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Gastroenterology, 2016; 150(6):1305-1318e8

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Published at: <http://dx.doi.org/10.1053/j.gastro.2016.02.028>

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**4 March 2021**

<http://hdl.handle.net/2440/129864>

## THE INTESTINAL MICROENVIRONMENT AND FUNCTIONAL GASTROINTESTINAL DISORDERS

**Short Title:** Microenvironment & FGID

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**Abbreviations:** 5-HT, 5-hydroxytryptamine, serotonin; 5-HTTLPR, 5-HT-transporter-gene-linked polymorphic region; AA, amino acid; BA, bile acid; BAM, bile acid malabsorption; CCK, cholecystikinin; CH<sub>4</sub>, Methane; CO<sub>2</sub>, carbon dioxide; CRF, corticotropin releasing factor; DCA, deoxy cholic acid; EC, enterochromaffin cell; FD, functional dyspepsia; FGF, fibroblast growth factor; FGID, functional gastrointestinal disorder; FODMAPs, fermentable oligosaccharides, disaccharides, monosaccharides, and polyol; FXR, farnesoid x receptor; GI, gastrointestinal; H<sub>2</sub>, Hydrogen; H<sub>2</sub>S, Hydrogen Sulphide; HLA, Human leukocyte antigen; IBAT, apical sodium-dependent ileal bile acid transporter; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; IBS-C, irritable bowel syndrome with constipation; IBS-D, irritable bowel syndrome with diarrhea; IBS-M, irritable bowel syndrome with mixed bowel habit; IFN, Interferon; IL, interleukin; JAM, junctional adhesion molecules; KYNA, kynurenic acid; MLCK, myosin light chain kinase; MUC, mucin; NCGS, non-celiac gluten sensitivity; NGF, nerve growth factor; PAR, proteinase activated receptors; PDS, post-prandial distress syndrome; SCFA, short chain fatty acids; SERT, serotonin reuptake transporter; SIBO, small intestinal bacterial overgrowth; SLC6A4, solute carrier family 6 (neurotransmitter transporter), member 4; TJ, tight junction; TLR, toll-like receptor; TNF, tumor necrosis factor; TPH, tryptophan hydroxylase; TRPV, transient receptor potential cation channel, subfamily V; ZO, zonula occludens

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**Conflict of interest:** None to declare.

**Author Contributions:** The working team was led by EQ and GB. All working team members contributed equally to the manuscript.

**Acknowledgements:** The authors would like to thank Maria Raffaella Barbaro for editing the manuscript.

## **ABSTRACT**

For decades, interactions between the enteric neuromuscular apparatus and the central nervous system have served as the primary focus of pathophysiological research in the functional gastrointestinal disorders. The accumulation of patient reports, as well as clinical observations, has belatedly led to an interest in the role of various luminal factors and their interactions with each other and the host in functional gastrointestinal disorders. Most prominent among these factors has been the role of food. As a consequence, while not always evidence-based, dietary interventions are enjoying a renaissance in irritable bowel syndrome management. Not surprisingly, given its exploration in many disease states, the gut microbiota has also been studied in functional gastrointestinal disorders; data remain inconclusive. Likewise, there is also a considerable body of experimental and some clinical data to link functional gastrointestinal disorders pathogenesis to disturbances in epithelial barrier integrity, abnormal entero-endocrine signaling and immune activation. These data provide growing evidence supporting the existence of micro-organic changes, particularly in subgroups of patients with functional dyspepsia and IBS. However, their exact role in the complex pathophysiology and symptom generation of functional gastrointestinal disorders needs to be further studied and elucidated particularly with longitudinal and interventional studies.

**Keywords:** microbiota; bile acids; serotonin; immune system; food; irritable bowel syndrome; functional dyspepsia

## INTRODUCTION

Though the focus of studies on the pathophysiology of functional gastrointestinal disorders (FGIDs) has been largely on the enteric neuromuscular apparatus and its central connections through the gut-brain axis, the potential importance of the luminal environment was noted many decades ago in the first descriptions of FGID-type symptoms developing *de novo* in the aftermath of an enteric infection.<sup>1</sup> Clinical experience informed us of the importance of food as a symptom precipitant yet, up until very recently, little research had been performed on interactions with diet and/or the products of digestion in the FGID sufferer. As the complexities of the human microbiota are increasingly understood, the possibility that microbe-host interactions, including immune and metabolic responses, might be relevant to the FGIDs has emerged. How any one or a combination of these luminal factors interact with each other and with the host is a subject of considerable research interest and putative pathophysiological mechanisms have been postulated (Figure 1).

These will be explored further in this review. Caveats that may limit the outcome of the present review must be acknowledged. Although we aim to refer to human studies, animal data could be mentioned when instrumental to better understand the role of microenvironmental factors in FGID. As most studies have been conducted in patients suffering from functional dyspepsia (FD) and irritable bowel syndrome (IBS), we will address only marginally other FGIDs. Pharmacological and other interventional approaches involving the intestinal microenvironment will not be systematically reviewed here.

## FOOD

There is increasing recognition that dietary factors may play a major role in the etiology and the pathogenesis of symptoms in both FD and IBS. Their impact could be mediated through direct interactions between dietary components and mucosal receptors that may have been sensitized to these stimuli, or via down-stream events triggered by dietary components, such as the release of gut hormones, changes in epithelial morphology, the generation of immune responses or altered signaling between the gut and the brain.

Dietary factors that reportedly trigger symptoms include eating patterns, as well as specific foods and/or food components. Only a few small studies have evaluated the direct effects of administering specific foods or nutrients on symptom provocation. No intervention studies have evaluated the impact of targeted dietary changes on symptom improvement.

Although patients with IBS have long associated their symptoms with food ingestion, a focused scientific and clinical interest in the potential role of food in IBS has emerged only recently.

### Role of Diet

No major differences have been found in eating patterns between FD patients and controls, although limited evidence suggests that patients eat fewer meals per week, and tend to eat more smaller meals/snacks, than controls.<sup>2</sup> While up to 80% of patients report that fatty foods/meals induce their symptoms, and ~30% exclude fried foods to avoid symptoms, many other foods are also reported to induce symptoms.<sup>3</sup> Data on dietary nutrient composition in FD are limited and inconsistent; possibly because some patients modify their diets in an attempt to alleviate symptoms. The only available prospective study, in FD patients, noted trends towards lower fat and energy intakes and direct relationships between, on the one hand, postprandial fullness and fat and energy intake and on the other, between bloating and fat intake.<sup>2</sup> Wheat- and carbohydrate-containing foods have been identified as triggers for symptoms, and FD patients frequently report symptoms on exposure to milk and dairy products, although their role remains unclear. Data on fiber intake in FD are inconsistent.

The majority of IBS patients associate ingestion of a wide range of foods with symptoms, particularly abdominal bloating and pain.<sup>4</sup> Patients frequently report making dietary adjustments, including reduced consumption of milk products, wheat products, alcohol and certain fruits or vegetables that are high in poorly absorbed short-chain carbohydrates and sugar alcohols (e.g. onions) and an increased intake of other fruits high in fermentable oligosaccharides, monosaccharides and polyols (FODMAPs) (e.g. grapes and pears).<sup>5</sup> Data on such dietary adjustments in IBS are not consistent. Many IBS patients report symptoms in

response to wheat-containing products, reminiscent of the sensitivity to gluten that characterizes celiac disease, despite negative celiac serology and normal small intestinal morphology; a phenomenon that has been termed “non-celiac gluten sensitivity” (NCGS). Subsets of patients also report symptoms following consumption of milk and dairy products, or spicy foods. The view that a lack of dietary fiber was the main cause of IBS has largely been revised. While soluble fiber may have some beneficial effects, insoluble fibers, including bran, appear to be neither of benefit, nor harm.

### **Provocation of Symptoms**

Prospective studies have only evaluated the effects of fat on symptoms. While equicaloric high-fat and high-carbohydrate yogurt-based meals both increased FD symptoms, pain, fullness and nausea were greatest after the high-fat meal.<sup>3</sup>

Studies that manipulate dietary constituents provide further insights. Ingestion of a high-FODMAP diet worsened symptoms (abdominal pain, bloating, excessive flatus) in patients, compared with healthy controls or a low-FODMAP diet. Moreover, in patients on a low-FODMAP diet, blinded re-challenge with fructose and/or fructan, but not glucose, exacerbated symptoms.<sup>6</sup> The role of gluten in IBS remains uncertain. While one recent study in patients with diarrhea-predominant IBS and “NCGS” found an improvement in symptoms on a gluten-free diet, and their relapse when gluten was re-introduced in a blinded fashion, another study was unable to confirm gluten-specific, as distinct from FODMAP-related, effects on symptoms.<sup>7</sup> In another study, exposure to gluten increased stool frequency and altered gut barrier function; mainly in IBS-D subjects who were human leukocyte antigen (HLA)-DQ2 or DQ8 positive. While some studies have found an improvement in symptoms on a milk- or dairy-free diet, these trials were often not blinded. Intolerance may also exist towards other components of milk. Acute ingestion of hot chili powder, in a capsule with a meal, increased abdominal pain and burning in IBS patients, compared with healthy controls.

### **Potential Mechanisms**

The limited research that has been performed suggests that symptoms generated by food ingestion in FD may be due to exaggerated signals originating in the upper GI tract, including gastric hypersensitivity to distension, small intestinal hypersensitivity to fat, and hypersensitivity to the effects of gut hormones (particularly cholecystikinin), acid, capsaicin and the products of colonic fermentation.<sup>3</sup>

Several factors could contribute to the pathophysiology of food-related symptoms. An enhanced phasic colonic motor response to food ingestion and colonic hypersensitivity to distension may both contribute to a non-specific increase in abdominal symptoms postprandially in IBS. FODMAPs are osmotically active and increase water content in the intestinal lumen. They are rapidly fermented to hydrogen (H<sub>2</sub>), carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), short chain fatty acids (SCFAs) and lactate. Such responses could be exaggerated in IBS; the resulting distension of the intestinal lumen may exacerbate visceral hypersensitivity. Gluten may cause a mild immune response in IBS patients, associated with exaggerated responses in enteric and sensory nerves and compromised intestinal barrier function.<sup>8</sup> Some of the adverse reactions attributed to “gluten” may reflect a hypersensitivity to wheat or intolerance to FODMAPs. GI symptoms attributed to wheat (the largest dietary fructan source) may also relate to FODMAPs, rather than gluten.<sup>7, 8</sup> Thus, the term “wheat intolerance or sensitivity” may be more appropriate than NCGS. A high prevalence of autoimmune disease amongst patients with wheat sensitivity has been described.<sup>9</sup> Lipids may exacerbate IBS symptoms through modulation of distal gut motor functions and sensitivity. An increase in the density of sensory fibers expressing transient receptor potential cation channel, subfamily V (TRPV)-1 receptors in IBS patients with visceral hypersensitivity may enhance transmission of pain signals, including those generated by spicy foods. Recently a role for TRPV4 has also been proposed as a possible pathway of pain transmission in patients with IBS (see below).<sup>10</sup>

### **Translational Research**

Prospective studies evaluating the effects of dietary interventions in FD are urgently required.

While recent studies have reported beneficial effects of a low-FODMAP diet on symptoms,<sup>11</sup> stool habits and quality of life, studies were small in size and further evidence is required to determine whether a low-FODMAP diet is better than a standard diet in controlling symptoms in IBS. Furthermore, the observed effects on the reduction of fecal commensal bifidobacteria, and detrimental effects on gut microbiota composition,<sup>12</sup> require further investigation. A gluten-free diet improves IBS symptoms and reduces bowel frequency and intestinal permeability.<sup>13</sup> <sup>14</sup> The gluten-specificity of these effects remains to be established. Comprehensive dietary counseling, including the adoption of healthy eating habits, avoidance of foods rich in FODMAPs, insoluble fiber and artificial sweeteners, replacing wheat with spelt products, and the importance of ingesting dairy products, has been reported to be associated with a significant reduction in IBS symptoms, including abdominal pain, diarrhea (in IBS-D) and constipation (in IBS-C), and a marked improvement in the quality of life.<sup>15</sup>

## THE MICROBIOTA AND ITS METABOLIC INTERACTIONS

When food enters our intestine the undigested components are utilized by the intestinal microbes, collectively called the GI microbiota. The microbiota is dominated by bacteria belonging to the phyla Firmicutes, Bacteroidetes and Actinobacteria. These microbes inhabit the various regions in the GI tract of which the colon is most densely populated. The microbiota has a major impact, not only on processes that occur in the GI tract but also on systemic functions, and thus plays a key role in our overall health.

### Impact of Diet and Lifestyle on Microbiota

It is evident that lifestyle and diet are crucial determinants of microbiota composition and function in humans. Comparative studies have demonstrated huge differences in microbiota composition between human populations in Western and those in developing countries and suggested that these are based on lifestyle and long-term dietary pattern differences.<sup>16</sup> Short-term dietary changes have also been shown to impact the composition of the microbiota. To date, such alterations have been shown in intervention studies which involve quite drastic changes in diets while more subtle, short-term dietary interventions have, in general, only a minor impact on the microbiota composition.<sup>17</sup> The impact of diet on the microbiota may be direct, through changes in its composition or total energy supply, or indirect via the induction of changes in intestinal transit time or intraluminal pH. Of note, the impact of diet on the microbiota is also highly dependent on the intestinal location. For example, the conversion of complex indigestible carbohydrates is the driving force for the microbiota in the colon, while the microbiota in the small intestine is largely driven by the fast uptake and conversion of sugars that are likely derived from digested dietary polysaccharides.<sup>18</sup>

## Microbiota Metabolism of Dietary Substrates in Functional Gastrointestinal Disorders

### Carbohydrate

Complex dietary components can be converted by the microbiota to a wide variety of metabolites which may involve cross-feeding and syntrophic interactions between individual microbes.<sup>17, 19</sup> Which metabolites are produced and in what quantities, is dependent on the dietary components. The fermentation of complex carbohydrates, such as fibers and resistant starches, results, in general, in the production of short chain fatty acids (SCFAs), notably acetate, propionate and butyrate; on a Western diet approximately 300 mmol of SCFA are produced daily. Since SCFAs are fuels for our intestinal cells and serve as signaling molecules, they are considered as beneficial, particularly butyrate and propionate. Butyrate can be produced by a wide variety of bacteria, and most well-known are *Faecalibacterium prausnitzii*, *Eubacterium rectale*, *Eubacterium hallii*, and *Roseburia intestinalis*.<sup>19</sup> Sources for butyrate production include sugars, lactate, acetate, as well as amino acids, such as lysine.<sup>20</sup> This allows butyrate producers to engage in metabolic cross-feeding interactions with organisms that convert complex food components. Propionate fermentation occurs via three distinct pathways, of which the succinate pathway is the most commonly utilized route in the gut, mainly performed by *Bacteroides spp.* and *Veillonella spp.*<sup>19</sup> Acetate can be produced by a wide variety of microbes in the gut from fermentation of carbohydrates in a so-called mixed

fermentation with lactate or other SCFAs such as propionate. Acetate may also be generated via reductive acetogenesis, the reduction of carbon dioxide with  $H_2$ , a process that is estimated to be responsible for one third of total acetate production in the intestine.

A few human studies suggest a role for SCFAs in FGIDs and imply that nerves are involved. For example, a reduction in abdominal pain in IBS patients administered sodium butyrate was observed.<sup>21, 22</sup> It was speculated that butyrate reduced the hypersensitivity of intestinal mechanoreceptors and altered neurotransmitter release, resulting in a reduction in luminal pressure and/or peristalsis. Others have observed higher levels of acetic acid, propionic acid and total organic acids in IBS patients, with higher acetic acid levels being associated with greater GI symptoms.<sup>22, 23</sup>

Carbohydrate fermentation also results in the production of  $H_2$  and  $CO_2$ , which are the main intestinal gases formed in the intestine by the microbiota. Whereas impaired handling of intestinal gases has been consistently described in IBS, the contribution of the microbiota to this phenomenon is far from clear.  $H_2$  is thought to inhibit fermentation, but can also serve as an energy source for a variety of microbes, including methanogenic Archaea, reductive acetogens and sulfate reducers.<sup>17-19</sup> The latter group produces sulfide, a toxic component that is regarded as harmful to our health. Potential sources of the required sulfate include dietary components and host-derived substrates, such as mucin (MUC). Although the relative volumes of intestinally derived gases excreted in the breath have been used to relate FGID symptoms to microbial fermentation rates *in situ* in the gut, this extrapolation is fraught with problems due to cross-feeding between different microbial populations, such as has been described above, resulting in altered relative concentrations of intestinal gasses that together determine the total volume.

Relatively little data is available on qualitative changes in gas composition in FGIDs. Increased  $CH_4$  production in constipation predominant IBS-C<sup>24</sup> is well known, but it is unclear whether this is a cause or effect. One study correlated  $CH_4$  with a higher motility index in IBS patients.<sup>25 24</sup> Hydrogen Sulphide ( $H_2S$ ) signals through multiple pathways, including nerves, but human studies are lacking to demonstrate a role.

#### Protein

Protein utilization requires protease activity, which is available in both man and microbes. Although less frequently studied than carbohydrate fermentation, microbial fermentation of protein is, in general, considered as potentially harmful to health, since AA fermentation can lead to toxic products such as amines and ammonia as well as N-nitroso, indolic, sulfur and phenolic compounds.<sup>26</sup> Potential sources of proteins for fermentation include diet and host-derived compounds. Although most proteins are digested and taken up by the small intestine, a high-protein diet could lead to the arrival of significant protein loads in the colon. Since microbes favor carbohydrate fermentation over protein fermentation, it has been speculated that low carbohydrate diets may promote protein fermentation in the intestine.

A recent study showed that concentrations of fecal proteases were higher in IBS-D patients compared to healthy controls, suggesting enhanced protein metabolism in the colon.<sup>27</sup> Remarkably, most of these proteases were of human origin. Nevertheless, it is conceivable that increased protease activity in the colon may lead to higher rates of AA fermentation.

#### Fat

Dietary fat content has also been negatively correlated with health status. In contrast, however, to carbohydrates and proteins, fat is not believed to reach the colon and be exposed to its microbiota in significant amounts since most is digested and absorbed in the small intestine. One indirect effect of dietary fat assimilation is its facilitation of the diffusion of bacterial components, such as lipopolysaccharide, across the epithelium, which could lead to low-grade inflammation, such as some have described in IBS.<sup>28</sup>

### **Microbiota Structure and Functional Gastrointestinal Disorders**

A recent report of the Rome Foundation on the microbiota in FGIDs provided an excellent overview of the importance of the microbiota in health and disease and, especially, in relation to FGIDs.<sup>29</sup> Figure 2 provides a schematic representation on the role of the intestinal microbiota in conversion of dietary components and their potential impact on the



pathophysiology of FGIDs. Several lines of evidence suggest the involvement of the intestinal microbiota in the pathogenesis of FGIDs in general and IBS in particular:

- 1) gastrointestinal (GI) infections are strong risk factors for the development of FD and IBS (see below);
- 2) fecal microbiota is substantially different in IBS and post-infectious IBS compared with healthy controls, and shows reduced microbiota diversity;<sup>29</sup>
- 3) innate and adaptive immunity directed to microbiota-derived molecules, including the expression of toll-like receptors (TLRs) in the mucosa, the production of human beta defensin-2 and antibodies to bacterial flagellin are substantially different in IBS compared with controls;<sup>29</sup>
- 4) some evidence indicates the existence of abnormal concentrations of fermentation end-products, such as SCFAs, which may participate in symptom production in some patients with IBS (see above); one recent study showed that total SCFA level was significantly lower in C-IBS patients than in D-IBS and M-IBS patients and healthy controls;
- 5) case-control studies show that systemic antibiotic use is a risk factor for the *de novo* development of FGIDs;<sup>30</sup>
- 6) in 43 RCTs the RR of IBS symptoms persisting with probiotics vs. placebo was 0.79 (95% CI 0.70-0.89), with positive effects on global IBS, abdominal pain, bloating, and flatulence scores.<sup>31</sup>

Nonetheless, major limitations still hamper the definition of the role of the microbiota in FGIDs. Indeed, there is no consensus on the nature of the microbial signatures that may be consistently (either positively or negatively) correlated to FGIDs. These inconsistencies may relate to several factors, including: 1) methodological differences, 2) variations in sample sources, 3) intrinsic variability between subjects, 4) differences in subject selection and definition of study populations, 5) overlap between the various FGIDs, and 6) differences in diet, therapy or other environmental exposures. It needs to be recognized that many studies described comparisons between different groups of subjects on the basis of a single fecal sample per subject, which only represents a snapshot of the microbiota and, as a result, such comparative analyses cannot differentiate between cause, consequence or coincidence. Given the large heterogeneity in the human population and the extent of microbial diversity, it is likely that many significant correlations are just coincidence. This may, in part, explain why there has been no consensus regarding whether a specific microbe or groups of microbes is associated with a given FGID.<sup>29</sup> Therefore, it is evident that longitudinal studies, involving repeated sampling of the microbiota, will be crucial to differentiate cause from consequence or coincidence. Such studies could include interventions with dedicated diets or dietary supplements, specific pharmacological interventions, or novel therapies such as fecal microbiota transplantation.

## BILE ACIDS

Bile acids (BAs) play a central and critical role in the digestion and absorption of fat and fat-soluble vitamins, and a highly efficient enterohepatic circulation ensures the conservation of secreted BAs; the primary means of BA conservation being active absorption via the apical sodium-dependent ileal BA transporter (IBAT) located on the apical surface of ileal enterocytes. BA absorption and secretion are closely linked through a feed-back loop which involves a number of receptors and mediators which ultimately impact on the rate-limiting enzyme in BA synthesis (Figure 3).<sup>32</sup>

BAs have a variety of physiological effects of relevance to the FGIDs; on motility, intestinal secretion, membrane permeability and visceral sensation<sup>33</sup> and act as important signaling molecules with effects well beyond the GI tract. As BAs repress bacterial growth in the intestine, the development of microbial enzyme pathways capable of deconjugating and transforming BAs is an important adaptive response by commensal bacteria.<sup>34</sup> In contrast, anti-bacterial and mucosal immune-stimulating effects of BAs play an important role in the prevention of small intestinal bacterial overgrowth (SIBO).<sup>35</sup>

Human physiological studies suggest a role for luminal BA signaling to enteric nerves in causing altered small bowel motility and increased sigmoid and rectal motility. In IBS-D, it was

estimated that as many as 10% of patients malabsorb BAs<sup>36 37</sup> and infusion of BAs in the colon disproportionately stimulated motility compared to controls. In idiopathic BA malabsorption (BAM),<sup>38</sup> phase III-induced neurogenic secretions were increased in the jejunum and prostigmine increased the colonic motility index, implying involvement of the enteric nervous system<sup>39</sup>. Genetic variants of the G protein-coupled bile acid receptor (TGR5), found on multiple cells including enteric nerves, have also been linked to transit time in patients with FGIDs.<sup>37</sup> Altered metabolism of BAs by colonic bacteria might also be involved, as constipation and increased transit time correlated with a reduction of colonic BAs, possibly the result of bacterial sulfation.<sup>40</sup>

## **EPITHELIUM AND MUCOSAL BARRIER**

The intestinal luminal-mucosal interface represents the first location where toxic and immunogenic particles face the scrutiny of the mucosa-associated immune system. Loss of molecular and functional integrity of the epithelial barrier could lead to activation of mucosal immune responses and set in motion events that are closely related to the origin and clinical manifestations of several FGIDs.

### **Molecular Structure**

The apical junctional complex keeps enterocytes tightly sealed and regulates paracellular permeability.<sup>41</sup> This complex is composed of tight junctions (TJs), adherens junctions and desmosomes. Intracellular (zonula occludens (ZO)-1, ZO-2 and ZO-3, and cingulin) and surface-membrane proteins (occludin, claudins, and junctional adhesion molecules (JAMs)) are major components of TJs.<sup>41</sup> Adherens junctions are mainly composed of e-cadherin, catenin and actin filaments.<sup>41</sup> Occludin seems to regulate the integrity of TJs while claudins determine their strength, size and ion selectivity, and JAMs their construction and assembly.<sup>42</sup> All are linked to actomyosin fibers by members of the ZO family and, in this way, control the opening/closing of TJs at paracellular spaces.<sup>43</sup> Zonulin transactivates the epithelial growth factor receptor via proteinase activated receptor 2 (PAR<sub>2</sub>) activation and reversibly regulates intestinal permeability.

### **Intestinal Permeability and Barrier Dysfunction**

The passage of molecules across the epithelium takes place mainly via two distinct routes: 1) the paracellular pathway, which allows small molecules (< 600Da) to diffuse through TJs and 2) the transcellular pathway, which facilitates the transit of larger particles via the processes of endocytosis or exocytosis. Rapid changes in permeability usually occur via myosin light chain kinase (MLCK)-mediated cytoskeleton contraction and endocytosis of TJ proteins. In contrast, more sustained changes in permeability involve the transcriptional modulation of TJ proteins, epithelial cell apoptosis and ultrastructural alterations in the epithelium.<sup>41</sup>

Acute stress either reduces net water absorption or increases jejunal secretion in healthy subjects through the parasympathetic nervous system and mast cell (MC) activation.<sup>44</sup> Moreover, higher background levels of stress have been related to decreased water secretion in healthy female volunteers exposed to cold pain stress.<sup>45</sup> Stronger stresses, like abdominal surgery, GI infections, hemorrhagic shock or intensive exercise, increase intestinal permeability. Corticotropin releasing factor (CRF) enhances transcellular uptake of macromolecules in the human colon via CRF-R1 and CRF-R2 receptors on sub-epithelial MCs.<sup>46</sup> Acute psychological stress also increases small intestinal permeability in humans; peripherally-administered CRF reproduces this effect and MC stabilization blocks the effects of both stress and CRF.<sup>47</sup>

In-depth reviews on the role of physiological and pathophysiological stimuli controlling gut barrier have been recently published.<sup>41, 48</sup> Vasoactive intestinal polypeptide regulates chloride secretion, MUC release, and paracellular permeability, partly through a direct effect on ZO-1. Substance P stimulates the release of pro-inflammatory cytokines and vasoactive mediators by macrophages, eosinophils, and MCs, contributing to chloride secretion, increased intestinal permeability and vascular leakiness. Nerve growth factor (NGF) has been involved in nerve- and mast cell-mediated, stress-induced barrier dysfunction. Both progesterone and estradiol

have been shown to reduce chloride secretion in intestinal epithelial cells whereas estradiol reinforced epithelial permeability and up-regulated JAM-A and occludin expression. Other mediators including CRF, leptin, and CCK may increase permeability while insulin-like growth factor, ghrelin, KdPT and glucagon-like peptide 2, may decrease intestinal permeability.<sup>41, 48</sup>

Various strains of *Vibrio cholera*, *Clostridium difficile* and toxin producing strains of *Escherichia coli* have shown to enhance intestinal permeability through direct TJ disruption, the production of toxins or proteases and the activation of the inflammatory cascade. In contrast, probiotics promote barrier integrity by increasing occludin, claudin 3, and ZO-1 and ZO-2 expression.<sup>49</sup>

Interferon (IFN)- $\gamma$  and tumor necrosis factor (TNF)- $\alpha$  induce barrier dysfunction through MLCK and claudin-2 up-regulation and down-regulation of occludin. Many other cytokines and proteases have effects on barrier function including interleukin (IL)-3, IL-4, IL-17, IL-22, and IL-26, IFN- $\alpha$ , IFN- $\beta$ , and transforming growth factors- $\alpha$ , and - $\beta$ .<sup>50</sup>

These aspects have been recently reviewed.<sup>48</sup> In predisposed individuals, gluten and other specific food components may lead to increased intestinal permeability through the zonulin pathway and MC-mediated enhancement of both passage routes. Whey proteins may improve barrier function by a transforming growth factor  $\beta$ -mediated increase in intestinal claudin-4 expression. Other nutritional products such as glutamine, butyrate, arginine, fatty acids, and prebiotics have been shown, to some extent, to exert a protective effect on the intestinal barrier.

Ethanol promotes separation of ZO-1 proteins, disassembly of actin and myosin filaments and MLCK activation. NSAIDs, methotrexate, tacrolimus, omeprazole, and corticosteroids may also enhance intestinal permeability whereas heparin, vitamin D, and larazotide may decrease permeability.

### **Mucosal Barrier and Functional Gastrointestinal Disorders**

There is little information on the status of mucus production in IBS other than isolated reports on the potential beneficial effects of probiotics or mesalazine on mucus quality and production, higher levels of trefoil factor 3 in the urinary IBS proteome, and increased expression of genes involved in the production MUC20 in the colon of IBS.

Enhanced intestinal permeability has been reported in FD and in subsets of patients with IBS (Table 1, Supplementary material) and linked to alterations in JAM-A, ZO-1, e-cadherin, claudins and occludin (Table 2, Supplementary material), and these changes associated to MC activation and clinical manifestations.

### **ENTEROENDOCRINE SYSTEM**

The entero-endocrine system, the largest endocrine organ, constitutes 1% of the gut epithelium. Fourteen cell populations including enteroendocrine cells and enteric nerves produce transmitter substances that signal to neighboring cells (paracrine), distant targets via the vascular system (endocrine) or through intrinsic/extrinsic nerves (neurocrine). These effector targets, in turn, control gut motility, secretion, sensation, absorption, vascular tone, microcirculation, immunity and cell proliferation (Figure 4; Table 3, Supplementary material).<sup>51-53</sup>

#### **Serotonin Metabolism and Receptors**

Serotonin (5-hydroxytryptamine, 5-HT), is a paracrine/neurocrine amine primarily contained in the gut (95%) and only minimally in the brain (5%). Serotonin is synthesized from tryptophan, in enterochromaffin cells (EC) (90%) and autonomic nerves (10%).<sup>54, 55</sup> Synthesis and release of serotonin involves conversion of dietary tryptophan to 5-hydroxy-L-tryptophan (catalyzed by tryptophan hydroxylase [TpH], isoforms, TpH1 [in gut] and TpH2 [in brain]),<sup>55-57</sup> granular packaging by vesicular monoamine transporter 1<sup>58</sup> and release, mainly determined by bowel wall distension, mucosal stroking, food, AAs, hypo- or hyper-osmotic solutions, glucose, galactose, adenosine, cholera toxin and chemotherapeutic agents.<sup>58, 59</sup> SCFA, which may be produced in increased amounts by intestinal microbiota fermentation of carbohydrate substrates, may also promote the release of serotonin.<sup>58</sup> An alternative metabolic pathway leads to the production of kynurenic acid (KYNA), and not serotonin, from tryptophan, and

results in reduced serotonin synthesis.<sup>60</sup> The serotonin re-uptake transporter (SERT) terminates serotonin action (Figure 4).<sup>56</sup>

In the gut, serotonin stimulates intrinsic primary afferent neurons (IPANs), which synapse in myenteric plexus with ascending and descending inter-neurons to evoke motility- and secretion-induced reflexes and also transmitting information to the brain.<sup>54, 56, 61</sup>

### Serotonin Metabolism in Functional Gastrointestinal Disorders

SERT hyperactivity may lead to increased reuptake of serotonin, hence reducing the effects of the amine on target tissues. In contrast, hypofunction of SERT may increase serotonin concentrations leading to gut hypercontractility, hypersensitivity, diarrhea and pain.<sup>59 56</sup> Accordingly, in cell lines, infection with enteropathogenic *E. coli* reduced SERT activity.<sup>62</sup> The SERT protein is encoded by a gene on chromosome 17q11 and is composed of 14 exons encoding 630 amino acids. Insertion/deletion of 44 base-pairs in 5-HT-transporter-gene-linked polymorphic region (5-HTTLPR), leading to reduced SERT expression, has been reported in IBS-D.<sup>63</sup> 5-HTTLPR (S/L) was more common than the S/S polymorphism in FD, particularly in the post-prandial distress syndrome (PDS).<sup>64</sup> In an Indian study, solute carrier family 6 (neurotransmitter transporter), member 4 (SLC6A4)-polymorphism and higher levels of 5-HT were associated with IBS, particularly post-infectious IBS and IBS-D.<sup>65</sup> The homozygous S genotype (reducing SERT expression) was more common in IBS-D.<sup>63</sup> A meta-analysis on 25 studies including 3443 IBS patients and 3359 controls, showed that the 5-HTTLPR mutation was associated with IBS-C but not with IBS-D and IBS with mixed bowel habit (IBS-M), and, particularly, among East Asians.<sup>66</sup> More studies are needed to clarify this issue.

The implication of serotonin in FGID has been previously reviewed.<sup>58, 67</sup> Data suggest that subgroups of patients with FGIDs show altered serotonergic signaling (Figure 4). Accordingly, the epigastric pain syndrome (EPS) subtype may have higher basal serotonin levels.<sup>64</sup> In contrast, some data suggest that subsets of patients with FD may have low basal and postprandial plasma levels of serotonin.<sup>68</sup> Studies suggest that IBS-D is associated with elevated, and IBS-C with reduced, serotonin plasma levels.<sup>58, 69</sup> In one study, both serotonin and KYNA levels were lower in the duodenal mucosa and higher in plasma in IBS, than controls, suggesting a contribution from the KYNA pathway.<sup>60</sup> As chili ingestion increases FGID symptoms and granisetron, a 5-HT 3-receptor antagonist, prevents it, serotonin is suggested as the mediator of chili-induced symptoms.<sup>70</sup> Though a low FODMAP diet has been shown to improve IBS symptoms,<sup>12</sup> data on its effect on brain and gut serotonin levels are lacking.

In post-infectious IBS (see below), altered EC cells number has been reported.<sup>59 71</sup> PI-IBS patients had higher rectal mucosal serotonin than non-PI IBS-D and non-diarrheal IBS. The entero-endocrine and immune systems are widely interconnected as suggested by the proximity of immune cells to EC cells.<sup>58, 67</sup> Furthermore, immune cells, including B and T lymphocytes, monocytes, macrophages, and dendritic cells express serotonergic receptors and mast cells, macrophages, and T cells synthesize serotonin from tryptophan.<sup>58</sup> Serotonin is chemotactic for dendritic cells, mast cells and eosinophils and may participate in the recruitment of these immune cells in the intestinal mucosa (see below).<sup>58</sup> Low-grade inflammation, such as has been detected in FGIDs may, in turn, contribute to altered serotonin synthesis and reuptake through changes in SERT expression.<sup>72 73</sup> Th1 responses generate IFN- $\gamma$  and TNF- $\alpha$  which inhibit SERT; Th2 responses, such as occur in parasitic infestations, stimulate IL-13, which increases EC numbers and TNF $\alpha$ , and also, therefore, inhibit SERT.<sup>74</sup> Efficacy for serotonergic agents, such as 5-HT3 receptor antagonists (alosetron, cilansetron, and ondansetron), including a large multicenter trial on ramosetron in female IBS-D patients with promising results,<sup>75</sup> and 5-HT4 agonists (cisapride, tegaserod and prucalopride) in the treatment of IBS-D and IBS-C, respectively, also provide evidence for a role for serotonin in the pathogenesis of FGIDs.<sup>55, 56</sup>

## IMMUNE SYSTEM AND NEURO-IMMUNE INTERACTIONS

### Post-Infectious Functional Gastrointestinal Disorders

The observation that FD and IBS can develop following an episode of acute infectious gastroenteritis supports the involvement of the immune system in the pathophysiology of FGIDs. The mean incidences of post-infectious (PI)-FD and PI-IBS following infection with diverse pathogens (bacteria, parasites or virus) are 9.6% and 10%, respectively, with an overall OR of 2.5 for the presence of a FGID at six months post-infection compared to controls.<sup>31-76</sup> Risk factors for PI-IBS include the severity and duration of the acute infection, female gender and psychological co-morbidity (e.g., hypochondriasis, neuroticism, depression, adverse life events, perceived stress, negative illness beliefs<sup>77</sup>), smoking, being a child at the time of the infection<sup>78</sup> (Figure 5). The pathogenesis of post-infectious FGIDs is multifactorial and involves both pathogen and host factors.<sup>77</sup> In PI-IBS, the colorectal mucosa shows increased infiltration of macrophages, mast cells and intraepithelial lymphocytes as well as PYY-containing enteroendocrine cells.<sup>71, 77</sup> The association of the TNF $\alpha$  SNP rs1800629 with PI-IBS supports the hypothesis of a genetic predisposition possibly contributing to increased epithelial permeability and an inability to resolve an acute inflammatory process.<sup>79</sup>

### Mucosal Immune Activation

Numerous studies have shown increased numbers of mucosal immunocytes (i.e., mast cells, eosinophils and T cells) in adult and pediatric patients with FD and IBS. Several participating factors have been claimed, including food allergy, an abnormal microbiota, BAM, and increased intestinal permeability. The magnitude of the inflammatory response is several-fold less than that seen in acute inflammation in inflammatory bowel disease (IBD). The wide overlap with healthy controls, possible geographic and dietary variation and lack of methodological standardization might explain the failure of some studies to confirm the presence of increased immune cells in FGIDs. The nature of the inflammatory process is also different from that seen in acute GI inflammation in IBD, with no involvement of neutrophils or frank tissue distortion.<sup>29, 80</sup> Eosinophils, usually linked to allergic reactions, have been associated with PDS and early satiety.<sup>81, 82</sup> Increased mast cell numbers have been detected in the stomach and duodenum of patients with FD,<sup>82</sup> in the esophagus of patients with non-cardiac chest pain<sup>83</sup> and throughout the gut in IBS-D and IBS-C,<sup>84</sup> particularly in females, in PI-FD and PI-IBS (for review, see <sup>80</sup>). Genetic factors such as the TNF $\alpha$  rs1800629 genotype<sup>79</sup> have also been implicated. Microbial molecular pattern-mediated activation of innate immunity suggests a pathogenic contribution of the gut microbiota.<sup>85</sup> TLRs are expressed on human submucosal and myenteric neurons<sup>86-88</sup> and altered TLR expression has been observed in IBS tissues. For example, TLR4 expression in colonic mucosal biopsies from IBS patients was increased, particularly in those with IBS-A.<sup>89</sup> TLRs 5 and 2 were also upregulated, while TLRs 7 and 8 were downregulated.<sup>89, 85</sup> Biopsy studies have provided evidence of epithelial permeability changes in IBS patients and bacterial proteases may play a role.<sup>90</sup> Thus, in some IBS patients there may be increased expression of TLRs and/or a disruption of the mucosal barrier and increased bacterial translocation resulting in increased TLR signaling and/or an abnormal immune response to luminal microbes.<sup>85, 89</sup>

### Impact of Immune Activation on Gut Sensorimotor Function

Supernatants obtained following incubation of mucosal biopsies from IBS subjects contained increased amounts of histamine, serotonin, polyunsaturated fatty acid metabolites<sup>80</sup> and proteases, including tryptase, trypsin.<sup>10</sup> The exact source of proteases remains unclear; they may originate from mucosal mast cells, gut bacteria<sup>91</sup> or pancreatic secretions.<sup>27</sup> In adoptive transfer experiments, biopsy supernatants from IBS subjects evoked abnormal functional responses in enteric and sensory nerves of recipient rodents<sup>92-94</sup> and human tissues.<sup>95</sup> These effects were, at least partly, related to immune and endocrine factors, including proteases, histamine and serotonin.<sup>72, 92, 94</sup> Application of biopsy supernatants to human or rodent tissues suggests that, in IBS, serine proteases, or PUFA metabolites, act respectively on PAR receptors<sup>92</sup> and TRPV-4<sup>10</sup>, to mediate visceral pain. In addition to these acute effects, a recent study suggested that the chronic release of immune metabolites could affect the structure of mucosal neural networks in IBS, i.e. increased neuronal density and outgrowth, as well as increased expression of mast cell NGF in the colonic mucosa of patients with IBS compared

with controls.<sup>96</sup> Mucosal supernatants of patients with IBS evoked increased neurite growth and expression of GAP43 (a key neuronal growth protein), when applied to primary cell cultures of rat myenteric plexus or to neuroblastoma cell line SH-SY5Y cultures.<sup>96</sup> Probiotics may have beneficial effects in IBS, through the modulation of immune function. Indeed, *Bifidobacterium infantis* 35624, but not strains of *Lactobacillus salivarius*, was able to reduce a systemic pro-inflammatory cytokine profile along with symptom improvement<sup>97</sup>.

### **Immune Activation and Symptoms**

Though several studies have demonstrated intestinal immune activation in FGIDs, reports of correlations with symptoms have been limited. Correlations were found between colonic mast cell density close to nerves and abdominal pain and bloating, and between bowel habit dissatisfaction, global IBS symptoms and circulating T cells.<sup>84, 98, 99</sup> In addition, immune activation featuring increased small bowel homing T cells has been associated with the intensity of pain, nausea and vomiting in FD.<sup>100</sup> Mucosal mast cells were associated with fatigue and depression, suggesting the potential role of psychological factors in the brain-gut-immune axis in IBS.

## **IMPLICATIONS FOR MANAGEMENT**

### **Diet and Food Components**

Food has long been recognized as an important precipitant of symptoms in FD and IBS. Exaggerated GI and colonic electrophysiological and motor responses to food ingestion have been extensively documented and a variety of hypotheses have been advanced to explain these responses, particularly in IBS. This process has also generated various diagnostic strategies which will be briefly reviewed here.

#### Food Allergy

Though up to 20% of the population and much higher proportions of IBS patients are convinced that they are allergic to certain foods and this is a major contribution to their problem, food allergy, traditionally denoted by an activation of IgE-mediated antibodies to a food protein, has not been linked convincingly to IBS pathogenesis and the status of IgG-based testing remains unclear.<sup>101</sup> Although confocal endomicroscopy studies suggests the existence of rapid morphological and functional changes in the epithelium and immune system of the small intestine after challenge with foods to which the patients reported intolerance, and symptomatic improvement following avoidance of these foods, this approach seems to be too cumbersome to be applied on a large scale.<sup>102</sup>

#### Food Intolerance

The contribution of lactose maldigestion to IBS depends on the prevalence of lactose maldigestion in the population studied. Furthermore, subjective reports of lactose intolerance correlate poorly with formal tests of lactose malabsorption rendering such tests of limited value in the evaluation of IBS.<sup>103</sup> Lactose intolerance should be identified in patients with FGID and milk products avoided accordingly.

Though IBS subjects appear, both subjectively and objectively, to be intolerant of fructose<sup>104</sup>, formal tests of fructose malabsorption failed to discriminate between IBS subjects and healthy controls.<sup>105</sup> Again, it does not seem possible to recommend testing in IBS or other FGIDs nor extensive elimination diets prescribed.

Sorbitol intolerance has also been reported in IBS and it is likely that sorbitol has an additive effect to fructose, with further exacerbation of symptoms.<sup>105</sup> Here again rates of intolerance and malabsorption do not tally, thereby, limiting the value of diagnostic testing or dietary advice.

#### Gluten intolerance/sensitivity

The relationships between celiac disease, “gluten-sensitivity” and IBS remain unclear with various studies reporting increased<sup>106</sup> or expected<sup>107</sup> rates of celiac disease among IBS subjects. The status of that entity which has come to be referred to as NCGS<sup>108</sup> is particularly unclear. Other than excluding celiac disease, and provide evidence of intolerance following double blind challenge, there are no currently validated diagnostic methods for diagnosing this entity.

## **The Microbiota and its Metabolic Interactions**

### SIBO, Fecal and Colonic Mucosal Microbiota

The status of small intestinal bacterial overgrowth in IBS remains highly controversial. Two factors contributing to variations in prevalence of SIBO among IBS subjects have been the test modality and diagnostic criteria used to diagnose SIBO.<sup>109</sup> While some patients with SIBO may present with IBS-type symptoms, it does not appear that SIBO is a major contributor to the pathogenesis of IBS, in general.<sup>110</sup> The lactulose breath test has shown poor diagnostic performance to detect SIBO. While the glucose breath test performs slightly better, routine testing for SIBO cannot be currently recommended.<sup>29</sup>

Though abnormalities in the fecal and colonic microbiota have been identified among IBS subjects and microbial signatures associated with certain demographic and etiological features in IBS, a fecal or mucosal microbial signal diagnostic of IBS or of an IBS sub-type or sub-population has yet to be validated.<sup>29</sup> Approaches aimed at modifying the microbiota, mainly with probiotics and non-absorbable antibiotics are now widely applied in clinical practice, particularly in patients with IBS, however, several questions remain to be elucidated, including, type of probiotics, dosage, relevant subgroups, therapeutic gain over placebo, treatment and retreatment schedules, as well as mode of action.<sup>29</sup>

### Bile Acids

Abnormalities in fecal BAs, as well as in serum markers of BA synthesis, have been reported in a sub-group of IBS-D,<sup>111</sup> and BAM may be responsible for a significant proportion of those with IBS-D.<sup>112</sup> The 23-seleno-25-homo-tauro-cholic acid (SeHCAT) test, the most widely employed and validated test for the diagnosis of BAM, is not universally available. Alternate approaches include the measurement of fecal BAs, or serum levels of 7  $\alpha$ -hydroxy-4-cholesten-3-one (C4), or a therapeutic trial of a bile salt sequestering agent.<sup>33, 40</sup>

## **Epithelium and Mucosal Barrier**

The usefulness of measures of barrier integrity (such as the lactulose-to-mannitol excretion ratio) in the diagnosis or assessment of FGIDs has not been established,<sup>113</sup> nor have the diagnostic role of *ex vivo* approaches on biopsies (Ussing chambers) and assays for molecular markers or surrogates of altered permeability (eg, endotoxin, anti-LPS antibodies, bacterial lactate, butyrate production, hemolysin test).<sup>48, 113</sup> The role of endoscopic endo-microscopy detecting rapid functional/structural mucosal changes following challenge with food allergens, although attractive, remain to be confirmed in future studies.<sup>102</sup> Strategies to modify mucosal permeability include the use of probiotics and dietary interventions although there is still uncertainty on the potential benefits.<sup>48</sup>

## **Enteroendocrine System**

While changes in basal or stimulated levels of a number of enteric hormones and neurotransmitters (such as post-prandial levels of 5-HT) have been described in IBS and other FGIDs, and manipulations of 5-HT metabolism have been shown to provoke symptoms, none have achieved the status of a diagnostic test. The use of drugs acting on serotonin agents remains a field of interest, now generating new potential therapeutic approaches for IBS-D with the study of older products with a new indication (eg, ondansetron)<sup>114</sup> or newer products (eg, ramosetron)<sup>75</sup> being tested in large clinical trials.

## **Immune System and Neuro-Immune Interactions**

That the engagement between luminal contents on one hand and the microbiota and the immune system on the other hand might be relevant to the pathogenesis of IBS is suggested by studies documenting the up-regulation of immune biomarkers and various members of the Toll receptor family in this disorder. However, given the variability in results between studies it is not possible at this time to employ measures of the mucosal or systemic responses in the diagnosis of an FGID or in the delineation of a subgroup thereof.<sup>115</sup> Two recent large placebo-controlled studies in IBS patients showed that mesalazine was not clinically superior over

placebo, although both studies suggested that subgroups, including PI-IBS, showed sustained responses.<sup>116, 117</sup>

With respect to gases released in the process of bacterial fermentation it should be noted that with some, but not complete, consistency, the detection of methane in the breath has been linked to the predominance of constipation in IBS.<sup>118</sup> The therapeutic implications of these findings remain unclear.

## CONCLUSIONS AND FUTURE DIRECTIONS

While the role of food and dietary components is, at last, attracting the attention it has long deserved, many questions persist. While dietary studies are challenging, only large-scale, prospective studies using validated instruments can provide much needed information on the food habits of FGID subjects and also address such questions as to what extent dietary preferences in FGID subjects reflect the subconscious exclusion of items to which they are intolerant. Of the multiple factors that have been proposed, intolerance to poorly absorbed dietary carbohydrates has emerged as a major contributor in IBS with the status of true food allergy and gluten sensitivity remaining unclear. Different mechanisms may be relevant to various subjects or subject groups. Long-term studies of dietary interventions will be important by defining, not just their beneficial effects on symptoms, but also identifying any negative consequences.

Though some tantalizing findings have been reported, studies of the gut microbiota and the host immune response in FGIDs have yielded variable and sometimes conflicting results: there is a need for longitudinal studies that include functional profiling of the microbiota, its metabolites (including gases and SCFAs) and related immunological responses in well-characterized IBS populations. Such information will be critical to guiding therapeutic strategies that aim to modulate the microbiota, its products and/or the immune response.

The signal transduction mechanisms that generate responses to BAs need to be delineated; i.e. whether they are receptor mediated, or generated by paracrine or non-specific effects. Above all, in relation to BAs, the current paucity of human studies needs to be addressed. Furthermore, microbiota-BA interactions in FGIDs need to be explored.

While functional and molecular alterations of factors involved in intestinal permeability may explain IBS pathophysiology and symptom generation and could yield innovative biomarkers valuable in both diagnosis and the assessment of therapeutic response in FGIDs, further studies, using validated and preferably non-invasive markers, are needed to more precisely define the mechanisms and functional consequences of these alterations and their primacy in a given FGID.

Well-designed studies on large numbers of patients and controls evaluating the enteroendocrine system, including changes in serotonergic responses, are also lacking in patients with FGIDs. Moreover, dietary, behavioral, pharmacological and gut-microbiota-directed manipulations of entero-endocrine responses are likely to be important approaches to the management of these disorders.



**FIGURE LEGENDS**

Figure 1. Schematic representation of the putative interplay between luminal and mucosal factors in FGIDs. Microenvironmental factors (e.g., food, microbiota, bile acids) may permeate in excess through a leaky epithelial barrier allowing amplification of signaling from the lumen to deeper mucosal and muscle layers, including overstimulation of the mucosal immune system. These factors may determine abnormal signaling to neural circuits (intrinsic primary afferent nerves and extrinsic primary afferent nerves), which in turn may affect intestinal physiology and sensory perception.

Figure 2. Overview of dietary components and metabolites produced in the GI tract, and their association with irritable bowel syndrome or its symptoms. \*means increased levels in irritable bowel syndrome patients. Increases in CH<sub>4</sub> and H<sub>2</sub> concentrations contribute to bloating and distension and intraluminal concentrations of bile acids and proteases will promote diarrhea. Irritable bowel syndrome (IBS); irritable bowel syndrome with diarrhea (IBS-D); irritable bowel syndrome with constipation (IBS-C); branched chain fatty acids (BCFAs).

Figure 3. Schematic representation of the interactions between the microbiota and bile acids as illustrated by a comparison of germ-free and normally colonized animals. The scheme shows increased activity and expression of cholesterol 7 $\alpha$ -hydroxylase and levels of taurine-conjugated  $\beta$ -muricholic acid in germ free mice. In contrast, the expression and activity of sterol 12 $\alpha$ -hydroxylase and cholic acid levels are similar in germ free and normally colonized mice. Taurine-conjugated  $\beta$ -muricholic acid is a natural antagonist of the farnesoid X receptor, which, in turn, may elicit reduced inhibition of rate-limiting enzyme cholesterol 7 $\alpha$ -hydroxylase in germ-free animals mice. In contrast, normally colonized animals, show a reduction in taurine-conjugated  $\beta$ -Muricholic Acid. This leads to increased activation of farnesoid X receptor in enterocytes, thus upregulating the fibroblast growth factor 15, which in turn suppresses cholesterol 7 $\alpha$ -hydroxylase in the liver. In addition, the microbiota affects intestinal bile acid metabolism and increased their excretion. bile acyl-CoA synthetase (BACS); cholesterol-4 (C4); cholesterol 7 $\alpha$ -hydroxylase (CYP7A1); cholic acid (CA); fibroblast growth factor receptor 4 (FGFR4); fibroblast growth factor 15 (FGF15); farnesoid X receptor (FXR);  $\beta$ -muricholic acid ( $\beta$ -MCA); taurine-conjugated cholic acid (TCA); deoxy cholic acid (DCA); lithocholic acid (LCA); sterol 12 $\alpha$ -hydroxylase (CYP8B1); taurine-conjugated  $\beta$ -Muricholic Acid (T $\beta$ MCA). Reproduced with permission from <sup>119</sup>

Figure 4. Mechanism of action of serotonin and its re-uptake in irritable bowel syndrome. 5-HT, serotonin; acetylcholine (ACh); gamma amino butyric acid (GABA); irritable bowel syndrome (IBS); irritable bowel syndrome with diarrhea (IBS-D); Irritable bowel syndrome with constipation (IBS-C).

Figure 5. Post-infectious IBS and related risk factors. Relative risk (RR).

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