



# Methodological advances in gastrointestinal tract physiology measurements: relevance to nutritional studies

cambridge.org/pns

The 57th Nutrition Society of New Zealand and 47th Nutrition Society of Australia Joint Annual Scientific Meeting was held at Massey University on 5th - 6th December 2023

Conference on Nutrition & Wellbeing in Oceania

Plenary 5: Gut Health

## Review Article

**Cite this article:** Biesiekierski JR, Yao CK, Tuck CJ, and Snelson M (2024). Methodological advances in gastrointestinal tract physiology measurements: relevance to nutritional studies. *Proceedings of the Nutrition Society*, page 1 of 13. doi: [10.1017/S0029665124007444](https://doi.org/10.1017/S0029665124007444)

Received: 1 May 2024  
Revised: 13 September 2024  
Accepted: 26 September 2024

**Keywords:** Gastrointestinal physiology; Gastrointestinal motility; Fermentation; Intestinal permeability

**Corresponding author:** Matthew Snelson;  
Email: [matthew.snelson@monash.edu](mailto:matthew.snelson@monash.edu)

Jessica R. Biesiekierski<sup>1,2</sup>, Chu K. Yao<sup>3</sup>, Caroline J. Tuck<sup>4</sup> and Matthew Snelson<sup>5,6</sup> 

<sup>1</sup>Department of Nutrition, Dietetics and Food, Monash University, Melbourne, VIC, Australia; <sup>2</sup>Human Nutrition Group, School of Agriculture, Food & Ecosystem Sciences, The University of Melbourne, Parkville, VIC, Australia; <sup>3</sup>Department of Gastroenterology, School of Translational Medicine, Monash University, Melbourne, VIC, Australia; <sup>4</sup>Department of Nursing and Allied Health, Swinburne University, Melbourne, VIC, Australia; <sup>5</sup>Hypertension Research Laboratory, School of Biological Sciences, Monash University, Melbourne, VIC, Australia and <sup>6</sup>Victorian Heart Institute, Monash University, Melbourne, VIC, Australia

### Abstract

The gastrointestinal (GI) tract plays a critical role in nutrition and the pathophysiology of disease, and there is an increasing variety of methodologies available for the assessment of various aspects of GI physiology. Advancements in assessment methods, including techniques to study gut motility, fermentation, permeability, and microbiota composition, have provided researchers with powerful tools to investigate the impact of diet on GI tract physiology and the microbiota-gut-brain axis. Mechanistic evidence from reverse translational studies, which apply findings from human studies to preclinical models in a 'bedside-to-bench' approach, have also enhanced our understanding of the bidirectional interactions and candidate signalling molecules among the diet-gut-brain relationship. Interpreting data from these advanced techniques and study designs requires a thorough understanding of their principles, applications, and limitations. This review aims to summarise the methodological advances in GI tract physiology measurements and their application in nutritional studies, focusing on gut motility, fermentation, and permeability. We will present examples of how these techniques have been utilised in recent research, discuss their advantages and limitations, and provide insights on their use and interpretation in research. Understanding the capabilities and limitations of these tools is crucial for designing robust studies and elucidating the complex interplay between diet and the GI tract. The scope of this review encompasses recent advancements in GI tract assessment methodologies and their implications for nutritional research, providing a comprehensive overview for researchers in the field.

The gastrointestinal (GI) tract plays a pivotal role in nutrition, serving as the interface between dietary intake and physiological functions. Its functions extend beyond digestion and absorption, as the GI tract also hosts a complex microbial community, known as the gut microbiota, which interacts with the host's immune and nervous systems. This intricate interplay, often referred to as the microbiota-gut-brain axis, has been implicated in various aspects of health and disease<sup>(1)</sup>. Diet is a key modulator of the microbiota-gut-brain axis, and understanding its impact is crucial for developing targeted nutritional interventions<sup>(2)</sup>. This review covers recent advancements in methodologies for assessing GI tract physiology, with a focus on gut motility, fermentation, and permeability, and their applications in nutritional research.

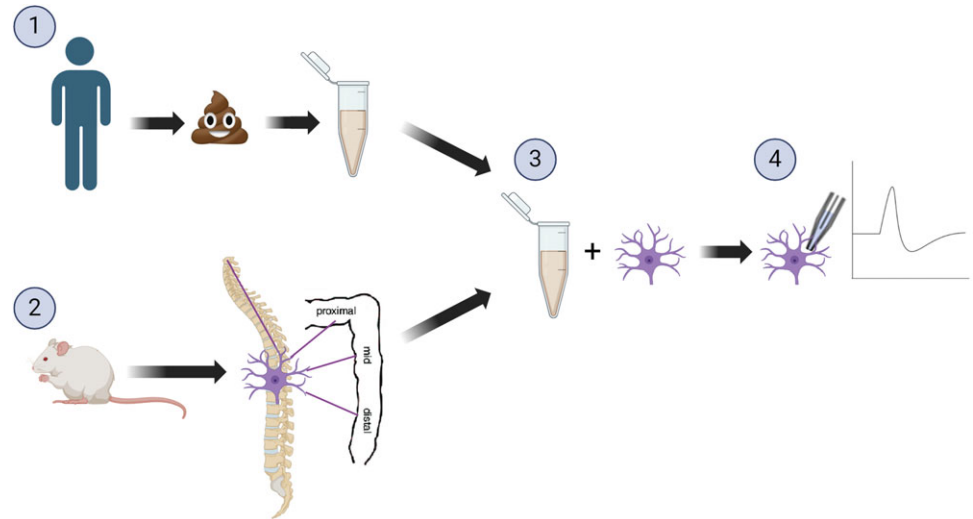
### Reverse translational studies to provide mechanistic insight

A reverse translational study, often referred to as a 'bedside-to-bench' approach, presents a unique opportunity to leverage insights from both human and animal trials, enhancing our clinical and mechanistic understanding. These studies involve collecting samples such as faecal, blood, or colonic biopsy specimens during human trials, which are subsequently utilised in *in vivo* and/or *ex vivo* experimental protocols. While reverse translational designs can be time and resource-intensive, they enable the conduct of mechanistic investigations that would be challenging to achieve solely through human studies. Importantly, by utilising human samples, reverse translational studies offer results that are more directly applicable to real-life scenarios compared to studies relying solely on animal models.

Given the relevance of the microbiome in gastrointestinal physiology, the use of human faecal samples applied to reverse translational studies is one way in which human gastrointestinal tract and gut-brain physiology can be more carefully studied. Human-to-mouse faecal microbiota transplantation (FMT) has been used to understand the impact of the

© The Author(s), 2024. Published by Cambridge University Press on behalf of The Nutrition Society. This is an Open Access article, distributed under the terms of the Creative Commons Attribution licence (<https://creativecommons.org/licenses/by/4.0/>), which permits unrestricted re-use, distribution and reproduction, provided the original article is properly cited.





**Figure 1.** Example of a reverse translation study (1) Human participants receiving a dietary intervention, faecal samples collected and subsequently the supernatant was collected. (2) The dorsal root ganglion was collected from mice, and (3) incubated with the human faecal supernatant. (4) Patch-clamp recordings were taken, to assess the effects of the faecal supernatants following dietary intervention on neuronal excitability. Image created with biorender.com.

gut microbiome in a range of conditions including depression<sup>(3)</sup>, inflammatory bowel disease<sup>(4)</sup>, and throughout pregnancy<sup>(5)</sup>. Following faecal collection from human participants, inoculation of the microbiome from the faecal samples to germ-free (without any resident microorganisms) mice allows for further understanding of the effects of the microbiome on behavioural studies, metabolome composition and immune markers. Recent guidelines for reporting on animal FMT studies have provided recommendations for protocol design and reporting which aims to improve the quality and reproducibility of studies using this technique<sup>(6)</sup>.

Reverse translational FMT protocol can be applied in nutrition studies by assessing or modifying the diet of human subjects before faecal sample collection. Additionally, the diet of the animals receiving the FMT can be adjusted to investigate diet-microbiota interactions. For example, one study using a dietary intervention modifying fermentable carbohydrate (FODMAP) intake in patients with irritable bowel syndrome (IBS) showed that symptom response related to the dietary change was associated with alterations to the metabolome and decreased urinary histamine levels<sup>(7)</sup>. Subsequently, a reverse translational design was used whereby faecal samples from IBS patients collected during the interventions were then applied *in vivo* to germ-free mice fed custom-designed low and high-FODMAP diets to investigate the role of the gut microbiota on visceral hypersensitivity (i.e. pain)<sup>(8)</sup>. An interaction between diet and the microbiota was observed, where histamine production may have induced intestinal mast cell activation. This finding suggests that microbiota-targeted therapies aimed at histamine could alleviate pain in a subset of IBS patients<sup>(8)</sup>. These insights, derived from the reverse translational approach, represent novel findings not previously demonstrated in human trials focused on diet.

Another method involves using human faecal samples in *ex vivo* models. This approach was employed in a study where patients with IBS underwent a randomised cross-over trial of low and high-FODMAP diets<sup>(9)</sup>. The supernatants from faecal samples collected in this trial were directly applied to mouse dorsal root ganglion (DRG) in patch-clamp recordings and to the lumen of mouse colonic tissue for afferent nerve recordings (Fig. 1). The aim was to investigate neurophysiological effects on pain signalling before and after dietary interventions. Reduced excitability of DRG neurones was observed with application of faecal supernatant from patients

reporting symptom improvement with the low FODMAP diet intervention, a response not seen with the high-FODMAP diet or colonic biopsy supernatant application. This was further explored through afferent nerve recordings, where intraluminal perfusion of faecal supernatant from before the dietary intervention increased markers of visceral sensitivity, an effect that was lost with application of faecal supernatant from the low FODMAP intervention. Additionally, the use of histamine antagonists successfully blocked these effects, providing mechanistic insights into the impact of altering dietary FODMAP intake<sup>(9)</sup>. These findings indicate that the interplay between diet and microbiota influences the production of luminal mediators that affect pain responses, highlighting insights not achievable through clinical study findings alone.

Although reverse translational designs utilising animal models offer distinct advantages, several critical design considerations must be addressed when employing such models in nutrition research. Firstly, there are notable physiological differences in the gastrointestinal tract among rodents, pigs, and humans that are essential for interpreting results accurately. For instance, when investigating changes in dietary intake of fermentable carbohydrates, it is crucial to consider the primary site of fermentation. In mice, fermentation predominantly occurs in the caecum, which is proportionally much larger compared to humans, in whom fermentation occurs in the colon<sup>(10)</sup>. Secondly, factors such as water source<sup>(11)</sup>, the diet the animals consume<sup>(12)</sup>, and even animal supplier<sup>(13–15)</sup> have been shown to have a pronounced impact on the microbiome profile which has implications for reproducibility of results across laboratories. Large variations exist between mouse chows available and subsequently used across laboratories, including diets made from purified ingredients *v.* grain-based diets often formulated using ground wheat and corn. In a comparative study of these two distinct types of diet on caecal microbiota, notable differences in both  $\alpha$ -diversity and  $\beta$ -diversity were observed<sup>(12)</sup>. An alternative approach to using commercially available animal diets involves custom-designed animal diets that more closely mimic human consumption. This can be achieved for both rodents<sup>(8,16)</sup> and pigs<sup>(17)</sup>, with pig diets offering a closer representation to human dietary patterns through the use of whole-food ingredients. Interestingly, one study found that sourcing mice from different suppliers had an even greater effect on the microbiome than diet<sup>(14)</sup>; thus to minimise supplier as a source of variation in the microbiome, it

has been recommended that, where possible, animals be purchased in one shipment from one supplier<sup>(18)</sup>. Thirdly, it is crucial that animal experiments consider dosages of test substrates that align with human consumption levels, rather than administering super-doses that exceed normal human dietary intake. This can be achieved through consideration of animal size and age alongside human dietary intake of the nutrient under investigation<sup>(19)</sup>, and should not be the maximal dose able to be administered as used in toxicology studies<sup>(20)</sup>. Lastly, animal model limitations, such as coprophagy (the ingestion of faeces) which occurs in many commonly used animal models including mice, rats, guinea pigs, rabbits and pigs, must also be considered<sup>(10)</sup>. Coprophagy is a normal part of animal physiology and is important for the absorption of nutrients such as vitamin K and certain B vitamins, which are produced by the gut microbiota<sup>(21)</sup>. Preventing coprophagic behaviour has been shown to alter animal physiology, particularly related to immunological tone<sup>(22–24)</sup>. As a result of coprophagic activity, the gut microbiota of co-housed animals tends to become similar over time, resulting in a cage effect<sup>(18)</sup>. One solution to mitigate this effect on experimental outcomes is to ensure that animals are only co-housed within the experimental group, and animals of different experimental groups are not co-housed together.

To fully leverage the potential of reverse translational study designs, enhanced multi-disciplinary integration of basic and clinical research is essential. Improved collaboration and understanding across these fields will lead to a more comprehensive grasp of the benefits offered by these combined techniques. Increasing the ability of clinicians, nutritionists, and dietitians to interpret fundamental scientific findings, and enabling discovery scientists to appreciate clinical relevance, including dietary manipulation, will result in higher-quality research outcomes.

### Gastrointestinal tract motility

The complex process of GI motility, which encompasses the coordinated contractions and relaxations of the smooth muscles along the GI tract, is essential for the proper digestion and absorption of nutrients, mixing of luminal contents with digestive secretions, and the efficient propulsion and elimination of waste products. Abnormalities in GI motility, characterised by altered patterns of contractility, tone, or synchronisation, can lead to not only motility disorders but also various disorders of gut-brain interaction, such as IBS, functional dyspepsia (FD), and gastroparesis<sup>(25)</sup>. These disorders not only significantly impair patients' quality of life but also place a substantial burden on healthcare systems worldwide, with increased healthcare utilisation, productivity loss, and associated costs<sup>(26,27)</sup>. Therefore, accurate assessment of GI motility is crucial for understanding the pathophysiology of these disorders, developing targeted interventions, and monitoring treatment response in both clinical and research settings including diet-specific effects.

Recent advancements in technology have led to the development of novel tools for assessing GI motility in humans, providing a more comprehensive and detailed understanding of GI function compared to traditional methods<sup>(28)</sup>. Some of these tools include high-resolution manometry (HRM), wireless motility capsule,<sup>(29)</sup> and MRI<sup>(30)</sup>. Table 1 summarises the advantages and limitations including both established and emerging techniques. The choice of method depends on the specific research question and the targeted GI tract region. Detailed discussion of the latest tools for assessing gastric motility are provided below.

### Gastric accommodation

Gastric function refers to the various activities and processes that occur within the stomach to aid in digestion and includes secretion of digestive enzymes for chemical digestion, gastric motility for mechanical digestion and gastric accommodation, which refers to the vagovagal-mediated reflex that occurs in the stomach during food intake. This reflex allows the stomach to relax and expand, providing a reservoir for the ingested meal before it is released into the small intestine in a controlled manner<sup>(46,47)</sup>. Impaired gastric accommodation is common in patients with FD and is associated with symptoms such as early satiety, postprandial fullness, and epigastric pain<sup>(48)</sup>. The migrating motor complex (MMC) is another important aspect of GI motility, characterised by a cyclical pattern of electrical and contractile activity in the stomach and small intestine during fasting, consisting of three main phases: quiescent, irregular contractions, and regular, high-amplitude contractions<sup>(49)</sup>. Control mechanisms for both gastric accommodation and MMC processes include hormonal and neural factors<sup>(49)</sup>. Disruptions in the MMC have been implicated in various GI disorders, such as small intestinal bacterial overgrowth, IBS and FD, as well as non-GI disorders including diabetes and obesity<sup>(49)</sup>.

### Intragastric pressure measurement

High-resolution manometry (HRM) is a catheter-based technique that provides detailed pressure measurements along the length of each GI region<sup>(50)</sup>. HRM provides a high spatial resolution and allows for the assessment of both the amplitude and propagation of peristaltic waves. This technique has revolutionised the understanding of GI motility disorders, particularly in the oesophagus, where it has become the gold standard for diagnosing and classifying motility disorders in clinical practice<sup>(51,52)</sup>. There are standardised HRM protocols to facilitate consistency and diagnostic reliability, detailing positions, manoeuvres, and importantly for oesophageal protocols, the bolus consistency<sup>(52)</sup>.

In research settings, HRM has been a valuable tool for investigating the effects of various interventions, particularly liquid nutrient challenges, on gastric function. The measurement of intragastric pressure (IGP) during nutrient infusion using HRM has emerged as a promising alternative to the gastric barostat for assessing gastric accommodation and motility,<sup>(53–55)</sup> having shown good reliability when compared to barostat and scintigraphy<sup>(56)</sup>. This technique involves nasally inserting a thin HRM catheter and positioning it along the stomach's length (from the fundus to the antrum and duodenum). The widespread use of HRM catheters in oesophageal manometry enhances its accessibility across various clinical and research settings. The protocol allows simultaneous measurement of IGP changes, nutrient volume tolerance and antral contractions during intragastric nutrient infusion, providing a comprehensive assessment of gastric function<sup>(53,54)</sup>.

For IGP measurements, participant preparation and data acquisition have been previously described, including in children and adults, healthy volunteers and different patient populations<sup>(53–58)</sup>. Participants lie semi-upright and can complete questionnaires (i.e. sensations of satiation and other subjective symptoms) or have blood samples taken throughout the experiment (e.g. concurrent hormonal measurements)<sup>(59–62)</sup>. Nutrient infusion is initiated after a stabilisation period (typically 10 min) at a low, constant rate (to avoid triggering secondary peristalsis)<sup>(63)</sup> until participants reach maximal satiation or report intolerable symptoms. Infusion should commence in late phase II of the MMC<sup>(49)</sup> to avoid

**Table 1.** Overview of gastrointestinal motility assessment: established and emerging tools

Tool	Key application (GI region)	Advantages	Disadvantages	Reliability/Accuracy	References
Barostat	Gastric accommodation, rectal sensitivity	Direct measurement of volume-pressure relationships	Invasive, time-consuming, somewhat limited availability	Considered the gold standard for assessing gastric accommodation and rectal sensitivity	(31)
High-resolution manometry (HRM)*	Oesophageal, gastric, duodenal, colonic, anorectal	Detailed pressure data, identifies motility patterns and can assess gastric accommodation	Invasive, requires skilled operator for placement	Gold standard for oesophageal and anorectal motility assessment	(32)
Body surface gastric mapping	Gastric myoelectrical activity	Non-invasive, provides data on gastric slow waves and dysrhythmias	Requires specialised equipment, limited data on clinical utility	Good correlation with invasive serosal mapping for assessing frequency and gastric activity	(33)
MRI	Gastric emptying, small intestinal transit, colonic transit	Non-invasive, provides detailed anatomical and functional data, no radiation exposure	Costly, time-consuming, requires specialised equipment and expertise	Good correlation with manometry and radiopaque markers for assessing orocecal, colonic and whole gut transit time	(34,35)
Breath stable-isotope test ( <sup>13</sup> C-octanoic acid breath test)	Gastric emptying	Non-invasive, well-tolerated, easy to perform	Indirect measure of gastric emptying, variability in breath sample collection and analysis, expense of <sup>13</sup> C substrate and MS analyses	Good correlation with scintigraphy	(36)
Breath hydrogen	Orocaecal transit time, small intestinal transit, small intestinal bacterial overgrowth	Non-invasive, widely available, easy to perform, low cost	Indirect measure of transit, variability in breath sample collection and analysis, false positives and reproducibility issues	Correlates with simultaneous scintigraphy however accuracy affected by many confounding factors including poor intra-individual test-retest reliability for lactulose breath testing for identification of SIBO	(37,38)
Wireless motility capsule	Gastric emptying, small intestinal transit, colonic transit	Non-invasive, provides data on whole gut transit	Capsule retention risk, limited availability, costly	Good correlation with scintigraphy and radiopaque markers for assessing gastric emptying and colonic and whole-gut transit	(39,40)
Scintigraphy	Gastric emptying, small intestinal transit, colonic transit	Non-invasive, direct visualisation of transit	Radiation exposure, limited availability, time-consuming	Considered the gold standard for gastric emptying assessment	(41,42)
Radiopaque markers	Colonic transit time	Non-invasive, widely available, easy to perform	Radiation exposure, provides limited information on segmental colonic transit and difficulty in specific anatomical location	Good correlation with wireless motility capsule for assessing colonic transit	(43,44)
Ultrasound	Gastric emptying, antral motility	Non-invasive, widely available, real-time assessment	Operator-dependent, limited visualisation of deep structures	Good correlation with scintigraphy for assessing gastric emptying	(45)

\*Includes HRM with impedance, or with pH-impedance.

interference with baseline measurements or strong phase 3 contractions. Participants can be easily blinded to the infusion contents, speed and volume to minimise psychological influences. Furthermore, the intragastric nutrient administration allows researchers to bypass any orosensory influences for food challenges. During infusion, the IGP exhibits a rapid decrease followed by a gradual recovery, indicating an initial gastric relaxation in response to nutrient infusion and a subsequent restoration of gastric tone<sup>(54,57,58)</sup>.

The advantages of IGP measurement using HRM is that it provides information on pressures in the lower oesophageal sphincter, proximal stomach, and distal stomach before, during,

and after the meal. As well as using IGP to assess gastric accommodation, the HRM recording also allows for calculation of gastric and antroduodenal motility<sup>(62)</sup>. Unlike the barostat, it does not interfere with the physiologically normal distribution of a meal within the stomach. However, like the barostat, HRM has limitations regarding invasiveness and laboratory setting measurements, which may not fully reflect real-world conditions. Participants must lie on a bed in a semi-upright position and the position of the HRM catheter (and any nasogastric tube) should be confirmed by fluoroscopy (real-time x-ray). In nutritional studies, HRM has been used to assess the effects of different liquid meal compositions or dietary components (e.g. peppermint oil,<sup>(57)</sup>

varying FODMAP concentrations,<sup>(64)</sup> nutritive *v.* non-nutritive sweeteners,<sup>(62)</sup> tastants<sup>(60,61)</sup> on gastric accommodation, motility, and satiety. However, the technique's sensitivity to detect subtle changes in gastric function induced by dietary interventions may be limited and measurements may be influenced by body position, respiration, and abdominal muscle activity, which should be considered when interpreting results<sup>(65)</sup>.

### Bioelectrical activity

Gastric electrical activity plays a central role in the organisation of gastric motility, particularly the slow wave, which is a propagating activation front that triggers the muscular contractions of peristalsis<sup>(66)</sup>. Body surface gastric mapping (BSGM) is a non-invasive technique that has been developed to overcome the technical limitations of previous electrogastrography<sup>(33)</sup> which lacks clinical utility<sup>(67)</sup>, and has been validated against invasive high-resolution serosal mapping<sup>(68)</sup>. BSGM measures the cutaneous dispersion of gastric myoelectrical potentials, arising from extracellular ion current flows during depolarisation and repolarisation of gastric tissues<sup>(69)</sup>. Recent advancements have enabled BSGM to provide valuable insights into the pathophysiology of altered gastric electrophysiology in the form of patterns of gastric dysrhythmias<sup>(68,70)</sup>.

The first commercially available BSGM test, the Gastric Alimetry™ System (Alimetry, New Zealand), involves a 30-minute fasted recording, followed by a provocative test meal (482 kcal oatmeal bar and nutrient drink), and up to 4 h where patients sit in a reclined chair<sup>(71–73)</sup>. Simultaneous digital symptom logging has recently enabled patient phenotyping alongside electrophysiology data<sup>(33)</sup>. Consensus working groups and other recent advancements in BSGM methods including the development of standardised test protocols, meals, output metrics, reference intervals, and disease classifications give promise for this technique to guide and inform clinical use<sup>(33)</sup>. However, as this technology is relatively new, some limitations include the somewhat inferred physiological basis of BSGM profiles and further data is needed to confirm relationships between gastric electrophysiology, motor activity, and symptoms<sup>(33,71)</sup>. In the context of future nutritional studies, ideally, BSGM should be able to be used to assess the postprandial response of gastric myoelectrical activity to different meal compositions and sizes, and importantly allow for varied meal completion. Currently, it is recommended that if less than 50 % of the test meal is consumed then BSGM data should be interpreted with caution<sup>(72)</sup>.

### Imaging

MRI has emerged as a powerful tool for evaluating GI motility, offering a non-invasive, non-ionising method<sup>(74)</sup>. Recent advances in MRI technology, such as faster acquisition times, improved resolution, and dedicated post-processing software, have enabled the visualisation and quantification of global and segmental GI motility<sup>(30)</sup>. MRI utilises the manipulation of hydrogen protons within the body using static and variable magnetic fields. Different pulse sequences generate contrast between tissue types, allowing for the assessment of various aspects of GI function, including gastric emptying, small bowel water content, and colonic volume<sup>(74,75)</sup>. Dynamic MRI, or cine MRI, involves acquiring a series of images to create movies of GI motion<sup>(30)</sup>. Quantitative analysis of these images using post-processing techniques, such as the dual registration of abdominal motion implemented in the

GIQuant software (Motilent Ltd, Ford, London), enables the assessment of global and local GI motility<sup>(76,77)</sup>.

Recent studies have demonstrated the utility of MRI in evaluating GI motility in healthy individuals and patients with various GI disorders<sup>(30)</sup>. Gastric MRI has also been used to assess the effects of different dietary components, such as FODMAPs, on GI physiology and symptom generation in patients with IBS<sup>(78)</sup> and uniquely interweaved with brain MRI to show how brain responses covary more extensively with GI symptom responses in IBS compared to gut responses<sup>(79)</sup>. Thus providing key data supporting the role of gut-brain axis dysregulation driving specific nutrient-induced symptom generation. However, MRI has some limitations, including supine positioning requirements, its high cost and time-consuming data processing<sup>(74)</sup>.

### GI tract and fermentation

A key function of the colonic microbiota that contributes to host health and disease is the fermentation of carbohydrates and protein, of which diet is a major source. Approximately 12–40 g of poorly absorbed and indigestible carbohydrates and 12 g of undigested proteins arrive in the colon from a typical Western diet<sup>(80–82)</sup>, contributing to the substrate pool for fermentation. Fermentation of these carbohydrates generally produces gases (carbon dioxide (CO<sub>2</sub>), hydrogen (H<sub>2</sub>) and methane (CH<sub>4</sub>)) and short-chain fatty acids (SCFAs; acetate, propionate and butyrate) with resulting acidification of luminal contents and rapid expansion of the microbial biomass<sup>(83)</sup>. However, as no poorly-absorbed or indigestible carbohydrates are created equal, the profile of metabolic end-products is highly dependent on the specific carbohydrate substrate(s) available to the microbes<sup>(84)</sup>. Conversely, fermentation of protein produces branched-chain fatty acids (BCFAs) and potentially toxic metabolites including ammonia, hydrogen sulphide (H<sub>2</sub>S) gas, phenols, indoles, and amines. These metabolic effects are also highly dependent on several factors including the type or amount of protein as well as the extent of carbohydrate fermentation<sup>(82)</sup>. Hence, to understand the fermentative profiles of the colonic microbiota in response to dietary interventions/manipulation in nutritional studies, there are a growing number of technological advancements that enable indirect or direct profiling of one or several of these metabolites simultaneously.

### *In vitro* fermentation systems

*In vitro* fermentation systems are an accessible, low cost and rapid assay for simulating colonic fermentation in the laboratory, replacing *in vivo*. techniques. These systems enable gas release, SCFAs and pH to be measured simultaneously, allowing an overview of the metabolic output of the gut microbiota to be profiled in response to diet. Examples of their utility include screening the fermentation characteristics of a large number of dietary substrates such as novel fibres<sup>(85)</sup> or food additives<sup>(86)</sup> to predict their physiological response *in vivo.*, examining interactions between dietary components (e.g. protein *v.* fibre or slowly fermentable psyllium *v.* readily fermentable inulin) on fermentation and investigating potential functional alterations of the gut microbiota in disease states. There are several *in vitro*. fermentation models that have been utilised with varying complexity ranging from *in vitro*. simulators such as the Simulator of the Human Intestinal Microbial Ecosystem (SHIME) model<sup>(87)</sup>, a 3-stage continuous system that mimics the differences in

microbial composition and activity across colonic regions to simple batch fermentation models that are static. The strengths and limitations of each method have been reviewed comprehensively elsewhere<sup>(88,89)</sup>. In the design of *in vitro* fermentation experiments, several aspects including faecal inoculum, substrates and duration of experimentation are highly influential in generating reliable results.

#### Faecal inocula

In the preparation of the faecal inocula, faecal samples from several participants are often pooled to obtain a representative diversity of the gut microbes in the colon. There is a growing body of evidence to suggest that the gut microbiota composition and metabolic responses can be heterogeneous in healthy individuals<sup>(90)</sup> as well as in disease states such as inflammatory bowel disease<sup>(91)</sup>. However, Aguirre *et al.*<sup>(92)</sup> demonstrated a similar cumulative production of SCFA between the pooled *v.* individual faecal samples, indicating a significant functional overlap but a higher biodiversity in the pooled samples. Interestingly, a large degree of inter-individual variability was seen in the production of BCFA, which was not present in the pooled samples. Therefore, the choice of pooling faecal samples or using individual inoculum may depend on the research question. Whether the faecal samples are collected fresh or frozen, is an important consideration, as the freeze-thaw process prior to the preparation of the faecal inocula has been shown to result in significant loss of specific gram-negative bacteria as well as other microbial communities<sup>(93)</sup> and favour the growth of bacteria within *Enterobacteriaceae* family<sup>(94)</sup>. Whilst freshly passed faecal samples are preferable to achieve optimal results, this may be impractical, and where freezing samples is unavoidable, cryopreservation with glycerol may attenuate unwanted effects on the microbial consortium<sup>(95)</sup>.

#### Substrates

The source of the dietary substrate can have significant implications for the fermentation result. Divergent fermentation characteristics of the same fibre substrate (arabinoxylans) but with slightly different chemical structures (i.e. from different classes of wheat) have been reported<sup>(96)</sup>, a finding that was also observed by So *et al.*<sup>(85)</sup> with two different sources of xylo-oligosaccharides (almond *v.* corn) and different products of Hi Maize resistant starch type 2. This could explain some of the inconsistencies seen in the literature on fermentation profiles of a fibre substrate but highlights the importance of detailing either fibre structure or source of substrate.

#### Experimental duration

The duration of the fermentation experiments is an additional aspect of consideration. The majority of *in vitro* experiments measure changes in fermentation kinetics over 24–48 h<sup>(97)</sup>. A rapid 4-hour faecal gas profiler has been proposed, as one study showed a 3-unit drop in pH following incubations with fibre substrates at 4 h, which can have a dramatic impact on the metabolic activities of the gut microbiota. This was demonstrated in a 48-hour *in vitro* study where the ability of microbes to ferment protein and generate phenols, a by-product of protein fermentation, decreased by 33 % at pH 5.5 *v.* pH 6.8<sup>(98)</sup>. Additionally, there may be differences in metabolic output in assays that are run for 24 h *v.* 4 h. For example, psyllium is ranked as a moderately fermentable fibre at 24 h based on its gas production profile<sup>(99)</sup>, but minimally fermentable at 4 h<sup>(85)</sup>. Therefore, batch fermentative experiments that are conducted over a prolonged duration, may have limited

physiological relevance to what occurs *in vivo*. Hence, to overcome this limitation, a rapid 4-hour faecal gas profiler using *in vitro* batch fermentation techniques was developed that enabled automated sampling of headspace gas release through an array of gas sensors that measure H<sub>2</sub>, CH<sub>4</sub>, H<sub>2</sub>S and CO<sub>2</sub> in real-time, with data communicated wirelessly to a computer for analysis<sup>(100)</sup>. This system has been demonstrated to be reliable and has good repeatability<sup>(101)</sup>.

#### *In vivo* measurements: indirect profiling via breath testing

Breath measurements of H<sub>2</sub>, CO<sub>2</sub> and CH<sub>4</sub> are highly attractive due to their ease of testing and patient acceptability. H<sub>2</sub> and CH<sub>4</sub> are produced specifically in response to microbial fermentation of carbohydrates, whereas CO<sub>2</sub> can diffuse into the gastrointestinal lumen from various chemical reactions in the GI tract (e.g. interactions between bicarbonate and stomach acid) but also from microbial fermentation. The premise of this test lies in the production of these gases in the colon that are then absorbed and travel to the lungs to be expelled in breath. Breath tests are usually performed to assess the malabsorption of carbohydrates such as in the assessment of lactose malabsorption, or the degree of fermentation in response to diets containing high or low fermentable carbohydrates such as FODMAPs. There are limitations with the use of breath hydrogen or methane assessment for carbohydrate malabsorption which may influence the interpretation of results. First, Yao *et al.* observed significant variability (8.5–345 %) in H<sub>2</sub>-producing capacity with breath testing performed on two separate occasions, at least 2 weeks apart following ingestion of 15 g lactulose, a non-digestible carbohydrate<sup>(102)</sup>. Such large variations could potentially be explained by the hastening of transit that occurs with ingestion of lactulose as has also been demonstrated previously, but could also highlight differences in hydrogen utilisation in other hydrogen disposal pathways. Second, breath H<sub>2</sub> and CH<sub>4</sub> are measured in parts per million and are poorly sensitive to low levels of gas production. For example, in a study using varying amounts of inulin, breath H<sub>2</sub> concentrations were not detectable when amounts of inulin less than 10 g were consumed<sup>(103)</sup>. Despite this, measurements of breath H<sub>2</sub> and CH<sub>4</sub> remain valuable in research settings to semi-quantitatively assess fermentative responses to a novel fibre or interactions between fibre combinations and fermentation (e.g. psyllium attenuating gas production of inulin<sup>(104)</sup>), or as indicators of compliance in dietary interventions that manipulate carbohydrate/fibre intake.

#### *In vivo* measurements: direct profiling

Differences in metabolic activities of the colonic microbiota across the different colonic regions were first described by Macfarlane *et al.*<sup>(105)</sup> following direct measurements of luminal contents of cadavers. Concentrations of SCFA were highest in the proximal colon<sup>(105,106)</sup>, indicating that carbohydrate fermentation is most active in this region. As fermentable carbohydrate supply decreases along the length of the colon, by-products of protein fermentation, such as BCFA and phenols, increase in concentration<sup>(105)</sup>, suggesting that protein fermentation is more active in the distal colon. Additionally, different fibre types, depending on the amount available and its fermentability can shape the metabolic profile in specific colonic regions. For example, oat bran, a fermentable fibre that is fermented at a moderate rate, raised total SCFA only in the caecum and not in the distal colon, suggesting that its fermentation is exhausted by the end of the proximal region<sup>(107)</sup>. In contrast,

wheat bran, being fermented at a slower rate, increases fermentation along the length of the colon<sup>(107)</sup>. Similarly, in a landmark study with pigs, supplementation with resistant starch increased butyrate production in the proximal colon, but the addition of wheat bran to resistant starch marked increased faecal SCFA concentrations, suggesting that fermentation was 'carried' to the distal colonic regions<sup>(108)</sup>. These concepts are of significant interest to nutrition studies where modulation of fermentation sites, as well as the delivery of metabolites to a specific colonic region, may have implications for optimising gut health.

Since the pioneering work of Macfarlane *et al.*<sup>(105)</sup>, technological advancements have evolved significantly with the advent of radiotelemetry devices including a wireless pH-motility capsule and more recently, intestinal gas using an Atmo gas-sensing capsule that can be performed in an ambulatory setting<sup>(29)</sup>. These telemetric capsules sample luminal contents via a semi-permeable membrane, enabling measurement of real-time changes in pH or gases directly at the site of fermentation<sup>(29)</sup>. Transit is also measured concurrently to enable localisation of the gastrointestinal region (e.g. stomach, small *v.* large intestine). These capsules therefore address current limitations of existing methods such as faecal SCFA measurements and breath H<sub>2</sub> that provide an overview of fermentation, or in the case of faecal SCFA, only reflect fermentative events in the distal colon.

The site and extent of fermentation at each colonic region was first characterised using the wireless pH-motility capsule in 33 healthy controls as well as in individuals with IBS<sup>(109)</sup>. Localisation of different colonic regions was performed by dividing the colon into quartiles based on transit time data. Using this approach, colonic pH levels were observed to be the lowest in the first quartile, confirming that fermentation was occurring maximally in the proximal region and pH increasing along the length of the colon. As a result, greater microbial fermentation was identified across all regions of the colon in patients with IBS compared to healthy controls. Subsequently, two studies<sup>(110,111)</sup> have utilised dietary fibre strategies to target changes in regional fermentation. First, direct measurements of pH using the wireless pH-motility device showed that feeding healthy controls high (13 g/d) amounts of fructans and resistant starch over a 24-hour period increased acidification of luminal contents along the length of the colon as well as in the distal colon compared to a low fibre diet (<2g)<sup>(111)</sup>. Second, tandem measurements of a gas-sensing capsule and the wireless pH-motility device saw differences in patterns of fermentation in response to diets supplemented with no fibre, a minimally fermentable or a combination of fibres<sup>(110)</sup>. For example, the wireless pH-transit capsule saw an increase in pH along the colon regardless of the fibre(s). In contrast, the gas-sensing capsule captured a significant increase in hydrogen in the last quartile of the colon, suggesting that fermentation has shifted distally compared to the no or poorly fermentable fibre where fermentation was mainly focused in the proximal colon<sup>(110)</sup>. Both these studies expanded our understanding of how diet can be manipulated to exert effects in specific colonic regions and the use of such telemetric technology to confirm the desired effects.

There are certain limitations with the selection of participants that may hinder the application of these ingestible capsules including the presence of strictures in patients with inflammatory bowel disease, previous abdominal surgery or those with difficulties swallowing large tablets<sup>(29)</sup>. Furthermore, the ingestion of these capsules follows a standardised protocol validated for transit measurements. This involves participants being fasted overnight before coming into the research centre to consume a low

energy, low-fat test meal containing 260 kJ with 2–3 % fat content. This is followed by a 6 h fast before the participant resumes usual activities. They will be instructed to wear a data receiver for the length of the capsule study until the capsule is passed<sup>(112)</sup>. Deviations in standardised protocol, either by replacing the standardised bar with a study meal that has a higher energy or fibre content<sup>(113)</sup>, or by shortening the fasting period<sup>(110)</sup> may affect whole-gut transit assessments, specifically delayed gastric emptying but does not affect capabilities to capture fermentative changes. Finally, the costs of purchasing equipment, transmission issues, and participant compliance are all factors that may influence the sample size or quality of data collected. Recently, the use of blue food dye has been utilised to assess whole-gut transit time; this methodology has several benefits in that it is low cost, low participant burden and does not require specialised equipment<sup>(114)</sup>. The emergence of direct profiling presents an exciting avenue for diet and gastroenterology research, providing indicators for which dietary strategies may target region-specific fermentative capacity.

### Intestinal permeability

The human GI tract is estimated to have a surface area of around 32m<sup>2</sup><sup>(115)</sup>. Across this surface, the GI tract must provide sufficient absorption of nutrients, electrolytes, and water from the lumen whilst also acting as a barrier against the entry of harmful substances, such as pathogens and toxins<sup>(116)</sup>. To achieve this barrier protection function, the GI tract is lined with a layer of epithelial cells, which are held together closely by tight junctions, adherens junctions, and desmosomes. In addition, this layer of cells contains goblet cells, which produce a thick layer of mucus, creating a physical barrier on top of the epithelial cell layer to prevent the gut microbiota from reaching the epithelial layer<sup>(117)</sup>. This mucus layer consists of a thick inner layer of mucus, located proximally to the epithelial layer, and a thinner outer layer of mucus. This thinner outer layer of mucus serves as a niche where gut bacteria that are mucus degraders, such as *Akkermansia muciniphila*, can reside and degrade mucin glycans<sup>(118)</sup>. Together this layer of epithelial cells and mucus layer act as a physical barrier, preventing the translocation of bacteria and bacterial components that inhabit the lumen.

Disruption of the intestinal barrier can permit the passage of microbial components, such as lipopolysaccharide, into the lamina propria and subsequently into the systemic circulation. Disruption of the intestinal barrier leads to the activation of immune response pathways in the intestinal tissue itself<sup>(119)</sup> and systemically<sup>(120)</sup>. Increased gut permeability is observed in many gut-related conditions such as ulcerative colitis and Crohn's disease<sup>(121)</sup>. Furthermore, disruption of the GI barrier has been implicated in the chronic low-grade inflammatory processes that are observed with many non-GI conditions, such as diabetes<sup>(122)</sup>, hypertension<sup>(123)</sup> and kidney disease<sup>(124)</sup>. There is increasing evidence for the role of GI permeability in the pathogenesis of many health conditions<sup>(122,125)</sup> and is also affected by nutritional factors such as parental nutrition<sup>(126)</sup>, degree of food processing<sup>(127)</sup>, dietary emulsifiers<sup>(128)</sup>, fibre<sup>(129)</sup>, fat<sup>(130)</sup> and protein<sup>(131)</sup> content.

### Transit across the gut barrier

Molecules in the lumen can pass across the GI epithelium through either paracellular or transcellular pathways, depending on the size, charge, and hydrophobicity of the molecule in question. Transcellular transport may be passive, where small compounds

**Table 2.** Overview of methods utilised for directly measuring intestinal permeability

Method	Principle	Advantage	Disadvantage	Reference
Ussing Chamber	Intestinal tissues are collected and mounted in an Ussing chambers and permeability assessed via molecular flux or TEER.	Allows assessment of permeability at specific sections of the GI tract.	Requires intestinal tissues, endoscopic biopsy in humans. Relatively time and labour-intensive, requiring specialised equipment.	(137)
FITC-Dextran Flux	The fluorescent FITC-Dextran molecule is gavaged orally, and fluorescence in the plasma indicating translocation from the gut lumen.	Non-invasive. Can be conducted with routine laboratory equipment.	As dextran-FITC is dosed by subject weight, the high cost is a barrier to application in humans and large animals.	(138)
Dual/Multi Sugar Test	An oral solution containing monosaccharide(s)/ disaccharide(s) is consumed, and the presence measured in plasma or urine. The ratio of lactulose to mannitol (or L-Rhamnose) is used to indicate small intestinal permeability.	Non-invasive and low cost.	Measurement of sugars requires HPLC or LC-MS system. Prolonged urine collection is required.	(139)
Polyethylene Glycols	Oral consumption of polyethylene glycols (PEGs) of differing molecular sizes, and measurement in urine or plasma	Non-invasive. Resistant to bacterial degradation, can assess whole gut permeability.	Range of molecular sizes of PEGs may be an issue for analysis. Prolonged urine collection is required.	(140)
<sup>51</sup> Cr-EDTA	Measurement of radiolabelled molecules in the urine, following oral administration.	Non-invasive. Resistant to bacterial degradation, can assess whole gut permeability.	Small dose of radiation for the participant, requires $\beta$ scintillation counter for measurement. Prolonged urine collection is required.	(141)

**Legend:** EDTA: Ethylenediaminetetraacetic Acid, FITC: Fluorescein Isothiocyanate, HPLC: High-Performance Liquid Chromatography, LC-MS: Liquid Chromatography-Mass Spectrometry, PEGs: Polyethylene Glycols, TEER: Transepithelial Electrical Resistance.

diffuse across the cell membrane; active, utilising cell surface receptors (e.g. PepT1 for the absorption of amino acids); or via endocytosis, where compounds such as larger peptides and proteins are enclosed in vesicles and absorbed. Importantly, this transcellular endocytosis appears to be one key mechanism by which bacterial lipopolysaccharide, and even whole bacteria themselves, can cross the intestinal epithelium<sup>(132,133)</sup>. The epithelial cells of the GI tract are kept in close contact by tight junctions, adherens proteins and desmosomes, with the tight junctions (e.g. occludin, zonula occludens-1, and claudin-2) playing an important role in regulating the ability for molecules to pass between epithelial cells, known as paracellular permeability<sup>(134)</sup>. This paracellular pathway can be divided into the pore pathway, which permits passage of molecules <8 Å in size and the leak pathway which allows molecules up to ~100 Å in size<sup>(135)</sup>. An in-depth description of these two pathways, has been recently published by Horowitz *et al.*<sup>(132)</sup>. When discussing intestinal permeability, it is the paracellular leak pathway and transcellular endocytosis that are most relevant, with the majority of research to date focused on paracellular mechanisms<sup>(136)</sup>.

### Measurement of intestinal permeability

Broadly, techniques for the assessment of intestinal permeability can be classified as either being a direct or indirect measurement. Direct, or functional, tests work on the principle of measuring the transit or flux of a molecule or group of molecules from one side of the epithelial barrier to the other, typically the luminal side to the basolateral side. A summary of the main methods utilised for directly measuring intestinal permeability, along with their respective advantages and disadvantages, is provided in Table 2. Indirect markers include the use of biomarkers in commonly collected samples such as plasma, that are purported to relate to intestinal permeability.

### Direct measurements of intestinal permeability

*In vivo* assessment of intestinal permeability is commonly conducted via the oral consumption of probes and the measurement of those probes in the plasma and/or urine. Commonly utilised probes include polyethylene glycols (PEGs), Fluorescein Isothiocyanate (FITC)-Dextran, Chromium-51-labelled ethylenediaminetetraacetic acid (<sup>51</sup>CrEDTA), mannitol, lactulose, L-rhamnose, sucralose and sucrose<sup>(136)</sup>. Tagesson *et al.*<sup>(142)</sup> first described the use of the fluorescent compound FITC-Dextran to measure intestinal permeability in 1978. This assay is widely used in small animal studies, including in mice<sup>(143)</sup>, rats<sup>(126)</sup> and poultry<sup>(144)</sup>. Dosing is typically done according to body weight or lean body mass<sup>(138)</sup>, and while this technique could be applied to larger animals including humans, it typically is not, likely due to the increased dose, and associated cost, that would be required. PEGs can span a range of sizes, with molecular weights between 400 and 4000 typically utilised in studies of intestinal permeability, which are then measured in urine or plasma by high-pressure liquid chromatography<sup>(140)</sup>. <sup>51</sup>CrEDTA has been used in rodents<sup>(145)</sup> and people<sup>(146–148)</sup> for the assessment of intestinal permeability, with the major disadvantage to this technique being the fact that participants do get exposed to a small amount of radiation<sup>(149)</sup>, although a novel protocol using non-radioactive <sup>52</sup>Cr-EDTA has recently been proposed<sup>(141)</sup>. <sup>51</sup>CrEDTA and PEGs are resistant to microbial fermentation and thus can measure whole-gut permeability<sup>(150)</sup>.

By far the most common method for assessing intestinal permeability *in vivo* in people is the dual sugar test. The dual sugar test utilises a monosaccharide (typically mannitol or L-rhamnose) and a disaccharide (typically lactulose) that is not metabolised in the small intestine. The smaller monosaccharide is capable of passing through the paracellular pore pathway, and its presence in the plasma or urine is related to the small intestinal surface area<sup>(135)</sup>. The disaccharide cannot pass through the pore pathway, instead, it



travels via the leak pathway, and thus its presence in blood or urine signifies an increase in permeability of the paracellular leak pathway, with this typically expressed as a ratio between the disaccharide and monosaccharide, i.e. the lactulose mannitol ratio<sup>(136)</sup>. Mannitol is a dietary contaminant, with <sup>13</sup>C mannitol and L-rhamnose being increasingly utilised to avoid the issues associated with the presence of (<sup>12</sup>C) mannitol in the diet<sup>(139,151)</sup>. The presence of these sugars in the urine up to two hours following oral consumption is considered to represent small intestinal permeability<sup>(140,152)</sup>, however, some studies do utilise urine collection periods of up to 5–6 h<sup>(153,154)</sup>. Whilst the majority of studies do measure the presence of these sugars in the urine, it has been suggested that serial plasma measurements provide greater sensitivity, particularly to transient changes in intestinal permeability that may be missed with bulk urine collection<sup>(155)</sup>. The probes utilised in the dual sugar test (lactulose, mannitol and L-rhamnose) are fermented by the colonic microbiota and while useful for assessing small intestinal permeability, are unreliable for colonic permeability.

It is increasingly recognised that there are vast differences between the small intestine and colon in terms of bacterial content, physiological functions and pH<sup>(156)</sup>. Thus, the dual sugar test has been expanded into the multisugar test, which utilises additional non-fermented saccharides, typically erythritol and sucralose, for measurement of colonic permeability<sup>(157,158)</sup>. Small intestinal permeability is assessed as per the dual sugar test, while colonic permeability is assessed by the sucralose to erythritol ratio or % sucralose recovery. Commonly urine that is collected between 5 and 24 h post ingestion is considered to be representative of the colon<sup>(152,153,159)</sup>, whilst other studies have used the period 8–24 h post ingestion<sup>(160,161)</sup>. This discrepancy in protocols may be related to the variation in small intestinal transit time<sup>(162)</sup>. The dual sugar, and increasingly the multi sugar, test of intestinal permeability are relatively non-invasive and cost-effective techniques for the assessment of permeability *in vivo*.

In addition to the above-mentioned *in vivo* direct permeability assessment, it is also possible to directly measure permeability *ex vivo* where intestinal tissue samples have been collected. These tissues may be collected as part of an endoscopic biopsy in people<sup>(163)</sup> or following dissection in animal studies<sup>(137,164)</sup>. The isolated section of intestinal tissue is mounted in an Ussing chamber and molecule probes such as PEGs or FITC-Dextran are placed on the luminal side of the tissue and flux to the basolateral side is measured to indicate epithelial permeability<sup>(137)</sup>. Additionally, a small voltage may be applied across the epithelial layer and the transepithelial electrical resistance (TEER) calculated, with lower TEER values indicating lower resistance and thus increased permeability across the barrier<sup>(165)</sup>. It is worth noting that TEER does not distinguish between the pore and leak pathways, as an increase in permeability through either pathway will lead to a reduction in TEER<sup>(136)</sup>. The Ussing chamber technique has the benefit of being specific to the section of the intestinal tract (e.g. duodenum, ileum, sigmoid colon, etc) where permeability is observed, however, it has the disadvantage of requiring an endoscopic biopsy in studies in people.

#### Indirect measurements of intestinal permeability

Whilst the direct measures described above and summarised in Table 2 involve the measurement of probes that pass from the luminal to the basolateral side of the gastrointestinal epithelium, indirect measures refer to biomarkers that may relate to permeability. The presence of lipopolysaccharide, from Gram-negative bacteria, in the blood has been widely used as a marker of

permeability, however, there are concerns that the limulus amoebocyte lysate assay used to measure it is not accurate at the lower levels observed with chronic low-grade inflammatory conditions<sup>(166)</sup>. Lipopolysaccharide binding protein (LBP) is an endogenous protein that is produced in response to LPS<sup>(167)</sup> and is commonly used as a marker of permeability. In a study comparing biomarkers with directly measured permeability, it was the only plasma biomarker to significantly correlate with the lactulose mannitol ratio, although it should be noted that the correlation coefficient ranged from  $r = 0.423$  in healthy-weight participants to  $r = 0.813$  in obese participants, suggesting that it may not be a highly reliable marker in all health states<sup>(154)</sup>. The two other plasma markers assessed in this study, zonulin and intestinal fatty acid binding protein (I-FABP), showed no correlation with the lactulose mannitol ratio<sup>(154)</sup>. Furthermore, there is evidence to suggest that commercially available zonulin kits may not be measuring zonulin, but another protein altogether<sup>(168,169)</sup>. An in-depth discussion regarding the plethora of purported intestinal permeability biomarkers has been published by Perez-Diaz-del-Campo *et al.*<sup>(170)</sup>. In summary, there is a need for more accurate biomarkers of intestinal permeability, with LBP being the current marker that appears to correlate best with directly measured permeability.

#### Conclusion

New technology and accessible tools will advance the identification of physiological biomarkers, enabling a better understanding of gastrointestinal function and dysfunction. Characterising motility, fermentation and permeability phenotypes that correlate with health and disease including specific disorders of gut-brain interaction will be crucial for developing targeted, individualised therapies. The ideal approaches should be non-invasive, easily applicable to large populations, highly sensitive and specific to reliably inform clinical management, and should be able to be accurately undertaken in a fed and physiological state.

**Acknowledgements.** The authors extend their gratitude to the attendees of the workshop titled “Design, Assessment, and Interpretation of Diet-Microbiome Interactions: Using Cutting-Edge Gut-Brain Studies as an Exemplar”, held during the 2023 combined Annual Scientific Meeting of the New Zealand and Australian Nutrition Societies, for their valuable insights and contributions.

**Author contributions.** M.S. conceptualised the manuscript. M.S., J.B., C.K.Y. and C.T. drafted, edited and approved the final manuscript.

**Financial support.** This work received no specific grant from any funding agency, commercial or not-for-profit sectors. JRB is supported by a National Health and Medical Research Council Emerging Leadership Fellowship (APP2025943). MS is supported by a National Heart Foundation Postdoctoral Fellowship (106 698). CK is supported by the Crohn’s Colitis Australia Angela McAvoy Scholarship and the International Organization for the Study of Inflammatory Bowel Diseases.

**Competing interests.** None.

#### References

1. Cryan JF, O’Riordan KJ, Cowan CS, *et al.* (2019) The microbiota-gut-brain axis. *Physiol Rev* **99**, 1877–2013.
2. Sandhu KV, Sherwin E, Schellekens H, *et al.* (2017) Feeding the microbiota-gut-brain axis: diet, microbiome, and neuropsychiatry. *Transl Res* **179**, 223–244.

3. Zheng P, Zeng B, Zhou C, *et al.* (2016) Gut microbiome remodeling induces depressive-like behaviors through a pathway mediated by the host's metabolism. *Mol Psychiatry* **21**, 786–796.
4. Jang H-M, Kim J-K, Joo M-K, *et al.* (2021) Transplantation of fecal microbiota from patients with inflammatory bowel disease and depression alters immune response and behavior in recipient mice. *Sci Rep* **11**, 20406.
5. Koren O, Goodrich JK, Cullender TC, *et al.* (2012) Host remodeling of the gut microbiome and metabolic changes during pregnancy. *Cell* **150**, 470–480.
6. Secombe KR, Al-Qadami GH, Subramaniam CB, *et al.* (2021) Guidelines for reporting on animal fecal transplantation (GRAFT) studies: recommendations from a systematic review of murine transplantation protocols. *Gut Microbes* **13**, 1979878.
7. McIntosh K, Reed DE, Schneider T, *et al.* (2017) FODMAPs alter symptoms and the metabolome of patients with IBS: a randomised controlled trial. *Gut* **66**, 1241–1251.
8. De Palma G, Shimbori C, Reed DE, *et al.* (2022) Histamine production by the gut microbiota induces visceral hyperalgesia through histamine 4 receptor signaling in mice. *Sci Transl Med* **14**, eabj1895.
9. Tuck CJ, Omar AA, De Palma G, *et al.* (2023) Changes in signalling from faecal neuroactive metabolites following dietary modulation of IBS pain. *Gut* **72**, 1678–1691.
10. Nguyen TLA, Vieira-Silva S, Liston A, *et al.* (2015) How informative is the mouse for human gut microbiota research? *Dis Model Mech* **8**, 1–16.
11. Barnett JA & Gibson DL (2019) H<sub>2</sub>O<sub>2</sub> no! The importance of reporting your water source in your in vivo microbiome studies. *Gut Microbes* **10**, 261–269.
12. Tuck C, De Palma G, Takami K, *et al.* (2020) Nutritional profile of rodent diets impacts experimental reproducibility in microbiome preclinical research. *Sci Rep* **10**, 17784.
13. Wolff NS, Jacobs MC, Haak BW, *et al.* (2020) Vendor effects on murine gut microbiota and its influence on lipopolysaccharide-induced lung inflammation and gram-negative pneumonia. *Intensive Care Med* **Exp** **8**, 47.
14. Rasmussen TS, de Vries L, Kot W, *et al.* (2019) Mouse vendor influence on the bacterial and viral gut composition exceeds the effect of diet. *Viruses* **11**, 435.
15. Tomkovich S, Stough JMA, Bishop L, *et al.* (2020) The initial gut microbiota and response to antibiotic perturbation influence clostridioides difficile clearance in mice. *mSphere* **5**, e00869–00820.
16. Tuck CJ, Caminero A, Jiménez Vargas NN, *et al.* (2019) The impact of dietary fermentable carbohydrates on a postinflammatory model of irritable bowel syndrome. *Neurogastroenterol Motil* **31**, e13675.
17. Kalantar-Zadeh K, Yao CK, Berean KJ, *et al.* (2016) Intestinal gas capsules: a proof-of-concept demonstration. *Gastroenterology* **150**, 37–39.
18. Muralitharan RR, Snelson M, Meric G, *et al.* (2023) Guidelines for microbiome studies in renal physiology. *Am J Physiol Renal Physiol* **325**, F345–F362.
19. Lai M, Chandrasekera PC & Barnard ND (2014) You are what you eat, or are you? The challenges of translating high-fat-fed rodents to human obesity and diabetes. *Nutr Diabetes* **4**, e135.
20. Committee ES (2011) Guidance on conducting repeated-dose 90-day oral toxicity study in rodents on whole food/feed. *EFSA J* **9**, 2438.
21. Savage DC (1986) Gastrointestinal microflora in mammalian nutrition. *Annu Rev Nutr* **6**, 155–178.
22. Aviles-Rosa EO, Rakhshandeh A & McGlone JJ (2019) Preliminary study: depriving piglets of maternal feces for the first seven days post-partum changes piglet physiology and performance before and after weaning. *Anim* **9**, 268.
23. Sha H, He X, Yan K, *et al.* (2023) Blocking coprophagy increases the levels of inflammation and depression in healthy mice as well as mice receiving fecal microbiota transplantation from disease model mice donors. *APMIS* **131**, 351–368.
24. Bo TB, Zhang XY, Kohl KD, *et al.* (2020) Coprophagy prevention alters microbiome, metabolism, neurochemistry, and cognitive behavior in a small mammal. *ISME J* **14**, 2625–2645.
25. Black CJ, Drossman DA, Talley NJ, *et al.* (2020) Functional gastrointestinal disorders: advances in understanding and management. *Lancet* **396**, 1664–1674.
26. Sperber AD, Bangdiwala SI, Drossman DA, *et al.* (2021) Worldwide prevalence and burden of functional gastrointestinal disorders, results of Rome foundation global study. *Gastroenterology* **160**, 99–114.e113.
27. Aziz I, Palsson OS, Törnblom H, *et al.* (2018) The prevalence and impact of overlapping Rome IV-diagnosed functional gastrointestinal disorders on somatization, quality of life, and healthcare utilization: a cross-sectional general population study in three countries. *Am J Gastroenterol* **113**, 86–96.
28. Fox MR, Kahrilas PJ, Roman S, *et al.* (2018) Clinical measurement of gastrointestinal motility and function: who, when and which test? *Nat Rev Gastroenterol Hepatol* **15**, 568–579.
29. Thwaites PA, Yao CK, Halmos EP, *et al.* (2024) Current status and future directions of ingestible electronic devices in gastroenterology. *Alim Pharmacol Ther* **59**, 459–474.
30. de Jonge CS, Smout AJPM, Nederveen AJ, *et al.* (2018) Evaluation of gastrointestinal motility with MRI: advances, challenges and opportunities. *Neurogastroenterol Motil* **30**, e13257.
31. Sarnelli G, Vos R, Cuomo R, *et al.* (2001) Reproducibility of gastric barostat studies in healthy controls and in dyspeptic patients. *Am J Gastroenterol* **96**, 1047–1053.
32. Kahrilas PJ, Bredenoord AJ, Fox M, *et al.* (2015) The Chicago classification of esophageal motility disorders, v3.0. *Neurogastroenterol Motil* **27**, 160–174.
33. O'Grady G, Varghese C, Schamberg G, *et al.* (2023) Principles and clinical methods of body surface gastric mapping: technical review. *Neurogastroenterol Motil* **35**, e14556.
34. Kirchoff S, Nicolaus M, Schirra J, *et al.* (2011) Assessment of colon motility using simultaneous manometric and functional cine-MRI analysis: preliminary results. *Abdom Imaging* **36**, 24–30.
35. Chaddock G, Lam C, Hoard CL, *et al.* (2014) Novel MRI tests of orocecal transit time and whole gut transit time: studies in normal subjects. *Neurogastroenterol Motil* **26**, 205–214.
36. Choi MG, Camilleri M, Burton DD, *et al.* (1997) [13C]octanoic acid breath test for gastric emptying of solids: accuracy, reproducibility, and comparison with scintigraphy. *Gastroenterology* **112**, 1155–1162.
37. Hammer HF, Fox MR, Keller J, *et al.* (2022) European guideline on indications, performance, and clinical impact of hydrogen and methane breath tests in adult and pediatric patients: European association for gastroenterology, endoscopy and nutrition, European society of neurogastroenterology and motility, and European society for paediatric gastroenterology hepatology and nutrition consensus. *United Eur Gastroenterol J* **10**, 15–40.
38. Szarka LA & Camilleri M (2012) Methods for the assessment of small-bowel and colonic transit. *Semin Nucl Med* **42**, 113–123.
39. Maqbool S, Parkman HP & Friedenberg FK (2009) Wireless capsule motility: comparison of the SmartPill® GI monitoring system with scintigraphy for measuring whole gut transit. *Dig Dis Sci* **54**, 2167–2174.
40. Kuo B, McCallum RW, Koch KL, *et al.* (2008) Comparison of gastric emptying of a nondigestible capsule to a radio-labelled meal in healthy and gastroparetic subjects. *Alim Pharmacol Ther* **27**, 186–196.
41. Tougas G, Chen Y, Coates G, *et al.* (2000) Standardization of a simplified scintigraphic methodology for the assessment of gastric emptying in a multicenter setting. *Am J Gastroenterol* **95**, 78–86.
42. Argenyi EE, Soffer EE, Madsen MT, *et al.* (1995) Scintigraphic evaluation of small bowel transit in healthy subjects: inter- and intrasubject variability. *Am J Gastroenterol* **90**, 938–942.
43. Sadik R, Abrahamsson H & Stotzer PO (2003) Gender differences in gut transit shown with a newly developed radiological procedure. *Scand J Gastroenterol* **38**, 36–42.
44. Rao SSC, Kuo B, McCallum RW, *et al.* (2009) Investigation of colonic and whole-gut transit with wireless motility capsule and radiopaque markers in constipation. *Clin Gastroenterol Hepatol* **7**, 537–544.
45. Holt S, Cervantes J, Wilkinson AA, *et al.* (1986) Measurement of gastric emptying rate in humans by real-time ultrasound. *Gastroenterology* **90**, 918–923.

46. Febo-Rodriguez L, Chumpitazi BP, Sher AC, *et al.* (2021) Gastric accommodation: physiology, diagnostic modalities, clinical relevance, and therapies. *Neurogastroenterol Motil* **33**, e14213.
47. Kindt S & Tack J (2006) Impaired gastric accommodation and its role in dyspepsia. *Gut* **55**, 1685–1691.
48. Tack J, Piessevaux H, Coulie B, *et al.* (1998) Role of impaired gastric accommodation to a meal in functional dyspepsia. *Gastroenterology* **115**, 1346–1352.
49. Deloosse E, Janssen P, Depoortere I, *et al.* (2012) The migrating motor complex: control mechanisms and its role in health and disease. *Nat Rev Gastroenterol Hepatol* **9**, 271–285.
50. Camilleri M, Bharucha AE, di Lorenzo C, *et al.* (2008) American neurogastroenterology and motility Society consensus statement on intraluminal measurement of gastrointestinal and colonic motility in clinical practice. *Neurogastroenterol Motil* **20**, 1269–1282.
51. Tack J, Pauwels A, Roman S, *et al.* (2021) European society for neurogastroenterology and motility (ESNM) recommendations for the use of high-resolution manometry of the esophagus. *Neurogastroenterol Motil* **33**, e14043.
52. Yadlapati R, Kahrilas PJ, Fox MR, *et al.* (2021) Esophageal motility disorders on high-resolution manometry: Chicago classification version 4.0©. *Neurogastroenterol Motil* **33**, e14058.
53. Janssen P, Verschueren S, Ly HG, *et al.* (2011) Intragastric pressure during food intake: a physiological and minimally invasive method to assess gastric accommodation. *Neurogastroenterol Motil* **23**, 316–322.
54. Janssen P, Verschueren S & Tack J (2012) Intragastric pressure as a determinant of food intake. *Neurogastroenterol Motil* **24**, 612–e268.
55. Carbone F, Vanuytsel T & Tack J (2017) The effect of mirtazapine on gastric accommodation, gastric sensitivity to distention, and nutrient tolerance in healthy subjects. *Neurogastroenterol Motil* **29**, e13146.
56. Carbone F, Tack J & Hoffman I (2017) The intragastric pressure measurement: a novel method to assess gastric accommodation in functional dyspepsia children. *J Pediatr Gastroenterol Nutr* **64**, 918–924.
57. Papanthanasopoulos A, Rotondo A, Janssen P, *et al.* (2013) Effect of acute peppermint oil administration on gastric sensorimotor function and nutrient tolerance in health. *Neurogastroenterol Motil* **25**, e263–e271.
58. Rotondo A, Janssen P, Mulè F, *et al.* (2013) Effect of the GLP-1 analog liraglutide on satiation and gastric sensorimotor function during nutrient-drink ingestion. *Int J Obes* **37**, 693–698.
59. Rotondo A, Masuy I, Verbeure W, *et al.* (2019) Randomised clinical trial: the DPP-4 inhibitor, vildagliptin, inhibits gastric accommodation and increases glucagon-like peptide-1 plasma levels in healthy volunteers. *Aliment Pharmacol Ther* **49**, 997–1004.
60. Deloosse E, Janssen P, Corsetti M, *et al.* (2017) Intragastric infusion of denatonium benzoate attenuates interdigestive gastric motility and hunger scores in healthy female volunteers. *Am J Clin Nutr* **105**, 580–588.
61. Deloosse E, Vos R, Corsetti M, *et al.* (2015) Endogenous motilin, but not ghrelin plasma levels fluctuate in accordance with gastric phase III activity of the migrating motor complex in man. *Neurogastroenterol Motil* **27**, 63–71.
62. Meyer-Gerspach AC, Biesiekierski JR, Deloosse E, *et al.* (2018) Effects of caloric and noncaloric sweeteners on antroduodenal motility, gastrointestinal hormone secretion and appetite-related sensations in healthy subjects. *Am J Clin Nutr* **107**, 707–716.
63. Janssen P, Vanden Berghe P, Verschueren S, *et al.* (2011) Review article: the role of gastric motility in the control of food intake. *Aliment Pharmacol Ther* **33**, 880–894.
64. Masuy I, Van Oudenhove L, Tack J, *et al.* (2018) Effect of intragastric FODMAP infusion on upper gastrointestinal motility, gastrointestinal, and psychological symptoms in irritable bowel syndrome vs healthy controls. *Neurogastroenterol Motil* **30**, e13167.
65. Welch RW & Gray JE (1982) Influence of respiration on recordings of lower esophageal sphincter pressure in humans. *Gastroenterology* **83**, 590–594.
66. O'Grady G, Du P, Cheng LK, *et al.* (2010) Origin and propagation of human gastric slow-wave activity defined by high-resolution mapping. *Am J Physiol Gastrointest Liver Physiol* **299**, G585–G592.
67. Schamberg G, Calder S, Varghese C, *et al.* (2023) Comparison of gastric Alimetry® body surface gastric mapping versus electrogastrography spectral analysis. *Sci Rep* **13**, 14987.
68. O'Grady G, Angeli TR, Du P, *et al.* (2012) Abnormal initiation and conduction of slow-wave activity in gastroparesis, defined by high-resolution electrical mapping. *Gastroenterology* **143**, 589–598.e583.
69. Du P, O'Grady G, Cheng LK, *et al.* (2010) A multiscale model of the electrophysiological basis of the human electrogastrogram. *Biophys J* **99**, 2784–2792.
70. Angeli TR, Cheng LK, Du P, *et al.* (2015) Loss of interstitial cells of cajal and patterns of gastric dysrhythmia in patients with chronic unexplained nausea and vomiting. *Gastroenterology* **149**, 56–66.e55.
71. Carson DA, O'Grady G, Du P, *et al.* (2021) Body surface mapping of the stomach: new directions for clinically evaluating gastric electrical activity. *Neurogastroenterol Motil* **33**, e14048.
72. Calder S, Schamberg G, Varghese C, *et al.* (2022) An automated artifact detection and rejection system for body surface gastric mapping. *Neurogastroenterol Motil* **34**, e14421.
73. Gharibans AA, Calder S, Varghese C, *et al.* (2022) Gastric dysfunction in patients with chronic nausea and vomiting syndromes defined by a noninvasive gastric mapping device. *Sci Transl Med* **14**, eabq3544.
74. Marciani L (2011) Assessment of gastrointestinal motor functions by MRI: a comprehensive review. *Neurogastroenterol Motil* **23**, 399–407.
75. Khalaf A, Hoad CL, Menys A, *et al.* (2018) MRI assessment of the postprandial gastrointestinal motility and peptide response in healthy humans. *Neurogastroenterol Motil* **30**, e13182.
76. Menys A, Taylor SA, Emmanuel A, *et al.* (2013) Global small bowel motility: assessment with dynamic MR imaging. *Radiol* **269**, 443–450.
77. Menys A, Hamy V, Makanyanga J, *et al.* (2014) Dual registration of abdominal motion for motility assessment in free-breathing data sets acquired using dynamic MRI. *Phys Med Biol* **59**, 4603.
78. Major G, Pritchard S, Murray K, *et al.* (2017) Colon hypersensitivity to distension, rather than excessive gas production, produces carbohydrate-related symptoms in individuals with irritable bowel syndrome. *Gastroenterology* **152**, 124–133.
79. Wu J, Masuy I, Biesiekierski JR, *et al.* (2022) Gut-brain axis dysfunction underlies FODMAP-induced symptom generation in irritable bowel syndrome. *Aliment Pharmacol Ther* **55**, 670–682.
80. Cummings JH, Englyst HN & Wiggins HS (1986) The role of carbohydrates in lower gut function. *Nutr Rev* **44**, 50–54.
81. Barrett JS, Geary RB, Muir JG, *et al.* (2010) Dietary poorly absorbed, short-chain carbohydrates increase delivery of water and fermentable substrates to the proximal colon. *Aliment Pharmacol Ther* **31**, 874–882.
82. Yao CK, Muir JG & Gibson PR (2016) Review article: insights into colonic protein fermentation, its modulation and potential health implications. *Aliment Pharmacol Ther* **43**, 181–196.
83. Cummings JH & Englyst HN (1987) Fermentation in the human large intestine and the available substrates. *Am J Clin Nutr* **45**, 1243–1255.
84. So D, Gibson PR, Muir JG, *et al.* (2021) Dietary fibres and IBS: translating functional characteristics to clinical value in the era of personalised medicine. *Gut* **70**, 2383–2394.
85. So D, Yao CK, Gill PA, *et al.* (2021) Screening dietary fibres for fermentation characteristics and metabolic profiles using a rapid in vitro approach: implications for irritable bowel syndrome. *Br J Nutr* **126**, 208–218.
86. Gerasimidis K, Bryden K, Chen X, *et al.* (2020) The impact of food additives, artificial sweeteners and domestic hygiene products on the human gut microbiome and its fibre fermentation capacity. *Eur J Nutr* **59**, 3213–3230.
87. Molly K, Woestyne MV, Smet ID, *et al.* (1994) Validation of the simulator of the human intestinal microbial ecosystem (SHIME) reactor using microorganism-associated activities. *Microb Ecol Health Dis* **7**, 191–200.
88. Ou JZ, Yao CK, Rotbart A, *et al.* (2015) Human intestinal gas measurement systems: in vitro fermentation and gas capsules. *Trends Biotechnol* **33**, 208–213.

89. Kalantar-Zadeh K, Berean KJ, Burgell RE, *et al.* (2019) Intestinal gases: influence on gut disorders and the role of dietary manipulations. *Nat Rev Gastroenterol Hepatol* **16**, 733–747.
90. Cantu-Jungles TM, Bulut N, Chambry E, *et al.* (2021) Dietary fiber hierarchical specificity: the missing link for predictable and strong shifts in gut bacterial communities. *mBio* **12**, e0102821.
91. Armstrong HK, Bording-Jorgensen M, Santer DM, *et al.* (2023) Unfermented  $\beta$ -fructan fibers fuel inflammation in select inflammatory bowel disease patients. *Gastroenterology* **164**, 228–240.
92. Aguirre M, Ramiro-García J, Koenen ME, *et al.* (2014) To pool or not to pool? Impact of the use of individual and pooled fecal samples for in vitro fermentation studies. *J Microbiol Methods* **107**, 1–7.
93. Aguirre M, Eck A, Koenen ME, *et al.* (2015) Evaluation of an optimal preparation of human standardized fecal inocula for in vitro fermentation studies. *J Microbiol Methods* **117**, 78–84.
94. Poppe J, Vieira-Silva S, Raes J, *et al.* (2023) Systematic optimization of fermentation conditions for in vitro fermentations with fecal inocula. *Front Microbiol* **14**, 1198903.
95. de Carvalho NM, Oliveira DL, Dib Saleh MA, *et al.* (2021) Preservation of human gut microbiota inoculums for in vitro fermentations studies. *Ferment* **7**, 14.
96. Tuncil YE, Thakkar RD, Arioglu-Tuncil S, *et al.* (2020) Subtle variations in dietary-fiber fine structure differentially influence the composition and metabolic function of gut microbiota. *mSphere* **5**, e00180–20.
97. Ou JZ, Yao CK, Rotbart A, *et al.* (2015) Human intestinal gas measurement systems: in vitro fermentation and gas capsules. *Trends Biotechnol* **33**, 208–213.
98. Smith EA & MacFarlane GT (1998) Enumeration of amino acid fermenting bacteria in the human large intestine: effects of pH and starch on peptide metabolism and dissimilation of amino acids. *FEMS Microbiol Ecol* **25**, 355–368.
99. Timm DA, Stewart ML, Hospattankar A, *et al.* (2010) Wheat dextrin, psyllium, and inulin produce distinct fermentation patterns, gas volumes, and short-chain fatty acid profiles in vitro. *J Med Food* **13**, 961–966.
100. Rotbart A, Yao CK, Ha N, *et al.* (2017) Designing an in-vitro gas profiling system for human faecal samples. *Sens Actuators B Chem* **238**, 754–764.
101. Yao CK, Rotbart A, Ou JZ, *et al.* (2018) Modulation of colonic hydrogen sulfide production by diet and mesalazine utilizing a novel gas-profiling technology. *Gut Microbes* **9**, 510–522.
102. Yao CK, Tuck CJ, Barrett JS, *et al.* (2017) Poor reproducibility of breath hydrogen testing: implications for its application in functional bowel disorders. *United Eur Gastroenterol J* **5**, 284–292.
103. Berean KJ, Ha N, Ou JZ, *et al.* (2018) The safety and sensitivity of a telemetric capsule to monitor gastrointestinal hydrogen production in vivo in healthy subjects: a pilot trial comparison to concurrent breath analysis. *Aliment Pharmacol Ther* **48**, 646–654.
104. Gunn D, Abbas Z, Harris HC, *et al.* (2022) Psyllium reduces inulin-induced colonic gas production in IBS: MRI and in vitro fermentation studies. *Gut* **71**, 919–927.
105. Macfarlane GT, Gibson GR & Cummings JH (1992) Comparison of fermentation reactions in different regions of the human colon. *J Appl Bacteriol* **72**, 57–64.
106. Cummings JH, Pomare EW, Branch WJ, *et al.* (1987) Short chain fatty acids in human large intestine, portal, hepatic and venous blood. *Gut* **28**, 1221–1227.
107. McIntyre A, Young GP, Taranto T, *et al.* (1991) Different fibers have different regional effects on luminal contents of rat colon. *Gastroenterology* **101**, 1274–1281.
108. Govers MJAP, Gannon NJ, Dunshea FR, *et al.* (1999) Wheat bran affects the site of fermentation of resistant starch and luminal indexes related to colon cancer risk: a study in pigs. *Gut* **45**, 840–847.
109. Ringel-Kulka T, Choi CH, Temas D, *et al.* (2015) Altered colonic bacterial fermentation as a potential pathophysiological factor in irritable bowel syndrome. *Am J Gastroenterol* **110**, 1339–1346.
110. So D, Yao CK, Gill PA, *et al.* (2023) Detection of changes in regional colonic fermentation in response to supplementing a low FODMAP diet with dietary fibres by hydrogen concentrations, but not by luminal pH. *Aliment Pharmacol Ther* **58**, 417–428.
111. Yao C, Burgell R, Taylor K, *et al.* (2017) Intestinal pH-transit profiling in patients with ulcerative colitis as a strategy to predict success of regional delivery of topically acting drugs. *J Gastroenterology Hepatol* **32**, 126–127.
112. Wang YT, Mohammed SD, Farmer AD, *et al.* (2015) Regional gastrointestinal transit and pH studied in 215 healthy volunteers using the wireless motility capsule: influence of age, gender, study country and testing protocol. *Aliment Pharmacol Ther* **42**, 761–772.
113. Wallace A, Eady S, Drummond L, *et al.* (2017) A pilot randomized cross-over trial to examine the effect of kiwifruit on satiety and measures of gastric comfort in healthy adult males. *Nutrients* **9**, 639.
114. Asnicar F, Leeming ER, Dimidi E, *et al.* (2021) Blue poo: impact of gut transit time on the gut microbiome using a novel marker. *Gut* **70**, 1665–1674.
115. Helander HF & Fändriks L (2014) Surface area of the digestive tract - revisited. *Scand J Gastroenterol* **49**, 681–689.
116. Buckley A & Turner JR (2018) Cell biology of tight junction barrier regulation and mucosal disease. *Cold Spring Harb Perspect Biol* **10**, a029314.
117. Cornick S, Tawiah A & Chadee K (2015) Roles and regulation of the mucus barrier in the gut. *Tissue Barriers* **3**, e982426.
118. Luis AS & Hansson GC (2023) Intestinal mucus and their glycans: a habitat for thriving microbiota. *Cell Host Microbe* **31**, 1087–1100.
119. Desai MS, Seekatz AM, Koropatkin NM, *et al.* (2016) A dietary fiber-deprived gut microbiota degrades the colonic mucus barrier and enhances pathogen susceptibility. *Cell* **167**, 1339–1353.e1321.
120. Di Vincenzo F, Del Gaudio A, Petito V, *et al.* (2024) Gut microbiota, intestinal permeability, and systemic inflammation: a narrative review. *Intern Emerg Med* **19**, 275–293.
121. Nalle SC & Turner JR (2015) Intestinal barrier loss as a critical pathogenic link between inflammatory bowel disease and graft-versus-host disease. *Mucosal Immunol* **8**, 720–730.
122. Snelson M, de Pasquale C, Ekinci EI, *et al.* (2021) Gut microbiome, prebiotics, intestinal permeability and diabetes complications. *Best Pract Res Clin Endo Metab* **35**, 101507.
123. Snelson M, Vanuytsel T & Marques FZ (2024) Breaking the barrier: the role of gut epithelial permeability in the pathogenesis of hypertension. *Curr Hypertens Rep* **26**, 369–380.
124. Meijers B, Farré R, Dejongh S, *et al.* (2018) Intestinal barrier function in chronic kidney disease. *Toxins* **10**, 298.
125. Bischoff SC, Barbara G, Buurman W, *et al.* (2014) Intestinal permeability—a new target for disease prevention and therapy. *BMC Gastroenterol* **14**, 189–189.
126. Purandare S, Offenbartl K, Weström B, *et al.* (1989) Increased gut permeability to fluorescein isothiocyanate-dextran after total parenteral nutrition in the rat. *Scand J Gastroenterol* **24**, 678–682.
127. Snelson M, Tan S, Clarke R, *et al.* (2021) Processed foods drive intestinal barrier permeability and microvascular diseases. *Sci Adv* **7**, eabe4841.
128. Chassaing B, Koren O, Goodrich JK, *et al.* (2015) Dietary emulsifiers impact the mouse gut microbiota promoting colitis and metabolic syndrome. *Nat* **519**, 92–96.
129. Cani PD, Possemiers S, Van de Wiele T, *et al.* (2009) Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. *Gut* **58**, 1091–1103.
130. Rohr MW, Narasimhulu CA, Rudeski-Rohr TA, *et al.* (2020) Negative effects of a high-fat diet on intestinal permeability: a review. *Adv Nutr* **11**, 77–91.
131. Snelson M, Clarke RE, Nguyen T-V, *et al.* (2021) Long term high protein diet feeding alters the microbiome and increases intestinal permeability, systemic inflammation and kidney injury in mice. *Mol Nut Food Res* **65**, e2000851.
132. Horowitz A, Chanez-Paredes SD, Haest X, *et al.* (2023) Paracellular permeability and tight junction regulation in gut health and disease. *Nat Rev Gastroenterol Hepatol* **20**, 417–432.
133. O'Donoghue EJ & Krachler AM (2016) Mechanisms of outer membrane vesicle entry into host cells. *Cell Microbiol* **18**, 1508–1517.

134. Campbell HK, Maiers JL & DeMali KA (2017) Interplay between tight junctions & adherens junctions. *Exp Cell Res* **358**, 39–44.
135. Zuo L, Kuo W-T & Turner JR (2020) Tight junctions as targets and effectors of mucosal immune homeostasis. *Cell Mol Gastroenterol Hepatol* **10**, 327–340.
136. Vanuytsel T, Tack J & Farre R (2021) The role of intestinal permeability in gastrointestinal disorders and current methods of evaluation. *Front Nutr* **8**, 717925.
137. Thomson A, Smart K, Somerville MS, *et al.* (2019) The Ussing chamber system for measuring intestinal permeability in health and disease. *BMC Gastroenterol* **19**, 98.
138. Voetmann LM, Rolin B, Kirk RK, *et al.* (2023) The intestinal permeability marker FITC-dextran 4kDa should be dosed according to lean body mass in obese mice. *Nutr Diabetes* **13**, 1.
139. Khoshbin K, Khanna L, Maselli D, *et al.* (2021) Development and validation of test for “leaky gut”; small intestinal and colonic permeability using sugars in healthy adults. *Gastroenterology* **161**, 463–475.e413.
140. Camilleri M (2021) What is the leaky gut? Clinical considerations in humans. *Curr Opin Clin Nutr Metab Care* **24**, 473–482.
141. von Martels JZH, Bourgonje AR, Harmsen HJM, *et al.* (2019) Assessing intestinal permeability in Crohn’s disease patients using orally administered 52Cr-EDTA. *PLoS One* **14**, e0211973.
142. Tagesson C, Sjö Dahl R & Thorén B (1978) Passage of molecules through the wall of the gastrointestinal tract. *Scand J Gastroenterol* **13**, 519–524.
143. Snelson M, Tan SM, Higgins GC, *et al.* (2020) Exploring the role of the metabolite-sensing receptor GPR109a in diabetic nephropathy. *Am J Physiol Renal Physiol* **318**, F835–F842.
144. Liu J, Teng PY, Kim WK, *et al.* (2021) Assay considerations for fluorescein isothiocyanate-dextran (FITC-d): an indicator of intestinal permeability in broiler chickens. *Poult Sci* **100**, 101202.
145. Ten Bruggencate SJM, Bovee-Oudenhoven IMJ, Lettink-Wissink MLG, *et al.* (2005) Dietary fructooligosaccharides increase intestinal permeability in rats. *J Nutr* **135**, 837–842.
146. Ten Bruggencate SJM, Bovee-Oudenhoven IMJ, Lettink-Wissink MLG, *et al.* (2006) Dietary fructooligosaccharides affect intestinal barrier function in healthy men. *J Nutr* **136**, 70–74.
147. Ainsworth M, Eriksen J, Rasmussen JW, *et al.* (1989) Intestinal permeability of 51Cr-labelled ethylenediaminetetraacetic acid in patients with Crohn’s disease and their healthy relatives. *Scand J Gastroenterol* **24**, 993–998.
148. Arslan G, Atasever T, Cindoruk M, *et al.* (2001) (51)CrEDTA colonic permeability and therapy response in patients with ulcerative colitis. *Nucl Med Commun* **22**, 997–1001.
149. Horton F, Wright J, Smith L, *et al.* (2014) Increased intestinal permeability to oral chromium (51Cr) -EDTA in human Type 2 diabetes. *Diabet Med* **31**, 559–563.
150. Bjarnason I, Macpherson A & Hollander D (1995) Intestinal permeability: an overview. *Gastroenterology* **108**, 1566–1581.
151. Grover M, Camilleri M, Hines J, *et al.* (2016) (13) C mannitol as a novel biomarker for measurement of intestinal permeability. *Neurogastroenterol Motil* **28**, 1114–1119.
152. Seikrit C, Schimpf JJ, Wied S, *et al.* (2023) Intestinal permeability in patients with IgA nephropathy and other glomerular diseases: an observational study. *J Nephrol* **36**, 463–474.
153. Wilms E, Jonkers D, Savelkoul HFJ, *et al.* (2019) The impact of pectin supplementation on intestinal barrier function in healthy young adults and healthy elderly. *Nutrients* **11**, 1554.
154. Seethaler B, Basrai M, Neyrinck AM, *et al.* (2021) Biomarkers for assessment of intestinal permeability in clinical practice. *Am J Physiol Gastrointest Liver Physiol* **321**, G11–g17.
155. Houghton MJ, Snipe RMJ, Williamson G, *et al.* (2023) Plasma measurements of the dual sugar test reveal carbohydrate immediately alleviates intestinal permeability caused by exertional heat stress. *J Physiol* **601**, 4573–4589.
156. McCallum G & Tropini C (2023) The gut microbiota and its biogeography. *Nat Rev Microbiol* **22**, 105–118.
157. van Wijck K, van Eijk HMH, Buurman WA, *et al.* (2011) Novel analytical approach to a multi-sugar whole gut permeability assay. *J Chromat B* **879**, 2794–2801.
158. Tatuca-Babet OA, Forsyth A, Udy A, *et al.* (2022) Use of a sensitive multisugar test for measuring segmental intestinal permeability in critically ill, mechanically ventilated adults: a pilot study. *J Parenter Enteral Nutr* **46**, 454–461.
159. Cao S, Shaw EL, Quarles WR, *et al.* (2022) Daily inclusion of resistant starch-containing potatoes in a dietary guidelines for Americans dietary pattern does not adversely affect cardiometabolic risk or intestinal permeability in adults with metabolic syndrome: a randomized controlled trial. *Nutrients* **14**, 1545.
160. Rao AS, Camilleri M, Eckert DJ, *et al.* (2011) Urine sugars for in vivo gut permeability: validation and comparisons in irritable bowel syndrome-diarrhea and controls. *Am J Physiol Gastrointest Liver Physiol* **301**, G919–928.
161. Camilleri M, Nadeau A, Lamsam J, *et al.* (2010) Understanding measurements of intestinal permeability in healthy humans with urine lactulose and mannitol excretion. *Neurogastroenterol Motil* **22**, e15–26.
162. Fischer M & Fadda HM (2016) The effect of sex and age on small intestinal transit times in humans. *J Pharm Sci* **105**, 682–686.
163. Vanheel H, Vicario M, Vanuytsel T, *et al.* (2014) Impaired duodenal mucosal integrity and low-grade inflammation in functional dyspepsia. *Gut* **63**, 262–271.
164. Clarke LL (2009) A guide to Ussing chamber studies of mouse intestine. *Am J Physiol Gastrointest Liver Physiol* **296**, G1151–1166.
165. Farré R, Fiorani M, Abdu Rahiman S, *et al.* (2020) Intestinal permeability, inflammation and the role of nutrients. *Nutrients* **12**, 1185.
166. Pearce K, Estanislao D, Fareed S, *et al.* (2020) Metabolic endotoxemia, feeding studies and the use of the limulus amoebocyte (LAL) assay; is it fit for purpose? *Diagn* **10**, 428.
167. Schumann RR, Leong SR, Flaggs GW, *et al.* (1990) Structure and function of lipopolysaccharide binding protein. *Sci* **249**, 1429–1431.
168. Ajamian M, Steer D, Rosella G, *et al.* (2019) Serum zonulin as a marker of intestinal mucosal barrier function: may not be what it seems. *PLoS One* **14**, e0210728.
169. Scheffler L, Crane A, Heyne H, *et al.* (2018) Widely used commercial ELISA does not detect precursor of haptoglobin2, but recognizes properdin as a potential second member of the zonulin family. *Front Endocrinol* **9**, 22.
170. Perez-Diaz-Del-Campo N, Castelnuovo G, Ribaldone DG, *et al.* (2023) Fecal and circulating biomarkers for the non-invasive assessment of intestinal permeability. *Diagn* **13**, 1976.