neighborhood Explorer" feature was used to investigate central nodes (in bold) in order to find molecules that directly interact with other molecules in the network. Most down-regulated genes associated with the regulation of intestinal barrier function linked to peroxisome proliferator-activated receptor alpha (*Ppara*) and hepatic nuclear factor 4 alpha (*Hnf4a*) genes which itself remained unchanged (not in bold). Genes or gene products are represented as nodes, and the biological relationship between two nodes is represented as an edge. All edges are supported by at least one reference from the literature. Color coding: red, up-regulated gene; green, down-regulated gene. The intensity of the colours specifies the degree of up- or down-regulation. Greater intensity represents a higher expression level. *Genes that are detected two or more times on the microarray.

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such as occludin and claudins (CLDNs), and modulates subcellular localization of proteins related to epithelial polarization (64). Some SLCs are also involved in oxidative stress defense and, together with CYP450s and GSTs, are PPAR α - or HNF4 α -dependently regulated. The biological interaction network generated from published microarray data (53, 60) shows decreased expression levels of these PPAR α - and HNF4 α -dependently regulated genes in the inflamed colon of *Il10*^{-/-} mice fed OA diet compared to C57 (wild-type) mice (Figure 3), whereas dietary EPA and AA, which reduced colon inflammation, had the opposite effect. Expression levels of genes coding for junction molecules (*Cldn8*, *Gja7* and *Tjp1*), the latter of which are cytoskeleton-associated molecules, remained unchanged in EPA- and AA-fed *Il10*^{-/-} mice compared to OA-fed *Il10*^{-/-} mice, but *Cldn8* and *Gja7* were decreased in abundance in OA-fed *Il10*^{-/-} compared to C57 mice (Figure 2).

Literature reports and the biological network presented here suggest that PUFAs can induce transcriptional regulators which might act in combination with their target molecules in defense against oxidative stress to maintain intestinal barrier function. Together these results provide insights of potential important roles for n-3 and n-6 PUFAs in maintaining/improving intestinal barrier function and thereby attenuating intestinal inflammation. Further studies that utilize post-genome technologies (including epigenomics, proteomics and metabolomics), and that take into account the type and/or amount of individual fatty acids, are required to further elucidate the molecular mechanisms of PUFAs. These may be of particular importance in developing therapeutic strategies that address intestinal tight junction barrier defects in IBD.

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