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REVIEW ARTICLE

Recent advances in measuring the effects of diet on gastrointestinal physiology: Probing the "leaky gut" and application of real-time ultrasound

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Abstract

There is a large pool of ideas in both mainstream and non-mainstream medicine on how diet can be manipulated in order to treat or prevent illnesses. Despite this, our understanding of how specific changes in diet influence the structure and function of the gastrointestinal tract is limited. This review aims to describe two areas that might provide key information on the integrity and function of the gastrointestinal tract. First, demystifying the "leaky gut syndrome" requires rational application and interpretation of tests of intestinal barrier function. Multiple ways of measuring barrier function have been described, but the inherent difficulties in translation from animal studies to humans have created misinterpretations and misconceptions. The intrinsic nature of intestinal barrier function is dynamic. This is seldom considered in studies of intestinal barrier assessment. To adequately understand the effects of dietary interventions on intestinal barrier function, background barrier function in different regions of the gut and the dynamic responses to stressors (such as psychological stress) should be assessed as a minimum. Second, intestinal ultrasound, which is now established in the assessment and monitoring of inflammatory bowel disease, has hitherto been poorly evaluated in assessing real-time intestinal function and novel aspects of structure in patients with disorders of gut-brain interaction. In conclusion, a more complete functional and structural profile that these investigations enable should permit a greater understanding of the effects of dietary manipulation on the gastrointestinal tract and provide clinically relevant information that, amongst other advantages, might permit opportunities for personalized health care delivery.

Introduction

Structural aspects of the gastrointestinal tract can be utilized to study the effect of changes in diet on gut health and physiology. In this second part addressing advances in methodologies in dietary research, intestinal barrier function and functional assessment using real-time intestinal ultrasound are discussed.

Measuring intestinal barrier function: Probing the "leaky gut"

The "leaky gut syndrome" is a popular, but poorly defined, concept that attributes dysfunction of the barrier between the gastrointestinal lumen and the circulation—with resultant increased exposure to potentially pro-inflammatory molecules—as the causative instigator of multiple chronic illnesses. One major hypothesis is that food provides the key to both causes and treatment. Indeed, there are many published reviews on how food influences intestinal permeability, examples being Usuda *et al.*,[1] Aleman *et al.*,[2] and Camilleri,[3] but the scientific basis of these effects in humans is shaky. Central to our understanding of whether food choice has a major role in intestinal permeability is how epithelial barrier function is measured and how results are interpreted.

The concept of a dynamic intestinal barrier. The backbone to the intestinal barrier is the intestinal epithelium, a single layer of cells joined together by tight junctions. It is semipermeable, permitting the absorption of molecules across it and allowing for immune sampling of the luminal contents.[2] A potentially important aspect often forgotten in studies of intestinal permeability is that the barrier is acutely dynamic and its relative permeability is influenced by many factors that include food components, drugs and "stress"; with stress representing a variety of conditions such as exercise, disease states and psychological stress.[3] The main implications are twofold. First, the measurement of barrier function at one point in time, without strict attention to such potential confounders, can be an inaccurate measure of resting barrier function. For example, the response to stress via corticotropin-releasing hormone in healthy subjects is markedly influenced by the status of the autonomic nervous system (in this case modulated by vagal nerve stimulation).[4] Second, the response to stressors may be as important or more important in disease states than the resting measures. For example, in first-degree relatives of patients with Crohn's disease, who are at higher risk of developing the disease themselves, have an exaggerated response of intestinal permeability to acute exposure to aspirin.[5]

Epithelial permeability pathways and their measurement. Passage of luminal molecules and/or bacteria through the intestinal epithelium in health can occur via three core pathways[3] as illustrated in Figure 1. Many ways of examining epithelial barrier function have been used and/or proposed (Fig. 2) with variable validity. Only selected methods of high relevance to humans will be discussed in this paper. The target substances and the location within the digestive tract where the permeation takes place is summarized in Table 1.

Permeation of exogenous probes from the lumen to the internal milieu. Often cited as the "gold standard" of non-invasive intestinal permeability measurements, the dual-sugar test takes advantage of the differential absorption of disaccharides and monosaccharides across the intestinal barrier.[6] The disaccharide is chosen for its absence of small intestinal hydrolysis and hence minimal absorption, its minimal metabolism once absorbed into the circulation and ready excretion into the urine. Lactulose is almost universally used. The monosaccharides are chosen on the basis of minimal, if any, presence in the diet, exclusive passive absorption properties, minimal hepatic metabolism after absorption and ready excretion in the urine. Rhamnose and ^{13}C-mannitol, but not mannitol, fit these criteria.[7] The concentration of sugars in the urine is proportionate to the amount absorbed across the intestine and are measured using high-performance liquid chromatography. The sugar concentrations are expressed as a ratio of lactulose to mannitol or rhamnose, with higher ratios indicating increased intestinal permeability. The use of the ratio rather than any sugar individually corrects for changes in probe concentrations related to variations in gastrointestinal transit time, surface area, and renal excretion factors. The urine dual-sugar test is largely a measure of changes within the paracellular route of intestinal permeability.

The duration of urine collection is critical. In 2 h, the small doses of sugars used are likely to be exposed to most of the small intestine with minimal amounts entering the colon. A 2-h urine collection is now accepted as indicative of small bowel intestinal permeability.[7] A 5-h urine collection has been often applied and said to be indicative of small and large bowel intestinal permeability. However, the rapid fermentation of lactulose in the colonic lumen will confound the results.[7] Results are prone to artifacts from urine spillage, fecal contamination and issues with sugar probe storage and probe ingestion, imprecise timing of urine collection, exercise, smoking, alcohol ingestion and use of non-steroidal anti-inflammatory agents. However, when the studies are performed strictly according to protocol, the results have excellent reproducibility.[7]

Measurement of colonic permeability requires different probes. ^{51}Cr-EDTA, polyethylene glycol and sucralose (which are supposed to not be absorbed or metabolized by colonic bacteria) with a 24-h urine collection have been successfully applied.[8–10] However, EDTA carries the burden of radioactivity and sucralose has some small intestinal absorption and may be present in the diet,[7] and all three probes require a full 24 h of collection. These factors dampen enthusiasm for these probes.

Permeation of endogenous probes. The concentrations of or responses to inflammatory bacterial structural elements have been applied to assess intestinal barrier function. Detection of bacterial DNA in culture negative blood samples has been utilized in metabolic diseases, inflammatory bowel disease and chronic liver disease with mixed results.[11–13] The difficulties encountered lie largely in the small amounts of circulating material, ease of sample contamination, heterogeneity in genetic

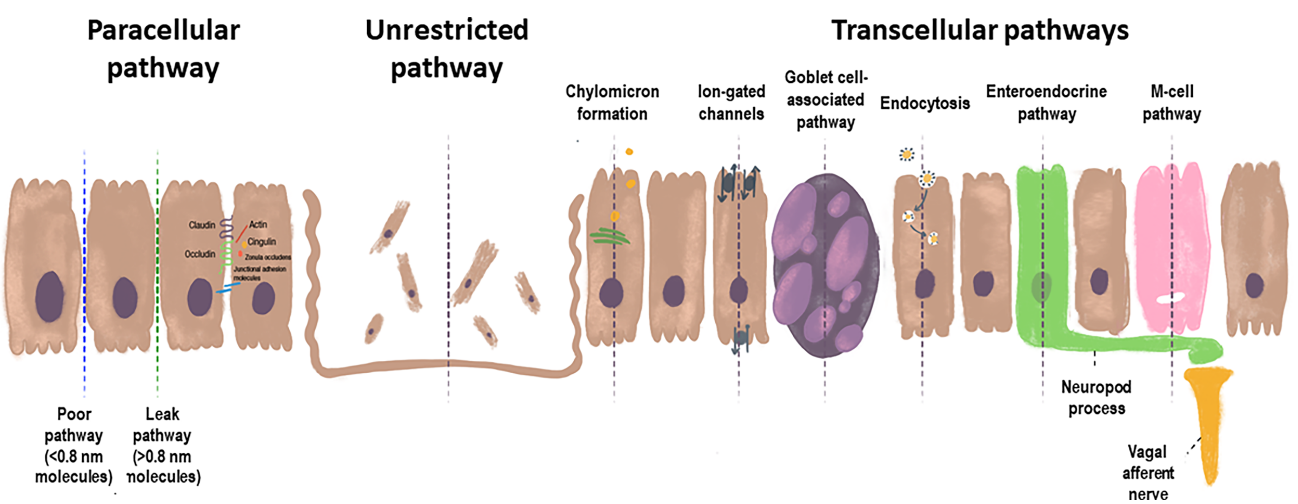

Figure 1 Multiple pathways involved in intestinal barrier function.

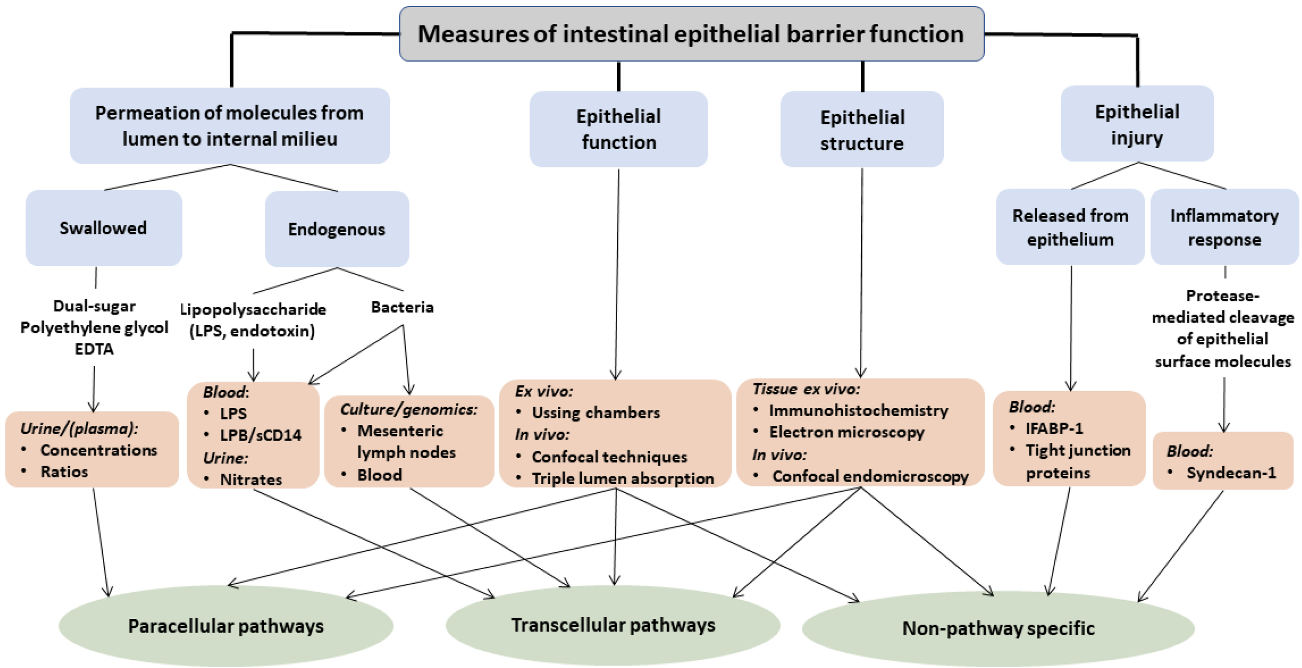

Figure 2 Measures of intestinal barrier function that have been described.

material probe selection and noise from the presence of human DNA within any given sample. Given these limitations, this method of measuring bacterial translocation is unlikely to proceed to widespread clinical use. Lipopolysaccharide (LPS, endotoxin), an integral component of gram-negative bacterial outer cell membrane, can physiologically permeate the epithelium via multiple pathways comprising paracellular "leak" pathway, transcellularly by endocytosis, through incorporation into triglyceride-rich lipoproteins (i.e. chylomicrons), with bacterial translocation and via the unrestricted paracellular pathway. Unfortunately, interpretation of circulating levels of LPS presents several challenges including the assays used, the risk of contamination and the fact that its serum concentrations physiologically increase post-prandially by up to 100% due to chylomicron formation with a high-fat diet.[14–16]

If the permeation of LPS across the intestinal epithelium is physiological (e.g. associated with chylomicrons), it may not incite an inflammatory response but is more likely to do so (referred to as "bioactive LPS") if secondary to a compromised epithelium. Bioactive LPS specifically binds to lipopolysaccharide-binding protein (LPB) and soluble CD14 (sCD14), resulting in a complex series of inflammatory responses that include induction of their synthesis. Indeed, their concentrations alter dynamically as a response to acute and chronic LPS exposure.[16,17] Chronically elevated exposure to bioactive LPS increases the concentrations of both, but acute changes may increase or decrease concentrations of either or alter the LPB:sCD14 ratio. Reproducibility of changes in LPB and sCD14 and effects of potential confounders such as circadian rhythms or food intake have, until recently,[18] have seldom been studied. What changes should be used as markers of changes to barrier function (particularly in acute modulatory studies) have varied and may depend upon the time-course and setting of the studies. Hence, care has to be taken in interpreting changes in LPB and sCD14.

Biomarkers of epithelial injury. Injured epithelial cells are likely to exhibit reduced functional capacity, and this would be anticipated to include impairment in intestinal barrier function. When the intestinal epithelium is injured or "under stress," molecules may be released from the cells into the circulation. While markers have been frequently used, studies of their methodological approaches, reproducibility and/or confounding factors have been limited. For example, commercially available assays of zonulin do not actually measure the zonulin molecule.[19] The circulating concentrations of intestinal fatty acid-binding protein (I-FABP), an intracellular protein expressed in epithelial cells of predominantly the small intestine, increase with major epithelial injury as in necrotizing enterocolitis, mesenteric ischemia and celiac disease,[20] but its response to more subtle injury, such as that associated with stress, is less predictable and its concentrations paradoxically tend to be lower with greater inflammation in patients with IBD.[18,21] Such performance together with observation of considerable diurnal variation and sensitivity to food intake[18] limit the value of I-FABP as a biomarker of changes in intestinal permeability.

Confocal laser endomicroscopy. This research technique that enables real-time microscopy of the intestinal mucosa via a fiberoptic endoscope has been a game-changer in helping to understand barrier function. Subjects are preloaded with intravenous fluorescein enabling barrier defects to be detected by fluorescein leakage and shedding of epithelial cells and, in acute

Table 1 Sites of selected measurement techniques that examine intestinal epithelial barrier function in and characteristics of permeating substances measured

Measure of permeability		Characteristics of permeating substance	Site
Permeation of exogenously delivered probes	Dual-sugar test (lactulose plus ^{13}C-mannitol or rhamnose)	Paracellular permeability allowing permeation of molecules <1 nm in size. Also disrupted in the case of diseases which allow for the unrestricted pathway (e.g. inflammation in the small or large bowel from infection, inflammation or ischemia)	Small intestine with 2-h urine collection. Longer or subsequently collected urine not validated for colonic permeability
	^{51}Cr-EDTA Polyethylene glycol		Small intestinal and colonic permeability with 24-h urine collection
	Sucralose		Colonic with timed urine collection after small bowel
Permeation of endogenous probes	Bacterial DNA	Methodologically challenging to ensure lack of contamination Transcellular and unrestricted pathways	Colon and small intestine
	Lipopolysaccharide (LPS) translocation	LPS translocates mainly transcellularly independently of or together with bacteria) or via unrestricted pathway	
	• LPS (endotoxin)	Challenging to measure without contamination and physiologically increases with chylomicrons	
	• Soluble CD14 • LPS binding protein	"Bioactive" (not physiologically absorbed) LPS reduces concentrations (mopped up) by acute exposure and synthesis is induced	
Markers of epithelial injury	Intestinal fatty acid-binding protein (I-FABP)	Measure of epithelial injury; likely allowing the passage of structures as large as bacteria, but confounded by diurnal variation and state of fasting	Small intestine
Direct endoscopic techniques	Confocal laser microscopy	Representation of areas of epithelial apoptosis, micro-erosions and increased permeability; best documented by pre-loading person with fluorescein allowing visualization	Small or large intestine—site chosen by endoscopist

exposure experiments, by increased density of intraepithelial lymphocytes.[22] With acute exposure to specific food proteins, barrier changes in the duodenum may be induced within a few minutes with dietary exclusion of the offending proteins apparently leading to improved clinical outcomes.[22–24] Such observations underline the dynamic nature of the intestinal mucosa/barrier and its potential importance in pathogenesis of illness.

Myths and partial truths in the effect of food on barrier function. Many stories of the effect of food on barrier function that appear to be widely supported[1,2] may have poor foundations in humans. For example, a high-fat diet comprising >70% of the diet consistently impairs barrier function in mice via multiple potential mechanisms, as reviewed elsewhere.[25] In humans, 49% fat may be the highest that can be practically achieved and tolerated by dietary change. At such levels, circulating LPS concentrations increase up to twofold.[14,15,26] However, evidence of this LPS being bioactive has not been shown[14,26] except in a small subset of healthy subjects in a single study.[15] Similarly, the concept that that dietary fiber improves intestinal barrier function (not usually defined as referring to the small and/or large intestine)[1] appears to be based upon the effect of butyrate, a product of fiber fermentation, on barrier function in cell lines in vitro, an observation first made by our group in 1997.[27] However, whether fiber has this effect in vivo is uncertain. In healthy men, dietary inulin supplementation reduced lactulose absorption as shown in 5-h urine collections (a test putatively of small intestinal and colonic permeability), but inulin-induced enhancement of bacterial fermentation of lactulose was not addressed as an alternative explanation.[28] In contrast, no change to excretion of non-fermented EDTA (as a more valid permeability probe for colonic permeability) was found with fructo-oligosaccharide supplementation.[8]

Conclusion on the application of markers of barrier function in dietary research. Naïve concepts and cures that populate discussions around the "leaky gut syndrome" need to be avoided as does confirmation bias in interpreting data. Healthy skepticism should be exercised in translating data from animal studies and in vitro systems to humans. The desire to define how foods affect barrier function is critical to developing preventive and therapeutic strategies. In probing the leaky gut, a trio of basic questions should be addressed—the background, fasting, resting and unstressed barrier function; the locoregional effects (e.g. small *vs*. large intestine); and the dynamic responses of intestinal permeability to stressors.

Gastrointestinal tract with ultrasound: Assessing structure and function

The widespread availability of affordable tools for accurately assessing the anatomy and physiology of the gastrointestinal tract is a significant unmet need in the management of gastrointestinal disorders. Most current diagnostic techniques employed in clinical practice focus on investigating the anatomy and macroscopic or microscopic changes of the gut, or specific features of gastrointestinal physiology, such as motility, secretion, and sensibility. These methods are valuable for distinguishing between organic and functional disorders but often prove ineffective in patients with concurrent disorders.

However, in recent years, significant efforts have been made to integrate anatomic imaging of the gut with its physiological assessment, particularly through non-invasive techniques such as magnetic resonance and intestinal ultrasound. Both of these methods can offer a detailed real-time evaluation of the morphology and function of the gut, particularly in terms of motility and content. Functional magnetic resonance is an expensive and relatively less accessible technique, primarily employed for studying gut function for scientific purposes, with limited use in clinical practice. On the other hand, ultrasound imaging is widely requested for abdominal complaints, especially as a first or preliminary investigation to detect or rule out organic diseases. Notably, abdominal ultrasound is requested in over 50% of patients with IBS,[29,30] mainly to exclude organic diseases, particularly IBD, owing to its high accuracy and cost-effective profile.[31,32]

Structure of the gastrointestinal tract. Intestinal ultrasound can assess the anatomy of most parts of the gastrointestinal tract, particularly the thickness and histological layers of the bowel walls (Fig. 3), mesenteric blood supply, and mural perfusion, both in fasting and postprandial phases.[32–34] The maximum bowel wall thickness (>3 mm), enlarged mesenteric lymph nodes, changes in mesenteric fat and bowel dilatation >25 mm (Fig. 4), are the main parameters in detecting organic diseases, especially IBD, and other less common gastrointestinal conditions.[32–38] However, IUS can provide also insights into the nature and quantity of bowel content, and offer an estimation and gross evaluation of functional properties of the gut such as motility and sensitivity.[32,37] In addition, along with the evaluation of normal and pathological findings of the gastrointestinal tract, IUS can also assess also morphological and functional changes biliopancreatic of the system.[39]

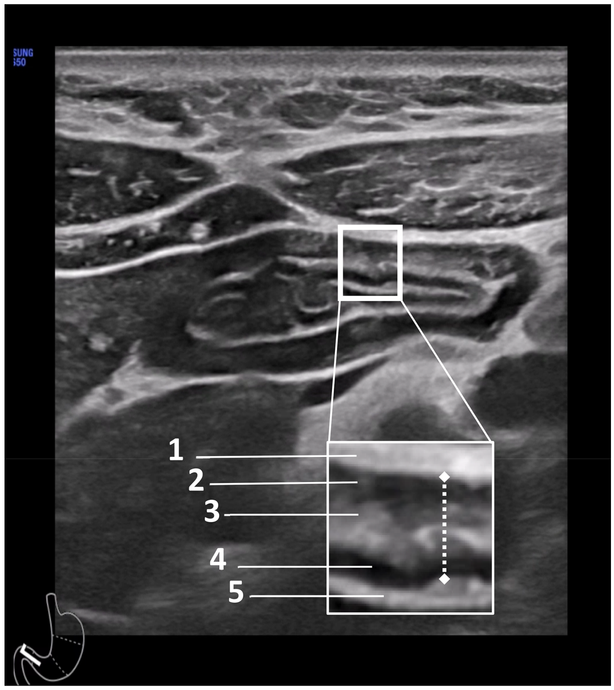

Figure 3 Ultrasound scan of the gastric antrum under fasting conditions. In the inset, a more magnified detail of the anterior gastric wall is visible, allowing for a better assessment of the maximum wall thickness (dotted vertical line) and the echostructure of the wall. Five layers are recognizable in the echostructure: (i) external hyperechoic layer (serosa); (ii) hypoechoic layer (muscularis propria); (iii) hyperechoic layer (submucosa);(iv) hypoechoic layer (mucosa); (v) hyperechoic layer (interface between lumen and mucosa). The stratification is identifiable across the entire gastric wall, as in all walls of the gastrointestinal tract.

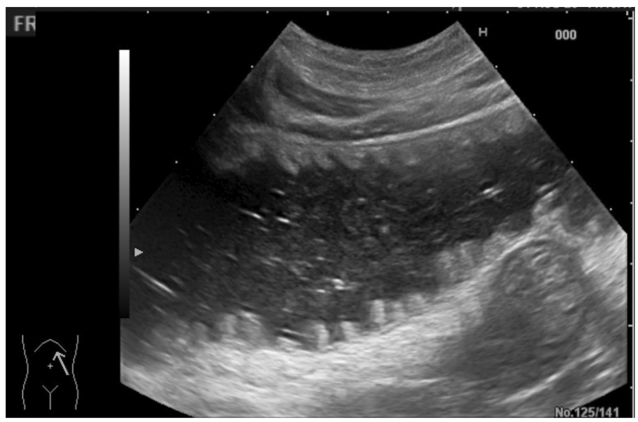

Figure 4 Dilatated small bowel (diameter >30 mm) with liquid content in a Crohn's disease patients with small bowel occlusion.

Function of the gastrointestinal tract. Most advantages of ultrasound stem from its capability to assess changes in the bowel in real time and monitor gut dynamics along with its content.[40] Indeed, several studies have demonstrated that ultrasound enables the evaluation of various parameters of gastric function, including gastric emptying, gastric accommodation, antral contractility, transpyloric flow, and intragastric distribution of meals.[41,42] Serial and standardized measurements of the gastric antrum up to 4–5 h post-meal have been utilized to assess gastric emptying time in dyspeptic patients, showing comparable accuracy to scintigraphy. The sonographic assessment of gastric emptying, as well as the sonographic evaluation of stomach accommodation and antral contraction, are somewhat laborious and time-consuming, and are currently not routinely employed in clinical practice. However, there is a growing interest in assessing gastric parameters (antral diameters and gastric volume) in emergency settings to predict enteral feeding intolerance and the risk of aspiration before elective surgery.[43,44]

The motility of the small bowel has been assessed by ultrasound in celiac disease, where increased small bowel peristalsis was detected.[39,45] More detailed information on sonographic motility of the small bowel has been provided in a study by Nylund *et al.*,[46] involving 121 healthy subjects. Intestinal ultrasound was able to detect jejunal motility in 52% and ileal motility in 62% of subjects during fasting. Postprandial motility, assessed 30 min after a standard meal consisting of 200 mL (300 kCal), was detected in 95% and 90% of subjects in the jejunum and the ileum, respectively. In subjects where motility was detectable both before and after the meal, the median number of contractions increased from 0 (IQR, 7) before the meal to 5 (IQR, 4) after the meal ($P = 0.038$) in the jejunal loops, and from 5 (IQR, 9) to 6 (IQR, 4) in the ileal loops ($P = 0.267$).

The potential of intestinal ultrasound lies not only in the real-time assessment of motility but also in detecting morphological and sensorial features of the gut. In IBS and uncomplicated diverticular disease of the colon (SUDD), intestinal ultrasound may reveal an increased thickening of the muscularis propria of the sigmoid colon compared with healthy subjects, likely indicative of its persistent contraction.[47,48] Additionally, ultrasound can demonstrate increased pain provoked by elective compression above the sigmoid colon, allowing discrimination of SUDD from other conditions. Importantly, the thickness of the muscularis propria correlates with pain evoked by compression only in SUDD patients.[47]

Intestinal ultrasound is capable of assessing features of intestinal content. In chronic constipation, ultrasound can detect hard stools within the colon, rectal fecal impaction, and megarectum (Fig. 5).[49] Most of these findings stem from studies conducted in geriatric and pediatric populations. In healthy adults, ultrasound, even when using a portable pocket-sized device, can identify fecal retention in the rectum when a rectal diameter >4.0 cm is assessed.[50] Fecal loading, evaluated by ultrasound, has been used as an indirect parameter of colonic function and constipation in adults.[51] Fecal loading and its consistency, assessed through rather complex scores not yet validated, have been proposed as parameters of colonic function. These scores, based on the transverse diameter of the colonic segments, the acoustic shadowing of the contents, and haustrations' appearance, show a good correlation with CT findings of stool and/or gas distribution, as well as with colonic transit time evaluated by radiopaque markers.[51]

Intestinal ultrasound can assess not only colonic fecal content but also its motility. Hussein *et al.* investigated spontaneous haustral activities and corresponding motor patterns in healthy subjects using colonic ultrasound.[52] Ultrasonography recordings of the ascending, transverse, and descending colon were able to identify three distinct rhythmic motor patterns: the 1 cycle/min and the 3 cycles/min cyclic motor patterns throughout the whole colon, and the 12 cycles/min cyclic motor pattern in the ascending colon. Whether these results will be confirmed without colonic preparation under physiological conditions, the potential to sonographically show rhythmic motor patterns associated with interstitial cells of Cajal-associated pacemaking activity would allow us to accurately identify and quantify colonic motility and dysmotility, providing useful insights for selecting appropriate therapy and monitoring its effectiveness.

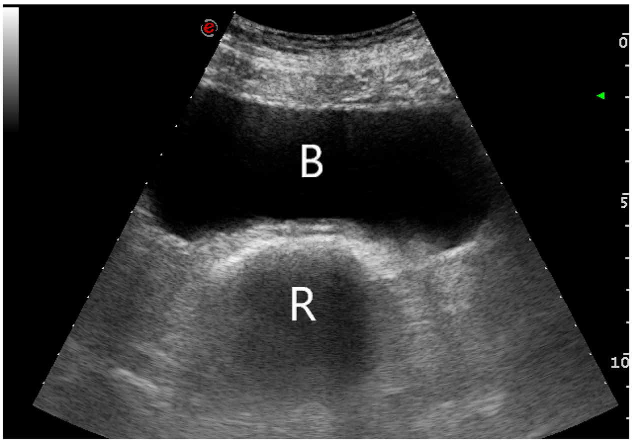

Figure 5 Enlarged diameter of the rectum (R) with fecal impaction in an elderly patient scheduled for proctosigmoidoscopy with unsuccessful preparation.

Ultrasound can assess and monitor gallbladder volume using the ellipsoid method, through serial standard measurements, both while fasting (range: 16–40 mm) and after a standard meal, defining a postprandial ejection fraction (usually >50%). Additionally, ultrasound can measure the diameter of the common bile duct, which enlarges after food intake. Dyskinetic gallbladder and common bile duct abnormalities may be observed in diabetic and obese patients, those with coeliac disease and gallstones, and in conditions such as biliary dyskinesia and Oddi sphincter dysfunctions. Abnormal gallbladder ejection fractions can help identify patients at risk of recurrence following gallstone dissolution with medical therapy, those at risk of gallstone formation (e.g. obese patients during rapid weight loss), and confirm the indication for cholecystectomy in patients with recurrent biliary colic and acalculous gallbladder disease.

In addition to these clinical indications, functional ultrasound of the gallbladder has a role in research for testing drugs and foods, showing good concordance with cholescintigraphy using 99mTc-HIDA ($r = 0.99$).[53,54]

Conclusion. The role of ultrasound in assessing the structure of the gastrointestinal tract is well established and currently used to detect, assess, and monitor gastrointestinal diseases, especially inflammatory bowel disease. Its role in assessing function and its

Table 2 Ultrasonographic findings, potential clinical indications, and research topics for functional ultrasound in digestive diseases

Region	Sonographic findings	Potential indications
Stomach	• Gastric emptying assessment • Gastric accommodation evaluation • Antral contractility evaluation • Transpyloric flow assessment • Intragastric distribution of meals evaluation	• Functional dyspepsia • Prediction of enteral feeding intolerance • Prediction of risk of aspiration before abdominal surgery
Small Bowel	• Detection of small bowel contractions • Assessment of small bowel diameter and quality of its content	• Small bowel obstruction • Small bowel bacterial overgrowth • Severity of small bowel strictures
Colon	• Sigmoid diverticula detection • Evaluation of colonic sensitivity and muscular hypertrophy • Assessment of fecal load • Evaluation of rectal fecal impaction and megarectum • Insight into spontaneous haustral activities	• Symptomatic uncomplicated diverticular disease • Irritable bowel syndrome • Constipation
Biliary tract	• Assessment of gallbladder postprandial ejection fraction	• Prediction of gallstone recurrence after medical therapy • Assessment of gallbladder emptying disorders in acalculous gallbladder

utility in evaluating functional disorders rely on preliminary evaluations. These observations demonstrate the potential of ultrasound in assessing functional disorders of the gut and biliary tract (Table 2) but warrant further confirmation and standardization. The translation of these findings into clinical practice requires additional efforts and investigations.

Overall conclusions

Addressing structural elements of gastrointestinal tract seems a key area of addressing the effects of diet. The evaluation of intestinal barrier function is challenging and affected by many physiological and methodological issues, which have hindered better understanding of the popular concepts of "leaky gut." Likewise, the application of ultrasound to evaluate gastrointestinal function via effects on gross structure is very appealing due to its non-invasive nature. While further refinement and evaluation of both aspects are required, they provide information on important aspects by which dietary manipulation might exert effects on disease processes and symptoms in the gastrointestinal tract.

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