Colonic and Anorectal Function in Defaecation and Continence

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Summary

"It is a sobering fact, for example, that in understanding the pathophysiology of the neural mechanisms of continence and defaecation…we have achieved only modest advances since the physiology was proposed by Gowers in 1877, and Denny-Brown and Robertson in 1935, and certainly very little more than was known over 40 years ago."

Associate Professor David Lubowski

'Colorectal Surgery: rigour and logic when treating pelvic floor disorders'

ANZ J Surg, 2012, 82(6):383-384.

In the 21st century, our understanding of human physiology is expanding at an exponential rate. Yet, many fundamental uncertainties remain regarding the physiology of the human colon, particularly in regards to motility and transit. This is a significant hindrance in how we implement and interpret diagnostic investigations and enact treatment modalities for common conditions in which disordered colonic motility may be implicated, such as faecal incontinence and constipation.

This aims of this thesis are to describe the functional colonic physiology and pathophysiology pertaining to continence and defaecation using a combination of clinical studies as well as laboratory-based in vivo and ex vivo human experiments. The two introductory chapters (**Chapters 1 & 2)** provide a review of the literature on colorectal neuromuscular physiology and describe the functional physiology of defaecation and continence. **Chapter 3** outlines the specific aims of each of the subsequent results chapters.

Clinical studies were initially performed to highlight the limitations in our current understanding and diagnostic evaluation of faecal incontinence (**Chapters 4 & 5**) and constipation (**Chapter 6**). The first two results chapters (**Chapters 4 & 5)** describe the discordance between symptom severity in faecal incontinence and tests of anorectal structure and sensorimotor function. These findings highlight the limitations in our diagnostic investigations and suggest that the severity of symptoms are not solely attributable to anorectal dysfunction.

The third results chapter (**Chapter 6**) involves an analysis of colonic manometry studies collected from children presenting to five international quaternary paediatric hospitals for the investigation of severe constipation. The majority of these children generated a colonic motor response ("high-amplitude propagating contractions" or HAPCs) and defaecated following pharmacological provocation with intraluminal bisacodyl. Despite this, these children still experience refractory symptoms. This indicates that defaecation requires more than just the ability to generate colonic motor patterns and that our

current approach to investigation and analysis cannot identify the causation of symptoms in the majority of these children.

In **Chapter 7**, I describe the first application of high-resolution impedance manometry in the human colon in vivo, providing a description of the functional role of colonic motility in gas transit. Both a meal and intraluminal gas insufflation resulted in a significant increase in gas in the distal colon, as well an increase in the prevalence of the "cyclic motor pattern". Despite provocation with gas insufflation into the distal colon, most participants reported no conscious urge to pass flatus. This suggests that colonic motility, and specifically the cyclic motor pattern, is related to the regulation of continence and evacuation. This additionally demonstrates that impedance manometry is a viable investigative tool for further studies of colonic function.

Finally, to investigate the physiology underlying the generation and modulation of colonic motor patterns, ex vivo human colonic experimental preparations were performed in **Chapters 8 & 9**. These findings demonstrate that propagating contractions in the human colon are likely to be primarily generated by myogenic mechanisms with additional neural modulation. The neuromuscular responses of colonic circular muscle to opioid agonists are also described in **Chapter 9**, with opioid use a widely recognised cause of constipation and altered colonic motility.

The collective interpretations of these findings and the implications for further research are discussed in **Chapter 10**.

Declaration

I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any university; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text.

Signed: 

Date: 27/1/2021

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Publications

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2. **Heitmann PT,** Mohd Rosli R, Maslen L, Wiklendt L, Kumar R, Omari TI, Wattchow DA, Costa M, Brookes SJH, Dinning PG. High-resolution impedance manometry characterizes the functional role of distal colonic motility in gas transit. *Neurogastro Motil* 2021; e14178.
3. Wattchow DA, **Heitmann PT**, Smolilo D, Spencer NJ, Parker D, Hibberd T, Brookes SJH, Dinning PG, Costa M. Postoperative ileus – an ongoing conundrum. *Neurogastro Motil* 2020; e14046.
4. *Mohd Rosli R, ***Heitmann PT,** Raghu K, Hibberd TJ, Costa M, Wiklendt L, Wattchow DA, Arkwright J, de Fontgalland D, Brookes SJH, Spencer NJ, Dinning PG. Distinct patterns of myogenic motor activity identified in isolated human distal colon with high-resolution manometry. *Neurogastro Motil* 2020;32(10).
   *R Mohd Rosli & PT Heitmann are equal first authors
5. **Heitmann PT,** Wiklendt L, Thapar N, Borrelli O, Di Lorenzo C et al. Characterization of the colonic response to bisacodyl in children with treatment-refractory constipation. *Neurogastro Motil* 2020;32(8).
6. **Heitmann PT**, Rabbitt P, Scloithe AC, Wattchow DA, Scott SM, Dinning PG. The relationships between the results of contemporary tests of anorectal structure and sensorimotor function and the severity of fecal incontinence. *Neurogastro Motil* 2020;32(11).
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Presentations

1. ‘The functional role of colonic motility in gas transit characterised by high-resolution impedance manometry’, Oral presentation, RP Jepson and Justin Miller Prize Presentations, Adelaide, November, 2020.
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11. ‘Can the results of anorectal investigations predict symptom severity in faecal incontinence?’, Poster presentation, Digestive Disease Week, San Diego, USA, May 2019.

Chapter 1: An Overview of Colonic Anatomy, Physiology, and Techniques to Record Colonic Motility

1.1 Outline

This chapter provides a review of the literature which forms the basis for the experiments and results chapters contained in this thesis. This includes an overview of the anatomy and physiology of the human colon, human colonic motility and transit, techniques used to record colonic motility and transit, the pathophysiology of colonic motility in functional bowel disorders, and the pharmacological modulation of colonic motility. Specific knowledge gaps in the literature have been summarised at the end of each section. While it is beyond the scope of this thesis to address all of these knowledge gaps, these were included as a means to highlight the limitations in our current scientific knowledge and pose research questions to be addressed both within this thesis as well as in future research in this field.

1.2 An Overview of the Anatomy and Physiology of the Human Colon

1.2.1 Anatomy

The human colon is a viscoelastic tubular organ located within the abdomen and pelvis(1). The colon is a segment of the gastrointestinal tract, beginning proximally at the ileocaecal junction and ending distally at the rectosigmoid junction. The colon is approximately 130cm in length in adulthood(2, 3), comprising several anatomical segments including the caecum, vermiform appendix, ascending colon, transverse colon, descending colon, and sigmoid colon. The luminal diameter narrows as the colon progresses distally; from approximately 60-80mm in the caecum to 25mm in the sigmoid colon(4). The colonic wall, akin to the elsewhere in the gastrointestinal tract, is comprised of four layers; serosa, smooth muscle (oriented in longitudinal and circular layers), submucosa, and mucosa.

The colon is enveloped in visceral peritoneum circumferentially and is affixed to the posterior abdominal wall via the mesocolon. The mesocolon is continuous with the mesentery of the small intestine from the duodenojejunal flexure to the mesorectum(5, 6) and is traversed by the neurovascular and lymphatic supply to the colon. The mesocolon of the ascending and descending colon is apposed to the retroperitoneum via Toldt's fascia(5, 6). In contrast, the transverse mesocolon and sigmoid mesocolon are approximately seven centimetres in length(3), allowing for greater mobility of their respective colonic segments.

The colon can be readily identified by several unique macroscopic features. These include;

- Taenia coli: three thick bands of longitudinal muscle visible from caecum to sigmoid colon, individually named the taenia libera, taenia mesocolica, and taenia omentalis. The taenia coli converge at the appendiceal base and rectosigmoid junction to form the continuous longitudinal muscle layers of the appendix and rectum respectively.

- Haustra: sacculations of the colonic wall which are separated by semilunar folds.
- Appendices epiploicae: globules of subserosal fat attached to the serosal surface of the colon.

Taenia coli are present in many species of herbivorous and omnivorous mammals, but not in carnivores(7). In primates, three taenia coli are most commonly observed, however one to four taenia coli are observed in different species(8). Some mammals, including the kangaroo and colobus monkeys, have taenia and haustrations in both the colon as well as the stomach(8). Other herbivorous mammals, including ruminants such as cows, have no taenia coli but instead have a capacious stomach which facilitates foregut fermentation(9), rather than hindgut fermentation which occurs in mammals with taenia coli.

The taenia coli are formed by interwoven layers of circular and longitudinal muscle(10). It is uncertain whether the haustra are formed passively by static contraction of the taenia coli and are fixed in position, or by dynamic circular muscle contraction forming mobile semilunar folds(11-13). The hypothesised function of the haustra is to retain digesta and increase transit time in order to facilitate fermentation and digestion of fibrous plant-based matter(7, 14). In a porcine animal model using concurrent video-fluoroscopy and implanted extraluminal strain gauge transducers, haustra appeared to permit rapid gas transit whilst prolonging transit of solid content(15).

The colon is derived embryologically from the midgut and hindgut regions of the primitive intestinal tube, receiving arterial supply from the superior and inferior mesenteric arteries respectively. The colon receives intrinsic neural innervation from the enteric nervous system, as well as extrinsic efferent innervation from the lumbar nerves (sympathetic) and the vagus and pelvic splanchnic nerves (parasympathetic; vagus nerve to proximal colon and pelvic splanchnic nerves to distal colon). The sympathetic neurons are organised in prevertebral ganglia; the coeliac, superior mesenteric, and inferior mesenteric ganglia(16). The parasympathetic neurons synapse in either the pelvic (hypogastric) plexus, or in the intramural myenteric plexus(17).

Afferent neural transmission from the colon occurs via viscerofugal/intestinofugal afferent neurons (IFANs)(18). Colonic distension may be detected by intraganglionic laminar endings, which have been described in the human rectum(19). IFANs have cell bodies in the colonic wall and relay sensory information to extrinsic centres including the prevertebral sympathetic ganglia(20), parasympathetic ganglia(21), as well as spinal cord and brainstem(22).

1.2.2 Physiology

The colon receives approximately 1500mL of liquid enteric content per day via the ileocaecal junction, of which less than one third of this volume remains in stool(23, 24). From the time of ingestion, transit through the oesophagus, stomach, and small intestine (≥7m in total length) occurs within several hours. Up to 12-30 hours, or 90% of total transit time(25), is spent traversing the 130cm length of the colon(26).

The functions of the colon include(27);

1. Mixing of contents.
2. Bacterial fermentation of carbohydrates.
3. Transmural exchange of fluid, electrolytes, and short-chain fatty acids.
4. Formation of solid stool.
5. Storage of contents prior to defaecation.
6. Evacuation of solid, liquid, and gaseous contents.

Coordinated motility patterns are integral to achieve these functions(11, 27), requiring the integrated actions of myogenic and neurohormonal mechanisms(11, 28). Much of the action, interactions, and integration of these systems has not been well established, particularly in humans(29).

1.2.3 Knowledge Gaps

- The action, interactions, and integration of neural, myogenic, and hormonal mechanisms in the generation and regulation of colonic motor function (addressed in **Chapter 8**).
- The formation and position of the colonic haustra; are haustra formed passively by static contraction of the taenia coli and fixed in position, or by dynamic circular muscle contraction forming mobile semilunar folds?
- The function of the taenia coli and haustra, particularly in regards to regulating the transit of solid, liquid, and gaseous intraluminal contents.

1.3 Human Colonic Motility: Myogenic, Neural, and Hormonal Control Mechanisms

1.3.1 Myogenic Control of Colonic Motility

Colonic smooth muscle exhibits spontaneous phasic activity initiated by the interstitial cells of Cajal (ICC)(30). Similar to cardiac myocytes, ICC are mesodermal rather than neural in origin(31, 32). The pacemaker action of the ICCs is driven by; (1) voltage-gated calcium channels which cause

depolarisation, and; (2) calcium-gated potassium channels which cause hyperpolarisation. The ICC are electrically coupled to adjacent myocytes via gap junctions(31, 33), allowing for the propagation of an oscillating membrane potential at a subthreshold level(34). This is the basis of "unitary" smooth muscle, whereby the muscle functions as a single functional unit or syncytium(35, 36). Local depolarisation elicits a junctional potential(34), causing muscle contraction in the absence of an action potential(34). The membrane potential of colonic smooth muscle can be modulated by mechanical stimuli, chemical stimuli, and/or neural activity(28). This can drive the membrane potential to a supra-threshold level, evoking an action potential.

There are several populations of ICC in the colonic wall which are responsible for differing frequencies of phasic contractility. These include those located in the submucosal plexus (ICC_{SM}), myenteric plexus (ICC_{MY}), and between the circular and longitudinal muscle (ICC_{IM})(23). Colonic "slow waves", at a frequency of 2-4 cycles/minute (cpm)(37), are driven predominantly by the ICC in the submucosal plexus (ICC_{SM})(38). Functionally, slow wave activity has been hypothesised to contribute to mixing of contents and slowing fluid transit(11). In human ex vivo preparations, slow waves persist in the presence of tetrodotoxin – a voltage-gated sodium channel inhibitor – further supporting the hypothesis of a myogenic origin(39).

When the submucosal plexus is excised from the specimen in in vitro studies, the circular muscle demonstrates tetrodotoxin-resistant, large-amplitude phasic contractions, at a frequency of 0.3-0.6cpm(38, 39). This activity has been labelled "slow phasic contractions" and attributed to the ICC_{MY}(39). Higher frequency, small-amplitude 'myenteric potential oscillations', at 8-30cpm, may also be generated by the ICC_{MY}(37, 40).

1.3.2 The Enteric Nervous System

1.3.2.1 Organisation and Classification of Enteric Neurons

The enteric nervous system is intrinsic to the gastrointestinal system, continuous from oesophagus to anus, and is estimated to contain 400-600 million neurons(21). The role of the enteric nervous system in colonic motor function may be best highlighted by diseases in which the system is absent or obliterated. Enteric neuropathies are characterised by dysmotility leading to severe constipation and gross colonic dilatation or "megacolon". Two notable examples are Hirschsprung's disease, caused by a congenital absence of myenteric and submucosal ganglia(41), and Chagas disease, in which *Tripanosoma cruzi* infection causes obliteration of enteric ganglia(42).

Enteric neurons are organised into two interconnected plexuses; (1) the myenteric (Auerbach's) plexus, located between the circular and longitudinal muscle layers of the gut wall, and, (2) the submucosal

(Meissner's) plexus, located in the submucosa. Enteric neurons can be broadly classified as intrinsic primary afferent neurons, excitatory/inhibitory efferent neurons, or ascending/descending interneurons(31). Collectively, these neurons form a sensorimotor loop, capable of functioning autonomously; first demonstrated by pioneering work on denervated canine colon by Bayliss and Starling(43). Enteric nervous system activity is additionally modulated by extrinsic sympathetic and parasympathetic innervation.

Much of our current understanding of the organisation of enteric neurons in the human colon has been derived using retrograde tracing and immunohistochemistry techniques(44-46). Using these techniques, enteric neurons can be identified via their neurochemical composition and by their direction and length of projection(46, 47).

Intrinsic primary afferent neurons (IPANs) comprise approximately 20% of all enteric neurons(48). IPANs are of Dogiel type II morphology (multiaxonal with a round/ovoid profile) and respond to chemical and mechanical stimuli to elicit local, intramural responses. Interneurons are the longest enteric neurons, up to 68mm in length(45), with the majority projecting in a proximal direction(49, 50). Interneurons mediate signalling from IPANs to motor efferent neurons to cause descending smooth muscle inhibition and ascending smooth muscle excitation.

Motor efferent neurons include both inhibitory and excitatory neurons, which innervate the colonic smooth muscle (musculomotor) and intestinal glands (secretomotor)(51). Excitatory neurons are more numerous, are of shorter length, and mostly project proximally, in contrast to inhibitory motor neurons, which mostly project distally(44, 45). These differences in polarity may serve a functional purpose in the peristaltic reflex (see **1.4.1 Transit of Liquid and Solid Luminal Contents**), in which excitatory neural pathways elicit muscular contraction proximal to the bolus, whilst descending inhibitory neural pathways elicit muscular relaxation distal to the bolus to enable antegrade transit of luminal content(43, 52). However, these differences in polarity do not readily describe the neural pathways which govern retrograde propagating contractions and retrograde transit.

1.3.2.2 Neurotransmitters

Acetylcholine is the predominant excitatory neurotransmitter in the human colon. Acetylcholine is likely to act by modulating calcium release from intracellular stores or calcium uptake into smooth muscle cells to elicit membrane depolarisation and excitatory junction potentials. Excitation can also occur via neural transmission which is resistant to hexamethonium (a nicotinic receptor antagonist), which may be mediated via tachykinin neuropeptides(53, 54).

Neurally-mediated inhibition in the colon occurs via hyperpolarisation of smooth muscle cells and inhibitory junction potentials, which involve the action of multiple non-adrenergic, non-cholinergic neurons(17, 55). The main neurotransmitters in inhibitory enteric neurons include nitric oxide, vasoactive intestinal peptide, adenosine triphosphate, and β-nicotinamide adenine dinucleotide(31, 56).

1.3.3 Hormonal Regulation of Colonic Motor Function

The gastroenteropancreatic endocrine system contains over 30 different cell types and utilises over 100 different messenger molecules(21), many of which may be involved in the regulation of colonic motility. It is difficult to separate the hormonal mechanisms from neural mechanisms in the regulation of motility due to their integration, hence the terminology of "neurohormonal" mechanisms. Endocrine and paracrine signalling is integrated with the enteric nervous system via enteroendocrine cells, formerly known as "enterochromaffin cells" due to their 5-HT content and reaction to chromaffin(57, 58).

Much of the literature regarding the neurohormonal regulation of colonic motility relates to the colonic response to a meal (see **2.6.6 Colonic Motor Response to a Meal**). The meal response (also described as the gastrocolic reflex or gastrocolonic reflex) describes the reflexive increase in colonic motility occurring at meal times. This has been proposed to be initiated in part by gastric distension and neuropeptide release, possibly including cholecystokinin, 5-HT, neurotensin, and gastrin(59). While still commonly used in current journals and textbooks, the terminology "gastrocolic reflex" is misleading as the colonic response to a meal can occur in the absence of gastric stimulation, evidenced by its preservation post-gastrectomy(60), and presence following the smell of food or verbal discussion of a meal(61). The colonic meal response is likely to be mediated by the central nervous system as it is absent in patients with spinal cord injury(62), most notably in the distal colon. Patients with spinal cord injury may still demonstrate a meal response in the proximal colon due to the action of the vagus nerves(63). Further evidence of a centrally-mediated neural origin for the meal response is the rapid nature of the response, occurring prior to or within seconds of starting to eat.

For many years, mucosal 5-HT production was thought to be pivotal to the generation of the peristaltic reflex (**1.4.1 Transit of Liquid and Solid Luminal Contents**). Whilst 5-HT is released from enteroendocrine cells in response to mechanical or chemical stimuli, the role of 5-HT in the generation of motility patterns has been refuted more recently(64). 5-HT may have a role in modulating motility, but does not appear to be essential to the generation of colonic motor patterns(65).

In addition to 5-HT, cholinergic stimulation of enteroendocrine cells can elicit the release of numerous other amines and peptides, including melatonin. While melatonin is commonly associated with being

the principal secretion of the pineal gland, there is estimated to be a 400 times greater concentration of melatonin in the gastrointestinal system(66). Exogenous administration of melatonin can modulate transit time, with lower doses associated with more rapid transit, and higher doses associated with slower transit(67-69). The mechanisms to account for these findings have not been established.

While the predominant paracrine action on colonic motility is mediated by the enteroendocrine cells, there are also important hormonal contributions to colonic motor function which occur more proximally in the gastrointestinal tract; principally pancreatic enzyme secretion and bile acid secretion and absorption. Luminal bile acids have been shown to increase colonic propagating contractions(70). This effect may be mediated by direct stimulation of myenteric neurons via the TGR5 receptor(71). Per rectal infusion of exogenous chenodeoxycholic acid provokes propagating contractions in the proximal colon, suggesting activation of long, recto-colonic reflex pathways(70). Rectal infusion of chenodeoxycholic acid also lowers the sensory threshold to rectal balloon distension(70) and increases stool urgency(72, 73), suggesting activation or sensitisation of rectal afferent neurons.

1.3.4 Knowledge Gaps

- The myogenic and neurohormonal mechanisms involved in the generation and regulation of colonic motor patterns (addressed in **Chapter 8**).
- The specific neurohormonal mechanisms which initiate the colonic meal response to account for how this response can occur rapidly following commencement of the meal and/or in the absence of gastric stimulation.
- A description and mapping of long recto-colonic and colo-colonic reflex pathways to explain motor responses in the proximal colon which occur in response to mechanical/pharmacological rectal stimulation.
- Comprehensive "mapping" of the enteric nervous system, including the distribution, neurotransmitter composition, and pathways of enteric neurons.

1.4 Colonic Motility and Transit of Luminal Contents

1.4.1 Transit of Liquid and Solid Luminal Contents

In 1899, Bayliss and Starling described the "Law of the Intestine", which detailed the peristaltic reflex in both the canine small intestine and colon to propel a bolus aborally(43, 52). In 1917, Trendelberg demonstrated similar propulsive, peristaltic activity using liquid distension of guinea pig ileum(74). More recently, the peristaltic reflex has been described as a coordinated sensorimotor response to sequential activation of neural circuits(11, 75). Mechanical distension of the gut wall activates

mechanoreceptors and, in turn, intrinsic primary afferent neurons. In response, activation of ascending excitatory neural pathways elicits muscular contraction proximal to the bolus, whilst descending inhibitory neural pathways elicit muscular relaxation distal to the bolus. Collectively, this creates a pressure gradient which facilitates antegrade bolus transit.

Whilst the peristaltic reflex accurately describes propagating activity in the presence of a bolus, distension alone may not generate contractile activity in the basal phase (see **2.5.1 Basal Phase**) or in response to small volumes of intraluminal content. The generation of colonic motor patterns in these instances may be more accurately described by the 'neuromechanical loop' hypothesis(75, 76), which is not a reflex but rather a graded response which adapts to the volume and consistency of intraluminal content. The character of intraluminal contents (solid, liquid, gas) is identified by mechanoreceptors and chemoreceptors in order to modulate the speed and amplitude of the response. It is not clear how the polarity of the response is determined to direct antegrade or retrograde transit.

In the human colon, there are several motor patterns which have been related to the transit of content (see **1.6 Colonic Motor Patterns**). The most studied motor pattern, predominantly related to antegrade transit of luminal contents, are colonic mass movements(77, 78) or high-amplitude propagating contractions(2, 23, 79, 80). Despite being the predominant focus of colonic motility studies to date, high-amplitude propagating contractions only represent <2% of all propagating colonic motor activity(2).

1.4.2 Colonic Gas Transit

The mechanisms and motility patterns which enable colonic gas transit have not been described. Under normal physiological conditions, the colon contains approximately 100-200mL of gas(81), which is predominantly composed of N_2, CO_2, H_2, and CH_4(81-83). Colonic gas is a combination of swallowed gas as well as gas produced by fermentation of carbohydrates by colonic microbiota(84). The majority of intraluminal gas is absorbed into the bloodstream or consumed by colonic microbiota, with the remainder evacuated as flatus(85). The average volume of flatus output from an adult human colon over a 24-hour period is approximately 700mL(86). Gas transit in the colon is highly efficient, with exogenous gas infusion into the proximal jejunum(87), ileum, or caecum(88) resulting in anal gas expulsion at comparable rate (1-30mL/min), equilibrating within less than 30 minutes of the infusion commencement(88).

Studies have modelled the luminal flow of content through the colon and anal canal using estimates of laminar flow (Hagen-Poiseuille equation)(89) and turbulent flow dynamics (Darcy-Weisbach equation)(90). The transit of gas is substantially different to that of liquid or solid colonic content. The

viscosity of water is 1.002cP, compared with air viscosity of 0.018cP. Given that the intraluminal length, diameter, and pressure will be identical for liquid or gaseous content, Hagen-Poiseuille's equation would dictate that the flow of gas will be 55 times more rapid than the velocity of fluid transit. Differences in transit time of solid, liquid, and gaseous luminal contents have been demonstrated in pigs, with more rapid gas transit preceding the slower transit of solid and liquid content(15). However, the assumptions in Hagen-Poiseuille's equation include that the luminal diameter is constant and circular and that the flow is laminar rather than turbulent – none of which are true in the human colon. The inherent limitations of these applications are the complexity of variables, including the viscoelastic nature of the colonic wall, mixed luminal content viscosity, and transient wall deformation(89).

1.4.3 Knowledge Gaps

- The functional relationships between specific colonic motor patterns and gas transit (addressed in **Chapter 7**).
- The functional relationships between specific colonic motor patterns and transit of solid and liquid luminal contents.
- The mechanisms which determine the polarity (antegrade/retrograde) of the motor response in the neuromechanical loop hypothesis.

1.5 Colonic Motility: Recording Techniques

1.5.1 Overview

Many techniques have been used to record colonic function, most of which assess either motility or transit(91). These include; sonography(92), magnetic resonance imaging(92), myoelectrical recordings(93), radio-opaque marker studies(94), radionuclide scintigraphy(95), ingestible tracking capsules(96-98), and colonic manometry(27, 99).

The assessment and understanding of human colonic motor function has been hindered by several factors. These include;

1. Most studies on colonic motor patterns have been performed using excised animal colon. There are substantial inter-species differences in the mammalian colon in both structure(100) and electrical activity(40, 101-103). This complicates comparisons between animal studies and humans. The human colon appears to share more similarities with the canine, porcine, or feline colon than conventional and more commonly used laboratory animals such as mice or guinea pigs(38).
2. The luminal diameter of the human colon is much wider than the colon of laboratory animals. Some authors have suggested that motility patterns that do not occlude the lumen may not be recorded

using conventional manometry catheters(104), particularly in the proximal colon where the luminal diameter is widest. However, a validation study (using both a software-based in silico model and in vitro models) demonstrated that this was not true(105). In that study, colonic manometry was capable of recording non-lumen occluding contractions, with the recorded data being most dependent on the viscosity of luminal content and the rate of colonic wall contractions.

3. Colonic motility patterns are not under voluntary control and are infrequent, particularly when compared with the frequency of muscle activity in other viscera (eg. myocardium). Transit time in the colon is also considerably slower than elsewhere in the gastrointestinal tract(23). Prolonged studies, typically 2-8 hours or longer, are therefore required to capture colonic motor patterns. This is not always feasible and, for some imaging techniques such as computed tomography or fluoroscopy, the radiation exposure from extended imaging is not acceptable for human studies.
4. Whilst the gastrointestinal tract is accessible via per oral/per anal intubation, these approaches are invasive, can be uncomfortable, and confer a small risk of viscus perforation.
5. Some form of bowel preparation (per rectal enema and/or full per oral bowel preparation) are required prior to colonoscopic insertion of a manometry catheter, which presumably alters the normal physiology and motility of the colon(106).
6. Analysis and interpretation of colonic manometry data is not standardised. Previously, analyses have been limited to either a motility index, area under the curve analyses, or descriptive, observational methods used to identify each individual pressure event(107). These approaches can be time consuming, introduce observer bias, largely ignore low-amplitude activity, and are difficult to quantify for comparisons pre- and post-intervention or between patients and healthy controls(108).

1.5.2 Colonic Manometry

Colonic manometry involves the recording of intraluminal pressure within the colon. Numerous techniques have been described, including balloon kymography, water-perfused or solid-state catheters, and low- and high-resolution catheters. Manometry catheters can be inserted using an antegrade approach via nasocolonic intubation or retrograde approach using per rectal or per stomal intubation(109). Techniques to guide catheter placement include colonoscopy, fluoroscopy, or cineradiology imaging(109).

The recording of intraluminal pressure is used as a surrogate measure of colonic smooth muscle activity. Initial prolonged studies of 24-hour duration used low-resolution catheters with individual pressure sensors spaced at ≥70mm(80, 100, 110-113). During those studies, basal motility patterns were recorded as well as motility patterns during sleep, awakening, and in response to meals and medications. However, the interpretation of low-resolution data can be problematic. Wide sensor spacing can

overlook contractions which propagate over short distances (<100mm) and can incorrectly label the direction of antegrade/retrograde propagation(114) (**Figure 1.1**). A study using high-resolution manometry (individual pressure sensors spaced at 10-25mm) also demonstrated that motor patterns which would have previously been described as "non-propagating" contractions in low-resolution studies(111, 112, 115, 116) usually consist of rhythmic contractions which propagate across a short segment of the colon(114).

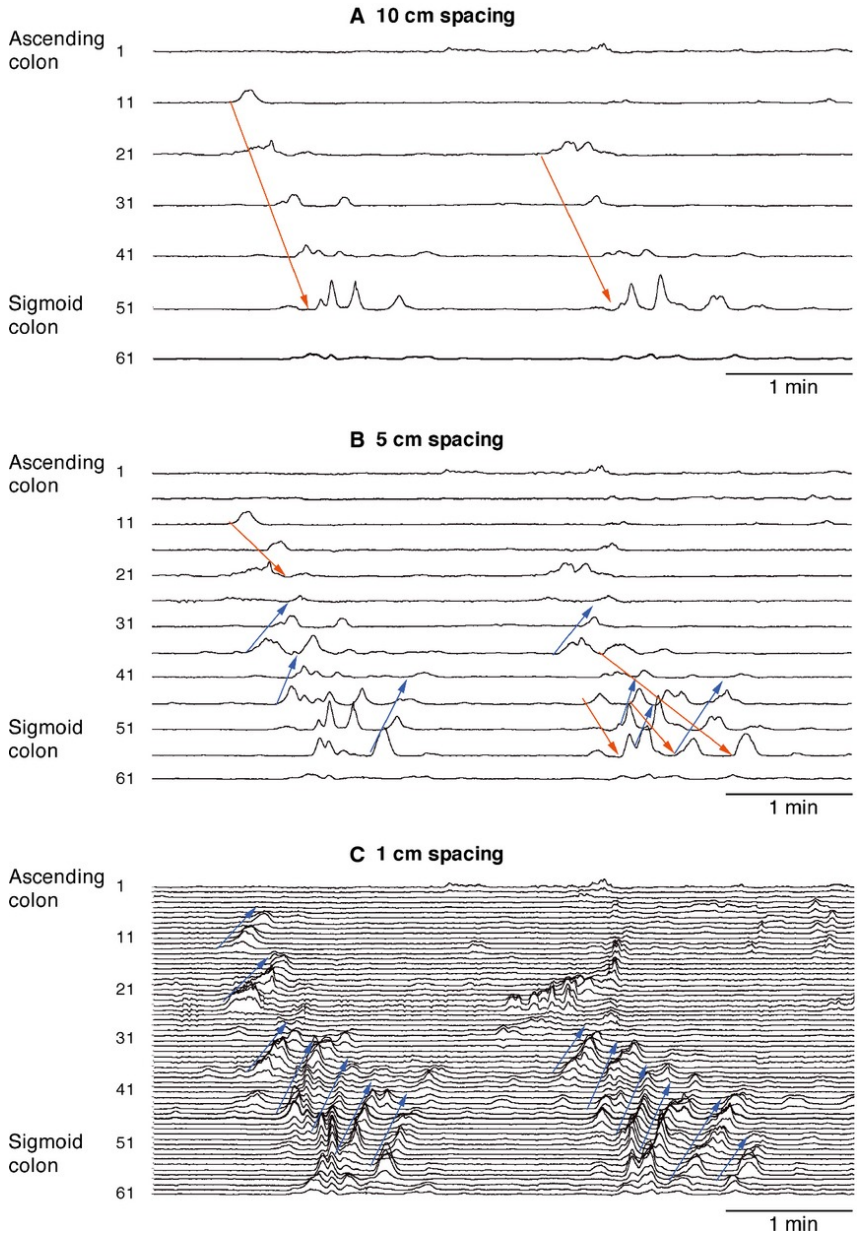

Figure 1.1 High-resolution colonic manometry data displayed with channels removed to replicate different sensor spacing; (A) 10cm, (B) 5cm, (C) 1cm. Propagating contractions which would be interpreted as antegrade when using 10cm spacing (A, red arrows) appear to be retrograde when re-assessed with 1cm spacing (C, blue arrows). Image source: Dinning PG, Wiklendt L, Gibbins I et al. Low-resolution colonic manometry leads to a gross mis-interpretation of the frequency and polarity of propagating sequences: initial results from fibreoptic high-resolution manometry studies. *Neurogastroenterol Motil.* 2013;25(10):e640-9.

As a result, high-resolution manometry has superseded low-resolution technology, which has increased the diagnostic yield in oesophageal motility disorders(117-119) and is also utilised in anorectal studies(120, 121). However, in colonic studies, several factors have hindered the uptake of high-resolution manometry. To date, the only clinical application of colonic manometry is the assessment of colonic neuromuscular function in treatment-refractory constipation(79, 122-124) and is only performed in specialist tertiary/quaternary centres (**Chapter 6**). Elsewhere, colonic manometry has been primarily utilised as a research tool (**Chapters 7 & 8**). There is no standardisation in procedure, analysis, and/or nomenclature(11).

Interpretation of colonic manometry data requires a considered understanding of the dynamic relationships between intraluminal pressure and colonic wall movement. A simplistic assumption would be that increasing pressure infers isotonic concentric muscle contraction and a reduction in luminal diameter. However, whilst pressure and luminal diameter are related, they remain independent metrics and there are multiple different contractile and pressure states that can occur. Additionally, there are passive states resulting in luminal and pressure changes which can occur independent of muscle action(125).

The relationships between changes in pressure, recorded by a manometry catheter, and changes in luminal diameter, recorded by concurrent video imaging, have been described using excised rabbit colon(76, 125). Diameter maps can be made from video imaging which detail all changes in diameter on a grey or colour scale. The manometry data is used to make a spatiotemporal map, similar to those commonly used in oesophageal and anorectal manometry recordings. These two maps can then be combined to create composite diameter/pressure maps(76). From these composite maps, all changes in pressure can be related to changes in diameter, allowing for the description of multiple distinct "mechanical states". These mechanical states are detailed below;

1. Isometric contraction/relaxation; in which muscle action against incompressible content (either the catheter or a lumen-occluding contraction) results in pressure changes without changes in luminal diameter (**Figure 1.2A**).
2. Auxotonic contraction/relaxation; in which muscle action against deformable content results in paradoxical changes in pressure and diameter (**Figure 1.2B**).
3. Isotonic contraction/relaxation; in which muscle action causes a change in diameter without a change in pressure (**Figure 1.2C**).
4. Passive changes; in which the muscle is inactive or quiescent and changes in either luminal diameter or pressure occur as a result of changes in the volume of intraluminal content (**Figures 1.2D-G**).

Volume changes in the absence of phasic activity have also been demonstrated by concurrent intraluminal manometry and electromechanical barostat recordings in the human colon(126). One

study, using concurrent colonoscopy and low-resolution manometry, demonstrated that luminal dilation can be associated with an increase in pressure(127). There may additionally be underlying motility patterns that do not alter intraluminal pressure sufficiently to be recorded by manometry, or tonic changes which facilitate propulsion of content(128). However, this is yet to be determined.

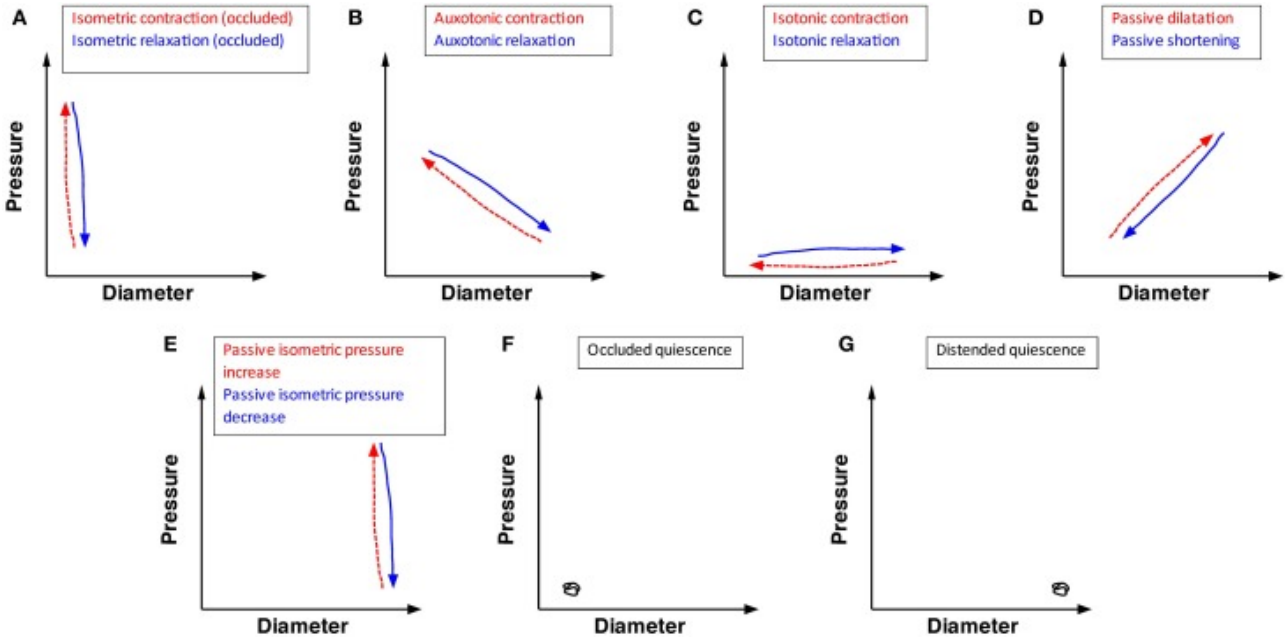

Figure 1.2 The mechanical states of the intestinal wall, described using relationships between pressure (y-axis) and diameter (x-axis). Image source: Costa M, Wiklendt L, Arkwright JW et al. An experimental method to identify neurogenic and myogenic active mechanical states of intestinal motility. *Front Syst Neurosci.* 2013;7(7):7. Licensed under Creative Commons Attribution.

1.5.3 Techniques Used to Record Intraluminal Pressure and Transit

1.5.3.1 Animal Studies

To detail the functional role of motility patterns, both colonic contractile activity and transit must be recorded concurrently. This has been achieved in animal studies in vivo using extraluminal strain gauge transducers and videofluoroscopy. In sheep, Bedrich & Ehrlein(129) demonstrated that propagating colonic contractions were associated with the transit of intraluminal content. The majority of antegrade propagating contractions in the proximal colon of sheep caused luminal narrowing without complete luminal occlusion. This was associated with; (a) antegrade transit of luminal contents ahead of the propagating contraction; (b) a central jet-like "backflow" through the narrowed lumen, and; (c) "backflow" at the cessation of the pressure wave. In combination, these movements were hypothesised to be important for the mixing and fermentation of digesta with the net result being gradual antegrade

transit over short distances. In the distal colon of the sheep, the characteristics of pressure and transit changed significantly. In this region, sustained segmenting contractions separated the luminal content into discrete pellets during gradual, antegrade transit.

Using a similar study performed in pigs, Hipper & Ehrlein(15) demonstrated coordinated ileocolonic propagating contractions which were associated with transit. Antegrade propagating contractions in the ileum and proximal colon were spatially and temporally associated with the antegrade transit of luminal contents. The velocity of transit was determined by the consistency of the content, with rapid gas transit preceding the slower transit of solid and liquid content. No retrograde transit was observed in pigs.

For these animal studies, implantation of the extraluminal strain gauge transducers required general anaesthesia, a midline laparotomy, and suturing of transducers onto the serosal surface of the colon. Wired connections to the transducers were tunnelled subcutaneously and exteriorised through the skin of the chest wall. While this technique provides a wealth of valuable data, the invasive nature of this approach is not suitable for human studies.

1.5.3.2 Human studies

In humans, there have been a number of studies that have used concurrent colonic manometry and cinefluorography or scintigraphy to assess the relationships between colonic intraluminal pressure and transit. The key findings from these studies have been summarised below.

Hardcastle & Mann performed a pioneering study in 1968(110), using balloon kymography (four water-filled latex balloons spaced at 50mm) and concurrent cineradiology in seven participants. They demonstrated that bisacodyl and oxyphenisatin stimulated antegrade propagating contractions which emptied the colon of content on cineradiology imaging. They referred to these contractions as "stripping waves", but they are likely to represent high-amplitude propagating contractions, which are known to be stimulated by bisacodyl (see **1.8.2 Assessment of Colonic Neuromuscular Function Using Bisacodyl**).

In 13 human participants, Torsoli et al.(130) used concurrent low-resolution manometry (2-4 channels spaced at 50mm) and cinefluorography to record intraluminal pressure and transit. Infusion of hyperosmolar glucose or bisacodyl into the small intestine was used to stimulate colonic motility. High-amplitude, lumen-occluding contractions propagating in an antegrade direction were spatiotemporally associated with the antegrade transit of content(130).

In 1997, Herbst et al.(131) similarly used concurrent low-resolution colonic manometry and radioisotope imaging to record motility patterns and transit in six patients with faecal incontinence and

six healthy volunteers. However, the imaging was infrequent, with single images performed at six hours, 21 hours, and then hourly for a further nine hours. As a result, real-time relationships between pressure and transit were not described. Rather, comparisons were made between patients and healthy volunteers based upon pressure and transit data separately.

Several studies have described concurrent colonic manometry and scintigraphy recordings in humans(132-137). Using these techniques, Dinning et al.(137) found that the vast majority of propagating sequences in the proximal colon (>90%) resulted in the transit of luminal content. However, only 45% of all recorded antegrade transit was related to a propagating contraction and, in some instances, antegrade propagating contractions were associated with retrograde transit of content(137). This may suggest that, at certain times, transit occurs in the absence of motility patterns. Other studies have also demonstrated a poor correlation between motor patterns and flow. However, it must be noted that the frequency of imaging in scintigraphy studies varies greatly, from 10-second to five-minute intervals(132-137). Even an imaging frequency of 10s, as used by Dinning et al.(137), may overlook a certain amount of tracer displacement which could account for some of the discrepancies between the recorded colonic motor patterns and transit.

Most recently, ingestible wireless motility capsules have been used to record whole gut and colonic transit(98, 138, 139). The SmartPill^{TM} is a capsule that contains a single pressure sensor, temperature sensor, and pH sensor. Using the physiological differences in pH between stomach, small intestine, and colon, the pH data is used to approximate the location of the device within the gastrointestinal tract(140). While there have been some attempts to relate the pressure recorded from the single sensor to motility within specific regions of the colon(138), the SmartPill^{TM} has no capability to discriminate which colonic region that the capsule is located in or provide information on propagation of contractions. In contrast, the electromagnetic capsule tracking system has the ability to be tracked in real-time throughout all regions of the digestive tract(98, 139, 141). This allows investigators to detail dwell time and antegrade/retrograde movement throughout all regions of the colon. However, unlike the SmartPill^{TM}, the magnetic capsules do not have pressure sensors and, as a result, cannot provide any information on pressure events which are associated with transit.

1.5.4 Impedance Manometry

In oesophageal and small intestinal studies, multichannel intraluminal impedance catheters have been used to record bolus transit(142-145). Electrical impedance is a measure in ohms (Ω) of the resistance to a current within a circuit. Impedance is altered by the conductivity of the media surrounding the circuit, increasing in the presence of a poorly conductive media, such as gas, or decreasing when immersed in a conductive media, such as liquid. Combined impedance and manometry recordings provide the capability to correlate motility patterns with bolus transit in real-time(146-149). As

swallowing is under voluntary control, and transit along the oesophagus occurs in seconds, videofluoroscopy has been used to validate the ability of impedance manometry to record bolus transit in the oesophagus(150, 151).

Due to the involuntary and infrequent nature of colonic smooth muscle activity, prolonged fluoroscopic imaging would be required to replicate this validation process in the colon. This would result in significant radiation exposure and is therefore not feasible for human studies. Colonic impedance manometry has been trialled in ex vivo animal studies. Costa et al.(76) used an impedance manometry catheter to record colonic motility in ex vivo specimens of rabbit colon. The preparation also allowed for real-time video recording of colonic wall motion. This study demonstrated a strong correlation between pressure increases and colonic contractions. In addition, the authors demonstrated a strong correlation between changes in impedance and changes in luminal diameter, thus indicating that impedance may be able to be used to determine when and where muscle contractions were occurring. Mohd Rosli et al.(152), also using ex vivo rabbit colon, demonstrated a characteristic admittance/pressure signature associated with colonic gas and liquid transit. Both studies indicated that impedance manometry may be promising tool for use in the human colon to detail pressure/transit relationships. To date, this has not yet been performed (**Chapter 7**).

1.5.5 Knowledge Gaps

- Standardisation of colonic manometry study protocols and automation of data analysis, to provide both a detailed characterisation and quantification of colonic motor activity.
- A characterisation of the relationships between intraluminal pressure and transit of intraluminal contents in humans. This would require the implementation of techniques which can concurrently record pressure and transit, such as high-resolution impedance manometry (addressed in **Chapter 7**).

1.6 Colonic Motor Patterns

1.6.1 Overview

Colonic motility exhibits diurnal variation, with suppression of colonic motility at night(80, 100, 111, 112, 115, 153-155). During the day, there are significant increases in colonic motor activity with several associated physiological stimuli. Two of the most notable stimuli for increasing colonic motility are morning awakening(80, 111, 153, 156) and meals(80, 111, 112, 132, 154, 157-159). Exercise and emotions such as stress, anxiety and anger can also increase colonic motility(160-164).

Several patterns of colonic phasic motor activity have been previously described, from simple type I, II, and III waves(165) to descriptions of the propagating or non-propagating nature of colonic contractions(80, 99, 111, 115). In this thesis, I will use the terminology described in a recent consensus paper(11), which was intended to standardise the terminology used in this field. The basis for the terminology reported in this consensus paper were the findings from recent studies using high-resolution colonic manometry(2, 166-168). The consensus paper labelled the four predominant colonic motility patterns as (**Figure 1.3**);

1. High-amplitude propagating contractions
2. The cyclic motor pattern
3. Single motor patterns (short and long)
4. Pancolonic pressurisations

1.6.2 High-Amplitude Propagating Contractions (HAPCs)

High-amplitude propagating contractions (HAPCs) are the most visually striking pattern when viewing a manometry recording. HAPCs have been defined by various characteristics, which have been detailed in a review on colonic manometry(27). In the recent consensus paper, HAPCs were defined as propagating contractions with an amplitude of >50mmHg, which propagate a minimum distance of 10-30cm, with a duration of 10-30s(11). HAPCs involve a series of propagating pressure waves which are of large amplitude, predominantly propagate in an antegrade direction, and most commonly originate in the proximal colon(2, 23, 79, 80). HAPCs are likely to correspond with the colonic mass movements initially reported on studies by Hertz and Holzknecht in the early 1900's(77, 78). They occur infrequently (4-23 times per 24-hour period in healthy adults(11)), most commonly on awakening, during the postprandial period, and prior to defaecation(111, 112, 131). HAPCs can also be elicited via mechanical stimulation including intraluminal colonic balloon distension, or chemical stimulation using intraluminal colonic/rectal bisacodyl, chenodeoxycholic acid, or short-chain fatty acids(112, 130, 136, 169, 170).

HAPCs are hypothesised to be generated via enteric excitatory motor neurons(80, 100). Bisacodyl, for example, is hypothesised to stimulate mucosal afferent nerves to increase pancolonic contractility via long colo-colonic reflex pathways(23). When bisacodyl is administered per rectum, HAPCs are generated in the proximal colon (see **1.8.2 Assessment of Colonic Neuromuscular Function Using Bisacodyl** & **Chapter 6**). Prior application of lidocaine, a sodium-channel antagonist, to the rectal mucosa can block the initiation of HAPCs by bisacodyl(110), further supporting a neural origin.

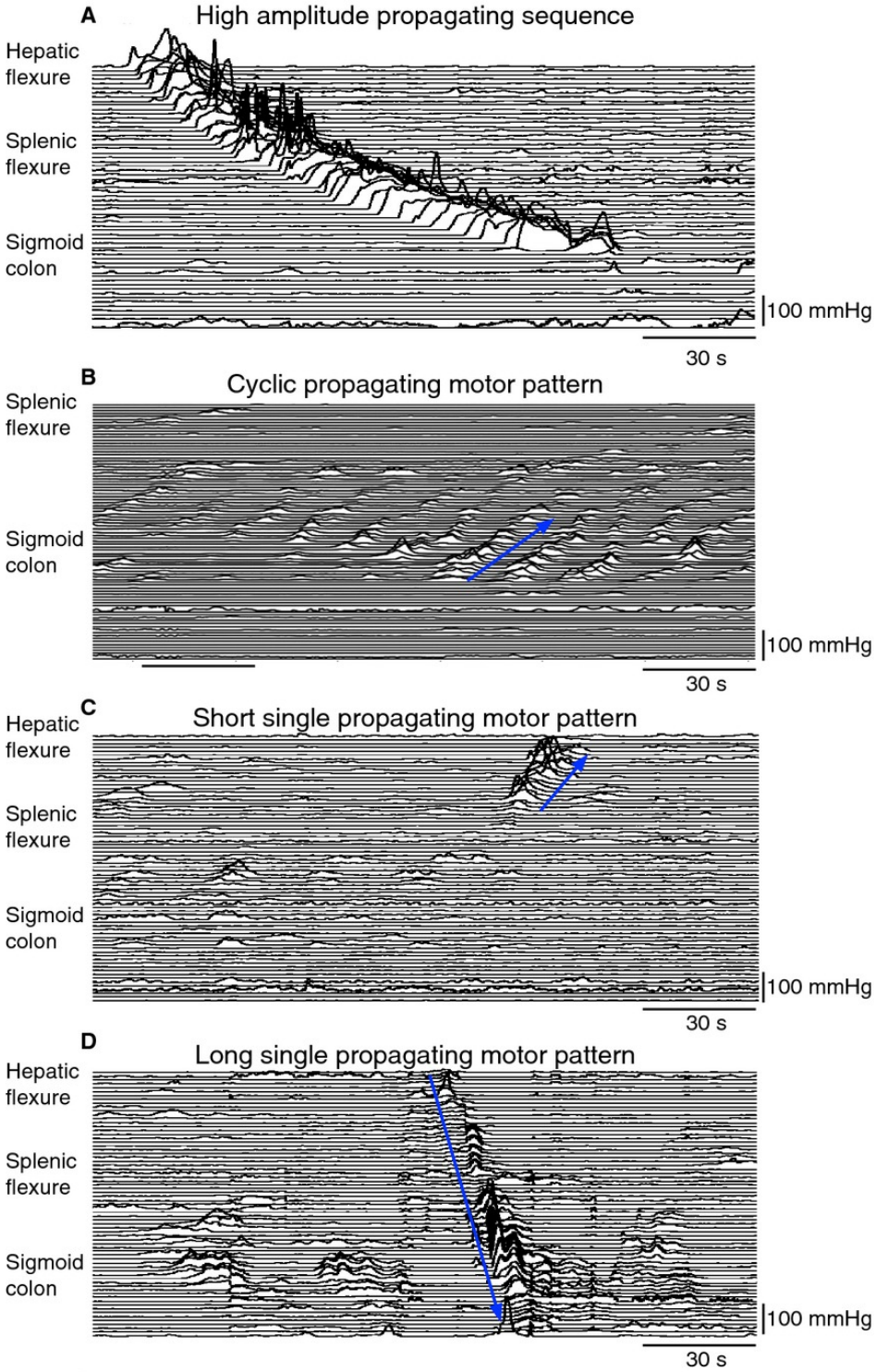

Figure 1.3 High-resolution colonic manometry recordings demonstrating; (A) a high-amplitude propagating contraction, (B) the cyclic motor pattern, and single motor patterns (short (C) and long (D)). Image source: Dinning PG, Wiklendt L, Maslen L et al. Quantification of in vivo colonic motor patterns in healthy humans before and after a meal revealed by high-resolution fiber-optic manometry. *Neurogastroenterol Motil.* 2014;26(10):1443-57. © 2014 John Wiley & Sons Ltd (Licence number #4881121289915)

1.6.3 The Cyclic Motor Pattern

The cyclic motor pattern consists of a series of rhythmic, propagating contractions that occur at a frequency of 2-10 cycles/minute (most commonly 2-8/min)(2), with one study also reporting a higher frequency between 11-20/minute(168). The cyclic motor pattern consists of low-amplitude pressure waves (23.1±21.4mmHg)(2)) that propagate in either an antegrade or retrograde direction.

In some high-resolution colonic manometry studies, the cyclic motor pattern is the most prevalent motor pattern seen in the human colon, comprising of ~70% of all colonic motor activity(2). The cyclic motor pattern is more prevalent during sleep(116, 171), general anaesthesia(172), and during the postprandial period(2). It can be stimulated by HAPCs(168) which may result in the antegrade propulsion of intraluminal content(116). The cyclic motor pattern is also more active during sacral nerve stimulation(173). The cyclic motor pattern is hypothesised to be myogenic in origin, representing the ICC-generated "slow waves"(2). Despite this, the increases in the cyclic motor pattern in response to the stimuli described above suggests that the cyclic motor pattern can additionally be augmented by neural innervation(2, 11).

The cyclic motor pattern is likely to represent previously described motility patterns including periodic colonic motor activity(174), periodic rectal motor activity(116), rectal motor complexes(171), and intermittent rectal motor activity(175). However, as the cyclic motor pattern can be found throughout the entire colon(112), the descriptions of "rectal" motor complexes or activity are not entirely accurate. The cyclic motor pattern in the distal colon is hypothesised to act as an "intrinsic colonic gatekeeper"(116) or a "rectosigmoid brake"(176). This hypothesis is supported by several findings in colonic disease states. For example; (1) sigmoid hypermotility causes a reduction in stool frequency in patients with ulcerative colitis(177) and, conversely, (2) patients with diarrhoea-predominant irritable bowel syndrome who report postprandial faecal urgency do not demonstrate the same postprandial increase in sigmoid motility which is seen in healthy controls(178-180). A reduction or absence of retrograde activity in the rectosigmoid has also been proposed to be contributory to faecal incontinence(173), which may explain why sacral nerve stimulation – which increases the cyclic motor pattern – has a therapeutic effect in this patient group(173, 181). To date, however, the functional role of the cyclic motor pattern in regulating rectal filling and continence has not been demonstrated (**Chapter 7**).

1.6.4 Single Motor Patterns

Single motor patterns are propagating pressure waves which are separated from other motor patterns by intervals of >60s. Single motor patterns are also hypothesised to be myogenic in origin, with their

location, frequency, and propagation modulated by neural innervation(2). Single motor patterns can additionally be stimulated by polyethylene glycol, an osmotic laxative, and linaclotide(182), a guanylate-cyclase 2C agonist which increases that luminal secretion of water, chloride, and bicarbonate.

Single motor patterns are described as short or long, depending on their length of propagation. Short single motor patterns propagate a distance of 7cm in either an antegrade or retrograde direction. Short single motor patterns are most commonly observed in the proximal or sigmoid colon. Long single motor patterns can propagate >40cm, predominantly commencing in the proximal colon and propagating in an antegrade direction. The functional role of single motor patterns in transit has not yet been described.

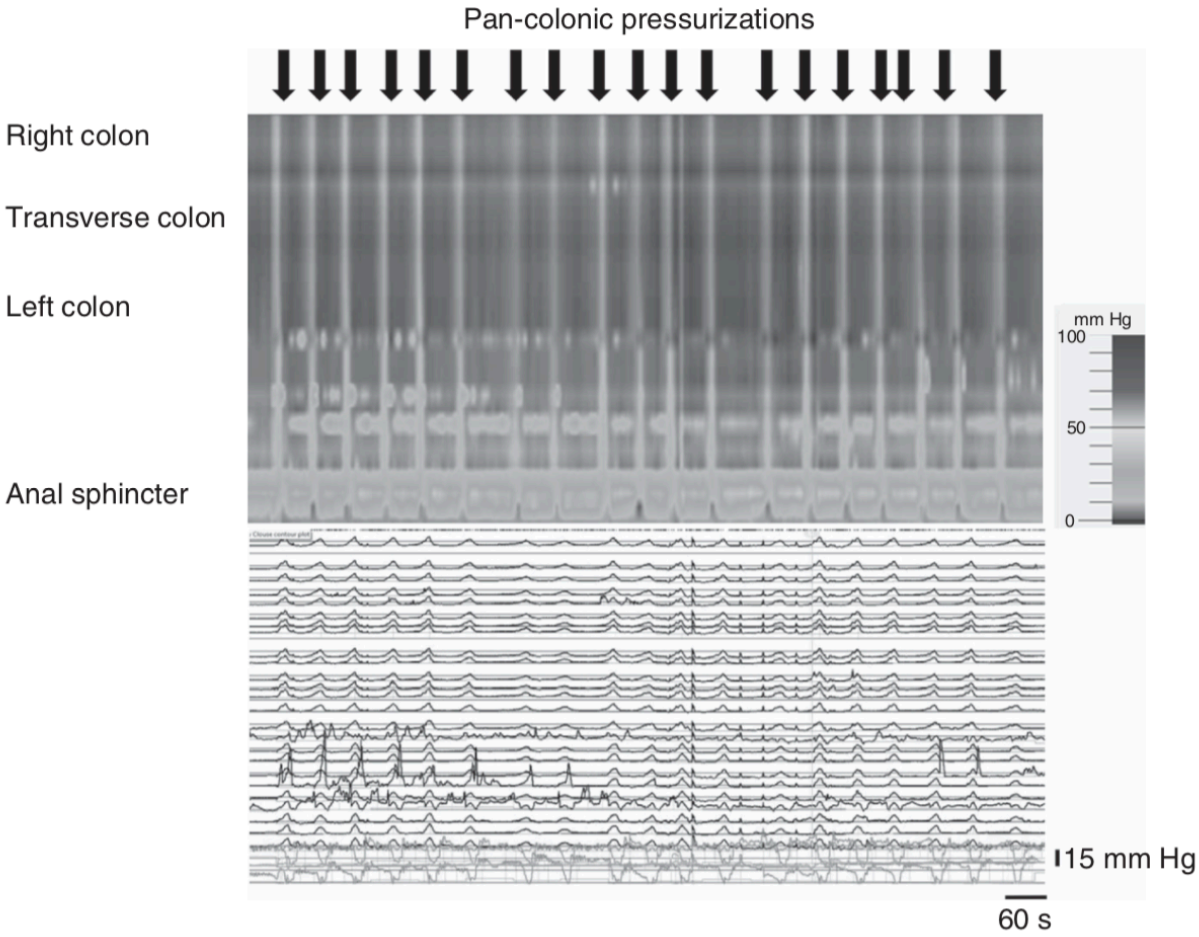

Figure 1.4 A high-resolution colonic manometry recording (top: colourmap, bottom: line tracing) demonstrating pancolonic pressurisations occurring at a frequency of ~1 cpm which are associated with anal sphincter relaxation. Image source: Corsetti M, Pagliaro G, Demedts I et al. Pan-colonic pressurisations associated with relaxation of the anal sphincter in health and disease: a new colonic motor pattern identified using high-resolution manometry. *Am J Gastroenterol.* 2017;112(3):479-489. © 2017 Wolters Kluwer Health Inc (Licence number #4881140585270)

1.6.5 Pancolonic Pressurisations

While synchronous pressure increases in the human colon had been described for many years, Corsetti et al.(166) described these events as "pancolonic pressurisations" (**Figure 1.4**). Pancolonic pressurisations are defined as a synchronous increase in pressure across all manometry channels, that are differentiated from artefact or abdominal wall strain by no concurrent change in abdominal wall electromyography(166). Pancolonic pressurisations are most frequent during meals, can be induced using prostigmine or bisacodyl, and can also be associated with anal sphincter relaxation, flatal urgency, and flatus(166, 167).

Synchronous pressure increases occurring across the distal channels at the termination of HAPCs have also been described(152, 166, 167, 183). These events are hypothesised to function as a means to maintain colonic wall tone and facilitate transit. This phenomenon may be associated with transit of content, as the lumen distal to a propagating contraction dilates to accommodate propulsion of content(152, 183). At this stage, however, no studies correlating motility to transit have been performed to evaluate the functional significance of synchronous pressurisations (**Chapter 7**).

1.6.6 Knowledge Gaps

- The functional relationships between specific colonic motor patterns and the transit of solid, liquid, and gaseous intraluminal contents (addressed in **Chapter 7**).
- The myogenic and neurohormonal mechanisms responsible for the generation of specific colonic motor patterns (addressed in **Chapter 8**).
- A characterisation of the proposed rectosigmoid "brake" function of the cyclic motor pattern. Specifically, to detail the functional role of cyclic motor activity in real-time during; (a) basal periods during which continence is maintained, (b) during rectal filling, and, (c) during defaecation.

1.7 Colonic Motility in Functional Bowel Disorders

1.7.1 Faecal Incontinence

Faecal incontinence is classified by the Rome IV criteria as the uncontrolled passage of stool, occurring at least twice in every four-week period for a minimum duration of three months(184, 185). Faecal incontinence is a common symptom, affecting 6-15% of the community(186-188), with symptoms likely to be under reported due to the associated embarrassment and shame(189).

1.7.1.1 Pathophysiology

The pathophysiology of faecal incontinence is varied and often multifactorial(190-192). Common causes include anal sphincter and/or pelvic floor injury(190, 193), altered rectal or anal canal sensation or compliance (hyper/hyposensitivity)(194, 195), and rectal/perianal diseases and surgery(196, 197). However, these mechanisms do not readily describe causation in all cases. Risk factors for developing faecal incontinence also include older age(188, 198-200), obesity, bowel disturbances (constipation, diarrhea, or abdominal pain)(199, 201), prior cholecystectomy(201), concurrent urinary incontinence(188, 200, 202-204), medical co-morbidities (diabetes, stroke, scleroderma, among others), and the use of psychiatric medications(198, 204).

Anal sphincter injury and/or anorectal dysfunction are the most studied causes of faecal incontinence and are the focus of diagnostic investigations(205-207). In men, iatrogenic injury to the neuromuscular integrity of the anorectum is implicated in pathogenesis, with over half of all men with faecal incontinence reporting prior anorectal surgery(196, 208). In women, obstetric anal sphincter injury is the most common mechanism of injury(190, 193). However, most women with obstetric anal sphincter injury at age 20-30 years do not develop symptoms of faecal incontinence until decades later(190), the reasons for which are unclear.

1.7.1.2 Diagnostic Investigations

Evaluation of a patient presenting with faecal incontinence must begin with a history and examination. The history should include the onset and duration of symptoms, precipitating events (eg. childbirth, anorectal surgery), volume (flatal incontinence, staining of underwear, or solid faeces), and associated symptoms such as urgency. Quantitative symptom scoring can also be performed, such as the Jorge-Wexner faecal incontinence severity score(205) or St Mark's incontinence severity score(209).

Examination should include a perianal and per rectal examination(210). This allows to clinician to visualise perianal conditions which may contribute to symptoms such as anorectal prolapse, anal gaping, fistula-in-ano, and/or prolapsing haemorrhoids. Perianal sensation and the integrity of the anocutaneous reflex should also be assessed. A digital per rectal examination is useful to assess for palpable anal sphincter defects (although it is uncommon to identify a sphincter defect by digital examination alone), the presence of an anorectal tumour, stricture, rectocoele, or impacted faeces which could contribute to overflow incontinence. The per rectal examination should also include dynamic manoeuvres, instructing the patient to bear down and squeeze or cough, to allow for assessment of pelvic floor descent and anal sphincter tone respectively. Flexible sigmoidoscopy or colonoscopy can also be performed to assess for underlying colorectal cancer or inflammatory bowel disease.

Before pursuing further investigation, a trial of conservative management may be undertaken in the first instance (see **1.7.1.3 Treatment**). For those who have refractory symptoms despite these measures, further anorectal physiology testing is warranted.

Anorectal physiology testing for faecal incontinence can include endoanal sonography, anorectal manometry, anorectal sensory testing, and pudendal nerve terminal motor latency (**Chapters 4 & 5**)(211). Collectively, these tests can determine the presence of a structural injury of the anal sphincter, neuromuscular dysfunction of the anorectum and/or pelvic floor, and the presence of pudendal nerve neuropathy. However, objective evidence of anorectal dysfunction in patients with severe incontinence is not always apparent, whereas some patients with a demonstrable anal sphincter injury experience only mild symptoms or are asymptomatic. For example, anal sphincter injury is reported in 27% of primiparous women(212, 213), yet less than one third of women with sphincter injuries report faecal incontinence postpartum(214). Conversely, 40% of patients presenting with faecal incontinence have normal anal sphincter morphology on endoanal sonography(215). Whilst lower anal canal resting and squeeze pressures have been associated with faecal incontinence when compared with healthy controls(216-218), there remains a considerable overlap in findings between these groups(219, 220). One study found that 9% of women and 18% of men with faecal incontinence had normal results on all routine anorectal investigations(221). The pathogenesis of symptoms in these patients is unclear.

1.7.1.3 Treatment

Despite a variety of options, symptom resolution following treatment occurs in <40% of patients(187). Conservative treatment options include continence pads, anal plugs, dietary modifications, stool-modifying medications (stool-bulking or anti-diarrhoeal agents), and/or pelvic floor physiotherapy and biofeedback therapy. The most common surgical options currently include anal sphincter repair(222), ileostomy/colostomy formation, and/or sacral nerve stimulation(223). Sacral nerve stimulation has been demonstrated to elicit an increase in the cyclic motor pattern in the distal colon(173, 181) which may have a functional a role in continence (see **1.6.3 The Cyclic Motor Pattern**).

1.7.1.4 The Functional Role of Colonic Motility in Continence

Colonic motility is involved in the normal physiology of defaecation, with high-amplitude propagating contractions temporally associated with defaecation(136, 137, 224). Beyond that, the role of colonic motility in defaecation is poorly understood and the functional role of colonic motility in continence has not been established. As mentioned above, some authors have hypothesised that the cyclic motor pattern in the distal colon may contribute to continence via action as an "intrinsic colonic gatekeeper"(116) or "rectosigmoid brake"(176) (**1.6.3 The Cyclic Motor Pattern)**. This hypothesis has, to date, not been further investigated in healthy volunteers or in patients with faecal incontinence.

Eight previous studies have recorded aspects of colonic or rectal motility in patients with faecal incontinence, the findings of which are summarised below. Collectively, the findings from these studies are conflicting and are confounded by the poor quality of several studies. Most critically, the functional relationship of colonic motility to continence and episodes of incontinence has never been assessed. Colonic manometry was used in six of these studies (low-resolution catheters in five studies(131, 225-228), and a high-resolution catheter in one study(173)) and impedance planimetry in two studies(229, 230). The low-resolution manometry catheters had sensors spaced at ≥10cm, which is likely to overlook a large proportion of propagating colonic motor activity(114). This would almost certainly overlook the cyclic motor pattern, which has a mean distance of propagation of <5cm(2).

In 1984, Keighley & Shouler(225) reported a higher motility index in the sigmoid colon in patients with faecal incontinence when compared with healthy controls. The functional significance of this is not clear. A motility index involves an area under the curve analysis using the mean amplitude of pressure data multiplied by a specified time duration. While this provides some ability to discriminate between periods of heightened activity and periods of quiescence, a motility index is a generalised measure of phasic activity that provides no characterisation of specific motor patterns. This is not particularly useful in characterising how colonic motor patterns were functionally related to periods of continence and, crucially, episodes when incontinence of faeces occurred. In addition, this data was recorded using a low-resolution manometry catheter (number and spacing of pressure sensors was not specified) positioned in the distal 15cm of the colon and rectum, which further limits the utility of these findings.

In 1997, Herbst et al.(131) used concurrent colonic manometry and radioisotope imaging to compare six patients with faecal incontinence to six healthy controls. They demonstrated no significant differences in the frequency or characteristics of colonic motor patterns or colonic transit between patients and healthy controls. The imaging of transit markers was infrequent (see **1.5.3.2 Human Studies**) and no analyses were performed on the associations between pressure and transit. As such, no description of the functional role of motility patterns in regulating rectal filling and maintaining continence was provided.

In 2000, Santoro et al.(226) used ambulatory anorectal manometry to evaluate the effects of amitriptyline on anorectal pressure and symptoms in 18 patients with faecal incontinence. Amitriptyline, a tricyclic antidepressant, acts on multiple receptors, altering the uptake of 5-HT and noradrenaline as well as antagonistic effects on cholinergic and histaminergic receptors. A four-week course of amitriptyline (per oral, 20mg, daily) resulted in a significant reduction in a faecal incontinence symptom severity score, as well as a reduction in the frequency and amplitude of rectal motor complexes (which are likely to be synonymous with the cyclic motor pattern, see **1.6.3 The Cyclic Motor Pattern**). They defined rectal motor complexes as rectal pressure activity which was neither

associated with the passage of flatus, defaecation, or internal anal sphincter relaxation. This definition specifically excludes motor patterns associated with defaecation or episodes of incontinence, which are the most critical moments to ascertain the functional role of motility in continence and defaecation. Given the multifocal actions of amitriptyline, it is difficult to ascertain whether the reduction in the symptom score was due to localised changes in anorectal function, altered motility and transit more proximally in the gastrointestinal tract, or actions at an autonomic or central nervous system level, including improvements in mental state.

Michelsen et al.(230) recorded concurrent impedance planimetry and anorectal manometry in 16 patients with faecal incontinence undergoing sacral nerve stimulation. This study demonstrated a reduction in postprandial rectal tone with sacral nerve stimulation, but no changes in antegrade or retrograde cyclic (2-6cpm) rectal contractions. Symptoms pre- and post-intervention were not assessed. Worsøe et al.(229) also used an impedance planimetry device, featuring five electrode pairs spaced at 20mm. They reported no significant differences in the prevalence or count of antegrade or retrograde cyclic (2-6cpm) propagating contractions before or after a meal when comparing 12 patients with faecal incontinence and 12 healthy volunteers. Again, no dynamic information at the time of defaecation or episodes of incontinence was provided.

In 2005, Chan et al.(227) recorded rectosigmoid manometry in healthy controls as well as in two groups of patients with urge faecal incontinence; (1) those with normal rectal sensation as determined by rectal balloon distension, and; (2) those with rectal hypersensitivity. When compared with the other subgroups, the rectal hypersensitivity subgroup demonstrated altered characteristics of rectal motor complexes (likely to be synonymous with the cyclic motor pattern, see **1.6.3 The Cyclic Motor Pattern**). These were of higher frequency, shorter duration, and lower amplitude when compared with the other subgroups. While these findings may suggest that patients with urgency and rectal hypersensitivity do have altered distal colonic motility, it is again not possible to ascertain the functional mechanisms which relate these altered characteristics to the manifestation of their symptoms. The manometry catheter used for this study featured six sensors spaced at 5cm, and therefore is likely to overlook the cyclic motor pattern, which has a mean distance of propagation of <5cm. Colonic motility was not recorded during defaecation or episodes of incontinence.

In 2010, Rodger et al.(228) performed low-resolution colonic manometry in the distal colon in 11 patients with faecal incontinence. These patients demonstrated a significantly higher count of propagating contractions during the pre-prandial period (but not in the post-prandial period) when compared with healthy control data. Definitions of specific motor patterns were based solely on amplitude data (either >5mmHg for a “low-amplitude wave”, and >50mmHg for “high-amplitude wave”). There was no further identification or characterisation of motor patterns, nor any description

of how motor patterns were related to symptoms of urgency or incontinence. Given the several notable limitations in the study methodology, it is difficult to draw meaningful conclusions from these findings.

The only study that has used high-resolution colonic manometry in patients with faecal incontinence was a 2013 study by Patton et al.(173) Colonic motility was recorded in 11 patients with faecal incontinence during a control period, a sham stimulation period, and a sacral nerve stimulation period. Sacral nerve stimulation was associated with a significant increase in the retrograde cyclic motor pattern, most notably in the left hemicolon. There was also a reduction in symptom severity scores during sacral nerve stimulation. These findings may support the hypothesis that distal colonic motility has a functional role in continence by acting as an "intrinsic colonic gatekeeper"(116) or "rectosigmoid brake"(116, 176) (see **1.6.3 The Cyclic Motor Pattern**). However, as with the other studies, there were no manometry recordings during episodes of incontinence.

In summary, previous descriptions of colonic motility in patients with faecal incontinence are conflicting. Two studies demonstrated no difference in colonic motility between patients with faecal incontinence and healthy controls(131, 229). Three studies implicated colonic hypermotility in symptom causation(225, 227, 228). Symptom improvement was elicited via; (a) a reduction in rectal motor complexes (likely to be synonymous with the cyclic motor pattern, see **1.6.3 The Cyclic Motor Pattern**) using amitryptline(226), yet also via; (b) an increase in the cyclic motor pattern using sacral nerve stimulation(173). Further studies using high-resolution colonic manometry to characterise the functional role of colonic motility in continence are required.

1.7.2 Constipation

The Rome IV criteria categorises constipation as either functional, opioid-induced, or as a subtype of irritable bowel syndrome with a predominance of constipation(231). Functional constipation is defined as difficult, infrequent, or incomplete defaecation, occurring for a minimum duration of three months(231). Diagnostic criteria additionally includes the acuity of onset, straining, stool consistency, sensation of incomplete evacuation, sensation of anorectal obstruction, and manual manoeuvres used to facilitate evacuation(231).

1.7.2.1 Pathophysiology

The pathophysiology of functional constipation has been broadly classified as; (1) normal transit; (2) slow transit, or; (3) disordered evacuation(99, 232). Describing a disease process as "functional" implies that there is no underlying organic cause. However, there have been several histopathological features reported to be associated with constipation(233). In slow transit constipation, for example, several studies have described reductions in the count and density of the ICC(234-241). It is not known

whether this is also present in those with normal transit constipation(242). Other features described in patients with constipation include reduced counts and density of neurons(243), ganglia, and glial cells(242), reduced thickness of the colonic circular muscle(233), as well as altered neurochemistry with reductions in levels of vasoactive intestinal peptide(244, 245), substance P(246), tachykinins(247, 248), and enkephalins(248). It is unclear whether these histological findings are causative, are the result of, or are rather an epiphenomena of constipation.

In both adults and children with constipation, colonic manometry studies have recorded several patterns of colonic dysmotility. These include; abnormal colonic contractile responses to meals and awakening(115, 249, 250), a reduction in the frequency of high-amplitude propagating contractions(79), a reduction in spatiotemporal coordination of colonic motility patterns(251-253), and a diminished meal response(254, 255).

In a unique study in four patients with slow transit constipation, high-resolution colonic manometry was performed in vivo pre-operatively, then repeated manometry recordings were made from the excised colon ex vivo immediately following a total colectomy(256). In vivo recordings demonstrated reduced propagating activity and a diminished meal response when compared with healthy controls. However, there were no differences between contractile activity in the ex vivo recordings when comparing patients and healthy controls. The apparent “normalisation" of colonic motor activity following surgical resection may implicate extrinsic neural innervation to the colon in the pathogenesis of constipation.

1.7.2.2 Diagnostic Investigations

Evaluation of a patient presenting with constipation must begin with a history and examination. The history should include the onset and duration of symptoms, the frequency of defaecation, stool consistency, as well as associated symptoms such as straining, abdominal pain, the sensation of incomplete evacuation, sensation of anorectal obstruction, and manual manoeuvres used to facilitate evacuation. The history should also include screening for any associated “red flag” symptoms which raise suspicion for colorectal cancer, such as per rectal bleeding, unintentional weight loss, and/or a family history of colorectal cancer. Quantitative symptom scoring can also be performed, such as the Cleveland Clinic constipation score(257).

Examination should include a perianal and per rectal examination(210). This allows to clinician to visualise perianal conditions which may contribute to outlet obstruction such as anal fissures, anorectal prolapse, and/or prolapsing haemorrhoids. A digital per rectal examination is useful to assess for the presence of an outlet obstruction caused by an anorectal tumour, rectocoele, intussception, or faecal impaction. The per rectal examination should also include dynamic manoeuvres, instructing the patient

to bear down, to allow for assessment of pelvic floor dyssynergia(258). Flexible sigmoidoscopy or colonoscopy can also be performed to assess for colorectal cancer, particularly in instances where "red flag" symptoms are identified.

A trial of conservative management (see **1.7.2.3 Treatment**) may be undertaken before pursuing further investigation. For those who have refractory symptoms despite these measures, further testing is warranted. This can include anorectal manometry, anorectal sensory testing, defaecography, colonic manometry (**Chapter 6),** and colonic transit studies.

1.7.2.3 Treatment

Conservative treatment options for constipation include increasing daily fluid and fibre intake, dietary modification, as well as modulation of bowel habit using stool bulking agents, laxatives, and/or enemas. Additional pharmacological agents available for the treatment of constipation include 5-HT_4 receptor agonists (eg. prucalopride), colonic secretagogues (eg. lubiprostone), and opioid receptor antagonists (eg. methylnaltrexone, alvimopan)(259). Surgical management can include techniques to address outlet obstruction (rectopexy, rectocoele repair, stapled transanal rectal resection), caecostomy/appendicostomy formation for administration of antegrade enemas, ileostomy/colostomy formation, or colectomy (subtotal colectomy with an end ileostomy or ileorectal anastomosis)(260).

1.7.3 Knowledge Gaps

- The role of colonic motility in the regulation of continence (addressed in **Chapter 7**).
- High-resolution colonic manometry studies in healthy controls and patients with faecal incontinence for detailed comparisons of colonic motor function/dysfunction.
- An assessment of the cyclic motor pattern in real-time during defaecation and episodes of faecal incontinence.
- A characterisation of the histological changes that occur in colonic smooth muscle in patients with constipation, and how these differ between normal transit and slow transit subgroups.
- The role of extrinsic neural inputs to the colon in the pathogenesis of slow transit constipation.

1.8 Pharmacological Modulation of Colonic Motility

1.8.1 Overview

Numerous pharmacological agents are available to modulate bowel habit. These include stool-bulking agents, laxatives, and anti-diarrhoeal medications. Laxatives act predominantly via one of two mechanisms; (1) an osmotic action to increase stool volume and bulk, increasing mechanical distension

of the colonic wall, or by; (2) a stimulant or prokinetic effect to increase colonic motility(259). The side effects of many common medications also alter normal bowel function (see **Chapter 2, Table 2.1**).

1.8.2 Assessment of Colonic Neuromuscular Function Using Bisacodyl

Bisacodyl, administered via oral tablet or enema, is a known stimulus for high-amplitude propagating contractions (see **1.6.2 High-Amplitude Propagating Contractions**). Colonic manometry with intraluminal bisacodyl infusion is used as a diagnostic modality to assess colonic neuromuscular function in children with treatment-refractory constipation(79, 122, 123, 261-263) (**Chapter 6**). In order to minimise the study duration, bisacodyl is used to provoke high-amplitude propagating contractions (HAPCs) and induce defaecation(264-269). Initiation of HAPCs following bisacodyl is considered to be a "normal" colonic response(264-269). A diagnosis of an "abnormal" bisacodyl response has a significant bearing on the clinical course of the child, as it can be an indication for surgical intervention(262, 264, 270-274).

Whilst categorised as a "stimulant" laxative, the exact mechanisms by which bisacodyl alters colonic physiology remain unclear. Bisacodyl is converted into an active metabolite *bis*-(p-hydroxyphenyl)-pyridyl-2-methane (BHPM) via esterase enzymes in the colonic mucosa(275) and there is minimal systemic absorption (276). Bisacodyl has been proposed to act via; (1) direct excitation of colonic smooth muscle via tetrodoxin-insensitive, nifedipine-sensitive pathways(277-279); (2) increased luminal secretion of electrolytes(277, 280, 281), and/or; (3) decreased water absorption(282). Rectal infusion of bisacodyl can stimulate high-amplitude propagating contractions in the proximal colon. Additionally, the action of bisacodyl can be blocked by a prior application of lignocaine to the rectal mucosa(110), further supporting the hypothesis of a neurally-mediated mechanism of action.

Previous studies have recorded colonic activity in response to bisacodyl using intraluminal balloon kymography and cineradiology(110), myoelectric recordings(283), radionuclide imaging(284), and high-resolution colonic manometry (250, 267, 285). The majority of these studies report only upon the ability of bisacodyl to induce high-amplitude propagating contractions, but provide no further characterisation of the colonic response to bisacodyl.

1.8.3 Opioid-Induced Constipation

Opioid receptor agonists can also alter colorectal function and defaecation. Synthetic opioids, such as loperamide, are used therapeutically to reduce the frequency of bowel motions in acute and chronic diarrhoeal illnesses, faecal incontinence, and to reduce ileostomy output in high output states(286, 287).

Opioid use for analgesia is also complicated by the common, undesirable side effect of constipation(288). Up to 45% of patients taking regular opioids report bowel motions less than three times weekly(289). To reflect this, an additional category was included in the Rome IV criteria for functional bowel disorders; opioid-induced constipation(231).

Opioids are associated with a reduction in the frequency and coordination of colonic propagating activity, increased non-propulsive activity, increased anal sphincter tone, and increased colonic transit time(231, 290-293). This results in reduced stool frequency, firmer stool consistency, straining, sensation of incomplete evacuation, sensation of anorectal obstruction, and manual manoeuvres may be required to assist defaecation.

The gastrointestinal tract contains μ, κ, and δ opioid receptors. Under normal physiological conditions, opioid receptors respond to endogenous ligands including enkephalins, endorphins, and dynorphins(294, 295). Opioid receptors act via G-protein coupling to inhibit adenylate cyclase and, in turn, reduce intracellular cyclic-AMP(296). This results in increased potassium permeability, hyperpolarisation of the cell membrane, and reduced excitability. This may suppress the action of enteric excitatory and inhibitory musculomotor and secretomotor neurons(295), which may contribute to the pathophysiology of opioid-induced constipation.

The colonic effects of opioids are likely to be mediated by modulation of both enteric and central nervous systems, but the mechanisms of action in humans are unclear (**Chapter 9).** Opioids and enteric neuromuscular physiology and pharmacology have mostly been described in animal studies(297-301), with only few descriptions in isolated human tissue specimens(302-305).

1.8.4 Knowledge Gaps

- The specific mechanisms of action of bisacodyl in stimulating high-amplitude propagating contractions and defaecation.
- A characterisation of the colonic response to intraluminal bisacodyl infusion for children with treatment-refractory constipation to inform interpretation of findings and subsequent clinical management (addressed in **Chapter 6**).
- The specific mechanisms by which endogenous and exogenous opioids cause alterations in human colonic neuromuscular function (addressed in **Chapter 9**).

Chapter 2: The Physiology of Human Defaecation Relating to Disorders of Continence and Evacuation

2.1 Statement

The content of this chapter has been published in *Nature Reviews Gastroenterology & Hepatology*.

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The co-authors have provided permission for the inclusion of the study in this thesis. The percentage contributions of each author to this study were as follows:

- Research design: **PH 25%**, CK 25%, PD 25%, MS 25%.
- Data collection and analysis: N/A
- Writing and editing: **PH 80%,** PV 4%, CK 4%, PL 4%, PD 4%, SMS 4%.

2.2 Abstract

The act of defaecation, while being a ubiquitous human experience, requires the coordinated actions of the anorectum and colon, pelvic floor musculature, as well as the enteric, peripheral, and central nervous systems. Defaecation is best appreciated through the description of four phases which are, temporally and physiologically, reasonably discrete. However, given the complexity of this process, it is unsurprising that disorders of defaecation are both common and problematic; almost everyone will experience constipation at some time in their life and many will suffer the indignity of faecal incontinence. A detailed understanding of the normal physiology of defaecation and continence is critical to inform management of disorders of defaecation. During the last decade, there have been significant advances in the investigative tools used to assess colonic and anorectal function. This review details the current understanding of the anatomy and physiology of defaecation and continence, pathophysiology of defaecation disorders, and considerations for further research in this field.

2.3 Introduction

Defaecation is a fundamental physiological process which results in the evacuation of faeces. Continence requires the voluntary control of defaecation. Both defaecation and continence are dependent upon a morphologically intact gastrointestinal tract and, additionally, the coordination and integration of multiple physiological systems including; (1) neural (principally the enteric nervous system, modulated by the peripheral somatic, autonomic, and central nervous systems); (2) muscular (smooth and striated); (3) hormonal (endocrine and paracrine), as well as; (4) cognitive (behavioral and psychosocial)(28, 128). Disorders of defaecation, such as constipation and faecal incontinence, are common, frequently co-exist(306-308), and incur a considerable burden of morbidity and healthcare expenditure(309-313). Constipation, for example, is the third most common presenting gastrointestinal symptom reported at outpatient clinics in the United States, with over three million estimated visits in 2009(312). The direct costs per patient for faecal incontinence and constipation are estimated to be between $1,594(314) and $7,522 per year(310). Furthermore, faecal incontinence and constipation frequently co-exist. Given the complexity of defaecation, a detailed understanding of normal physiology is critical to inform management of disorders of defaecation.

Since the most recent review article on this topic(315), there have been significant technological advances in the investigative tools used to assess colonic and anorectal function (**Box 2.1**) including; high-resolution colonic(2, 316) and anorectal manometry(317, 318), wireless capsule devices(98, 138), and magnetic resonance imaging (MRI) techniques(319-321). In this article, we provide an overview of the anatomy and physiology of defaecation and continence, pathophysiology of defaecation disorders (**Table 2.2**), as well as considerations for further research.

Box 2.1 Key Advances in Our Understanding of Human Defaecation

- Descriptions of transit occurring in the basal, pre-expulsive, and expulsive phases of defaecation using the magnetic tracking system.
- High-resolution manometric descriptions of anal canal pressure during resting, voluntary squeeze, and functional length measurements.
- High-resolution manometric characterisation of colonic motor patterns during the basal phase of defaecation.
- A description of the force vectors involved in active anal canal dilation during defaecation using MRI and video myogram.
- MRI volume assessments pre- and post-defaecation to assess colorectal ejection fractions of semi-solid and gaseous content.
- Descriptions of rectal intraganglionic laminar endings and rectal sensory afferent pathways.

2.4 Overview of Relevant Anatomy

2.4.1 Colon

The colon is a viscoelastic(1), tubular organ, beginning proximally at the ileocaecal junction and ending distally at the rectosigmoid junction (see **1.2 An Overview of the Anatomy and Physiology of the Human Colon**). The colon is approximately 130cm in length in adulthood(3), with a luminal diameter of 60-80 mm in the caecum, progressively narrowing to 25mm in the sigmoid colon(4). An elongated or redundant colon has been proposed as having a causative role in the pathogenesis of constipation(322, 323). Delayed colonic transit has been demonstrated with colonic elongation in mice(324), but it is unclear whether this has any functional significance in humans.

The colon receives intrinsic neural innervation from the enteric nervous system, extrinsic sympathetic innervation from the lumbar nerves, and extrinsic parasympathetic innervation from the vagus nerve (proximal colon) and pelvic splanchnic nerves, which collectively govern the sensorimotor function of the colon(128)**.**

2.4.2 Rectum

The rectum can be considered a specialised distal extension of the colon. Located in the pelvis, its high compliance (i.e. distensibility in response to filling) is necessary for accommodation of content immediately prior to defaecation (the rectal 'reservoir'). Although a number of landmarks have been used to define the upper border of the rectum, a recent consensus group suggested that the rectum

commences at the mesocolic-mesorectal transition, or the "sigmoid take off". This location corresponds to the convergence of the taenia coli into one continuous sheath of longitudinal muscle(325).

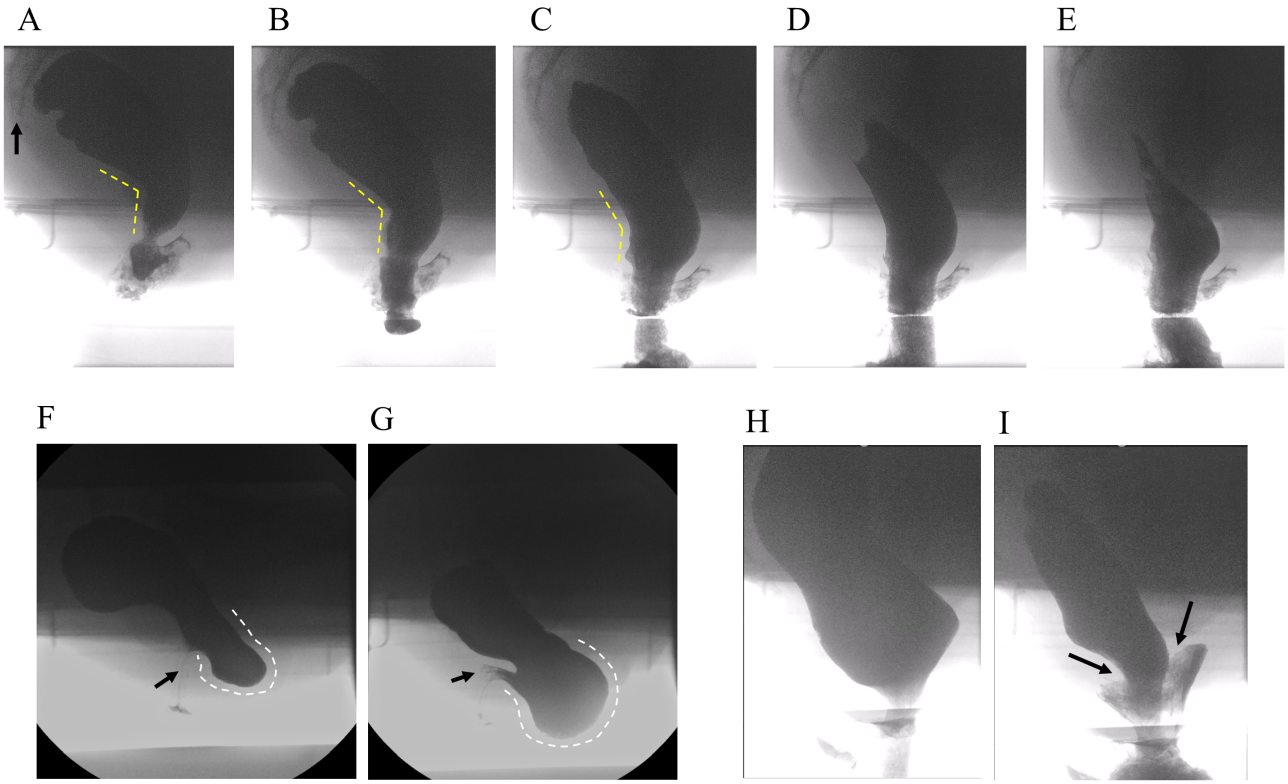

Figure 2.1 Barium (X-ray) defaecography images. Series A-E shows defaecation of neostool contrast in a healthy female subject (lateral view; sacrum arrowed in A). There is clear opening of the anorectal angle (dashed yellow lines: images A-C), with progressive dilation of the anal canal (images B-D), allowing rapid expulsion of the majority of contrast. Images F & G show the development of a huge retaining rectocoele (dashed white lines) in a patient complaining of difficulty in rectal evacuation, sense of prolapse, and post-defaecation leakage. Image F is at rest, and image G at mid evacuation. Marked distortion of the anal canal position can be noted (arrowed). Images H & I show the development of a striking full-thickness recto-anal intussusception (arrowed) in a patient presenting with coexistent faecal incontinence and tenesmus.

The rectosigmoid junction had been proposed to be the site of an anatomical sphincter; the *Sphincter of O'Beirne*(326). This was further substantiated by a thickened ring of muscle seen on some (but not all) cadaveric dissections(327), as well as a high pressure zone recorded at the rectosigmoid junction using low-resolution manometry(328, 329). However, other authors have refuted the existence of a sphincter at this location(330, 331).

The luminal wall of the rectum forms several transverse folds named the spiral rectal valves or *Valves of Houston*(332, 333). The valves can vary in number, position, and histological morphology(334). Most commonly, three valves are present, which individually do not encircle the full circumference of

the lumen, but are positioned in a spiral orientation relative to one another(333). Histologically, the valves can contain mucosa only, or mucosa as well as circular and longitudinal muscle(334). The spiral rectal valves are hypothesised to slow faecal descent during defaecation and to compartmentalise the rectum, which may assist in separating solid and gaseous content(334). However, no study to date has established their functional significance.

A rectocoele is a herniation of the anterior rectal wall into the posterior wall of the vagina. This may be a pathological finding associated with obstructed defaecation (**Figure 2.1**). However, rectocoeles are present in the majority of asymptomatic women and may, in some cases, represent a normal anatomical variant(335, 336). MRI defaecography demonstrated a rectocoele >2 cm in size in 62% of healthy women(337). Other studies using fluoroscopic defaecography demonstrated a rectocoele in 81% of women(338), or 93% of healthy nulliparous and parous women with a mean depth of 2.5cm(336). Nevertheless, a rectocoele of greater than 4cm in size can be considered to be truly pathological (i.e. does not occur in health), and occurs in >15% of patients with constipation(320). Vaginal delivery is associated with an increase in rectocoele size and prevalence(339).

A rectal intussusception is an invagination of the rectal wall which progresses distally during defaecation. Intussuscepta may also cause symptoms of obstructed defaecation, and can be classified according to the Oxford system from grade I (minor recto-rectal) to grade V (external prolapse)(340). Fluoroscopic defaecography studies have demonstrated an infolding of >3mm in 50% of healthy, asymptomatic volunteers(338) and a full-thickness intra-rectal intussusception in up to 20% of healthy volunteers(336). However, intussuscepta descending towards the anal canal are very uncommon in health(336, 338), with the prevalence of rectoanal (i.e. Oxford grade III and IV) intussusception and external rectal prolapse (i.e. Oxford grade V) on barium defaecography in patients with chronic constipation being 23.7% and 5.3% respectively(320).

2.4.3 Anal Canal

The rectum meets the anal canal at the pelvic hiatus(341). The lumen of the anal canal is shaped like an hourglass(342), with the middle third being the least distensible region(343). The length of the anal canal can be described in terms of the anatomical length(344) (dentate line to anal verge), surgical length(345) (anorectal junction to anal verge), or functional length(346) (a high pressure zone which exceeds the resting intraluminal rectal pressure). The anal canal is generally longer in males than females(317, 346, 347).

At rest, the anal canal is angulated at approximately 65-108°(336, 348) to the superior-inferior axis of the rectum, forming the anorectal angle(190). The puborectalis muscle forms a U-shaped sling around

the anorectal junction, further supporting the anorectal angle via resting tonic activity (postural reflex). This was hypothesised by Sir Allan Parks to be a physiological valve(349), though this was later questioned in subsequent studies(350, 351).

The anal canal contains vascular columns(352), *Columns of Morgagni*, and the haemorrhoidal plexus. The vascular columns are supported by the conjoint longitudinal muscle, or *Treitz's muscle*(353), which is a continuation of the longitudinal muscle of the rectum in the anal canal(354). Collectively, these contribute up to 10-20% of anal canal resting pressure(355).

The internal anal sphincter (IAS) is the continuation of the circular muscle layer of the rectum, forming a ring of smooth muscle which encases the anal canal circumferentially in a spiral orientation(356). The IAS is not under voluntary control, receiving autonomic innervation which mediates IAS relaxation via the release of nitric oxide from non-adrenergic, non-cholinergic neurons(357, 358). This differs to the conjoint longitudinal muscle which contracts in response to cholinergic stimulation(359). Due to the spiral orientation of the IAS, contraction causes shortening and narrowing of the anal canal and relaxation causes lengthening(356). Resting tone of the IAS may be neural or myogenic in origin(360). Resting IAS tone is responsible for the majority (70-85%) of anal canal resting pressure (190, 355, 361, 362). The IAS also exhibits phasic contractile activity (termed 'slow-waves' and 'ultra-slow waves') occurring at dominant frequencies of 16-18cpm and 1-3cpm respectively(363-366). Accordingly, the IAS should be considered as a phasically active muscle that generates tone, rather than the conventional description of a tonic muscle(366).

In contrast to the IAS, the external anal sphincter (EAS) is comprised of skeletal muscle under spinal and cortical control(367). The EAS makes a small contribution to anal canal resting pressure, but is largely responsible for generating maximal squeeze pressure and the voluntary control of continence(361). The EAS is further supported by the action of the transverse perineal and bulbospongiosus musculature, to create a "purse string" closure at the perineal body(368).

The anorectum receives both autonomic and somatic innervation. Preganglionic autonomic cell bodies are located in the intermediolateral column of the lumbosacral spinal cord, with cell bodies of sympathetic neurons originating from L_1-L_3, and parasympathetic neurons from S_2-S_4(17). Preganglionic sympathetic fibres synapse in the inferior mesenteric or hypogastric plexuses. Preganglionic parasympathetic neurons synapse either in the hypogastric plexus with the sympathetic neurons, or directly in the intramural myenteric plexus. Postganglionic autonomic fibres (both sympathetic and parasympathetic) innervate the anorectum via the hypogastric, sacral, and pelvic splanchnic nerves(293, 369). It has recently been shown that innervation to the IAS is also supplied by

nerve fibres coursing in the intersphincteric space; these are derived from the myenteric plexus of the distal rectum and via inferior rectal branches of the pelvic plexus(370-372).

The EAS and pelvic floor musculature receive dual somatic motor innervation from branches of the sacral nerves directly, as well as pudendal nerve branches (inferior haemorrhoidal/rectal and perineal nerves; S_{2-4})(373, 374). Pudendal nerve innervation of the EAS is bilateral, with unilateral pudendal nerve stimulation eliciting circumferential EAS contraction(375).

2.4.4 Pelvic Floor Musculature and Supporting Ligaments

The pelvic floor includes the striated muscles of levator ani (pubococcygeus, iliococcygeus, and puborectalis) and coccygeus(293, 344, 373), as well as the endopelvic fascia and ligamentous attachments, which support the pelvic viscera and provide attachments to the pelvic wall. The principal ligamentous support is afforded by the pubourethral ligament, uterosacral ligament and cardinal ligament(376, 377). The urogenital hiatus and rectal hiatus allow passage of pelvic viscera to the perineum, including the urethra and vagina anteriorly and rectum posteriorly. The neuromuscular integrity of the IAS, EAS, and pelvic floor are critical to continence(293), and are most vulnerable to structural traction injury during vaginal delivery or iatrogenic damage secondary to perianal/anorectal surgery(370, 378-380). Connective tissue disorders such as Ehlers-Danlos syndrome hypermobility type can also lead to laxity of pelvic floor ligaments resulting in descending perineum syndrome(381).

2.4.5 Knowledge Gaps

- Interactions and integration of myogenic, neural, and hormonal mechanisms in the control of defaecation and continence (addressed in **Chapter 8**).
- Neural pathways and reflex mechanisms that exist between the colon, rectum, anal sphincter, and pelvic floor musculature that control defaecation and continence.
- Functional role of the spiral rectal valves.
- Functional relationships between colonic length/redundancy and transit in humans.

2.5 Overview of Physiology: The Four Phases of Defaecation

The process of defaecation is best appreciated through the description of four phases (**Figure 2.2**), which are temporally and physiologically reasonably discrete(315):

1. Basal phase

2. Pre-expulsive phase
3. Expulsive phase
4. End phase

2.5.1 Basal Phase

2.5.1.1 Physiology

The basal phase describes the non-defaecatory state, during which the gastrointestinal system performs several homeostatic functions including(27):

1. Mixing of luminal content.
2. Propulsion of content distally for eventual expulsion.
3. Bacterial fermentation of carbohydrates.
4. Transmural exchange of fluid, electrolytes, and short-chain fatty acids.
5. Formation of solid stool.
6. Storage of contents prior to defaecation.

Coordinated motor patterns are essential to these functions(11, 27). The control of colonic smooth muscle involves the integrated actions of neural, myogenic, and hormonal mechanisms(11, 28). However, much of the action, interactions, and integration of these systems remains poorly understood(29). Continence, simplistically, is maintained by an intraluminal rectoanal pressure gradient, with tonic contraction of the anal sphincter resulting in an anal canal resting pressure which exceeds rectal pressure(346). The rectum is generally empty during the basal phase and only begins to fill during the pre-expulsive phase(293, 382).

2.5.1.2 Colonic Motility

The colon receives approximately 1500 mL of liquid enteric content (chyme) per day via the ileocaecal junction(23, 24). Mean colonic transit time is ~24 h, ranging between ~4 and 50 hours; this represents 70-80% of whole-gut transit time(98, 138). Digesta enters the caecum and moves aborally (outside episodes of mass movement or defaecation), with a net antegrade flow rate of approximately 1cm/hour(383), and is characterised by a series of “to-and-fro” motions. As demonstrated by studies tracking an ingestible magnetic capsule, regional transit time in the colon is not evenly distributed(139, 141) (**Figure 2.3**). When the location of the capsule is tracked in real-time, it can spend many hours in one region and move through an adjacent region in seconds to minutes(141). The motor patterns responsible for these movements may include low-amplitude propagating contractions, high-amplitude propagating contractions, the cyclic motor pattern, and colonic pressurisations.

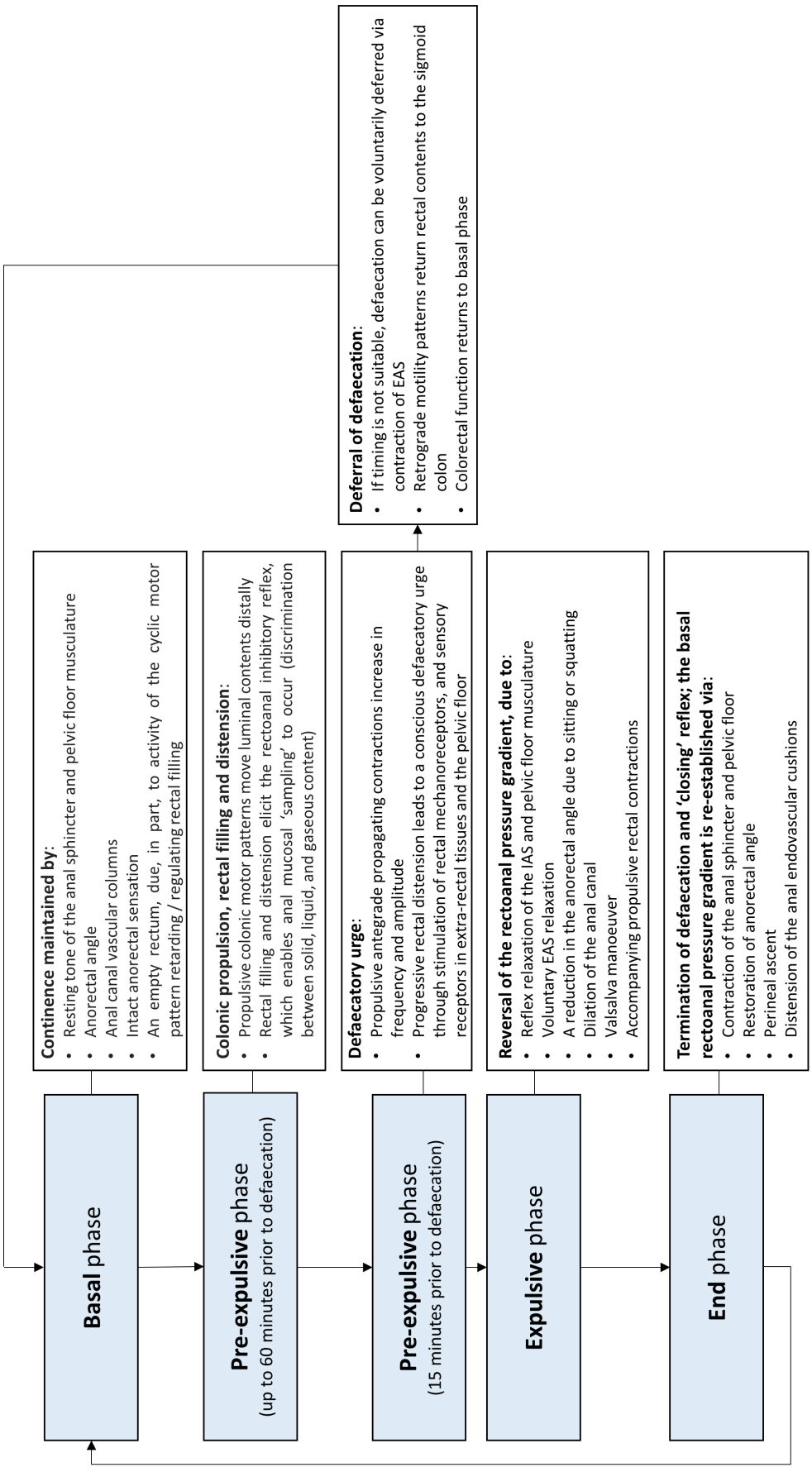

Figure 2.2 Flowchart depicting principal events occurring prior to and during defaecation.

Low- and high-amplitude propagating contractions, as recorded by intraluminal manometry, have both been temporally associated with luminal transit(136, 137) and are likely to be the motor patterns associated with the "fast antegrade" and "long fast antegrade" movements described in studies using the magnet tracking system(98, 141) (see **1.6 Colonic Motor Patterns**). Low-amplitude propagating contractions can also travel in a retrograde direction(2, 11). These have been associated with retrograde luminal transit and may aid in retarding flow of content through the colon, allowing for absorption and fermentation to occur(137).

Synchronous pressure increases across long colonic segments, or pancolonic pressurisations (**1.6.5 Pancolonic Pressurisations**), increase in prevalence during a meal and decrease immediately afterward(166, 167). These phenomena are associated with transient anal sphincter relaxations(166), which enable anal 'sampling' of intraluminal content (see **2.5.2.3 Anorectal Sensorimotor Activity**) and also with the flatal urge and expulsion of flatus(166, 167), which may partly explain the pooling of gas in the distal colon after a meal(384). An increase in synchronous colonic pressure waves in the distal colon had previously been shown with the administration of the acetylcholinesterase inhibitor neostigmine(385); findings confirmed by Corsetti et al. during high-resolution manometry recordings(166). Similarly, earlier studies using colonic barostat recordings demonstrated increased colonic wall tone in response to meals and neostigmine(386, 387). It has been hypothesised that colonic pressurisations are initiated by colonic distension(388) although their specific role in the expulsive phase of defaecation, if any, remains unknown.

Through conventional low-resolution colonic manometry studies, non-propagating activity was commonly reported to be the most prevalent motor pattern(111, 112). Such activity was proposed to facilitate mixing and transmural exchange of water, electrolytes, and short-chain fatty acids. A number of low-resolution manometry studies also identified non-propagating rhythmic activity in the rectum and labelled them 'rectal motor complexes'(171), 'periodic rectal motor activity'(116) or 'intermittent rectal motor activity'(175). However, the same motor pattern can be found throughout the entire colon(112) (see **1.6.3 The Cyclic Motor Pattern**).

With the introduction of high-resolution manometry, the majority of what was previously considered "non-propagating" has been shown to consist of rhythmic, propagating contractions, the majority of which occur over short distances at a frequency of 2-8/min(2) (see **Figure 1.1** and **1.5.2 Colonic Manometry**). This activity has subsequently been labelled the *cyclic motor pattern* and can be stimulated by a meal(2) (see **2.6.6 Colonic Motor Response to a Meal**), and high-amplitude propagating contractions(116, 168, 174). The cyclic motor pattern is active during sleep(116, 171), general anaesthesia(172), and can be activated by sacral nerve stimulation(173). As the cyclic motor pattern occurs at the same frequency as colonic "slow waves" (38, 389, 390), it is likely to be generated

by the interstitial cells of Cajal(38). In addition, given the increase in the cyclic motor pattern in response to physiological, pharmacological, and electrical stimulation, it can be modulated by neural pathways(11, 75, 388).

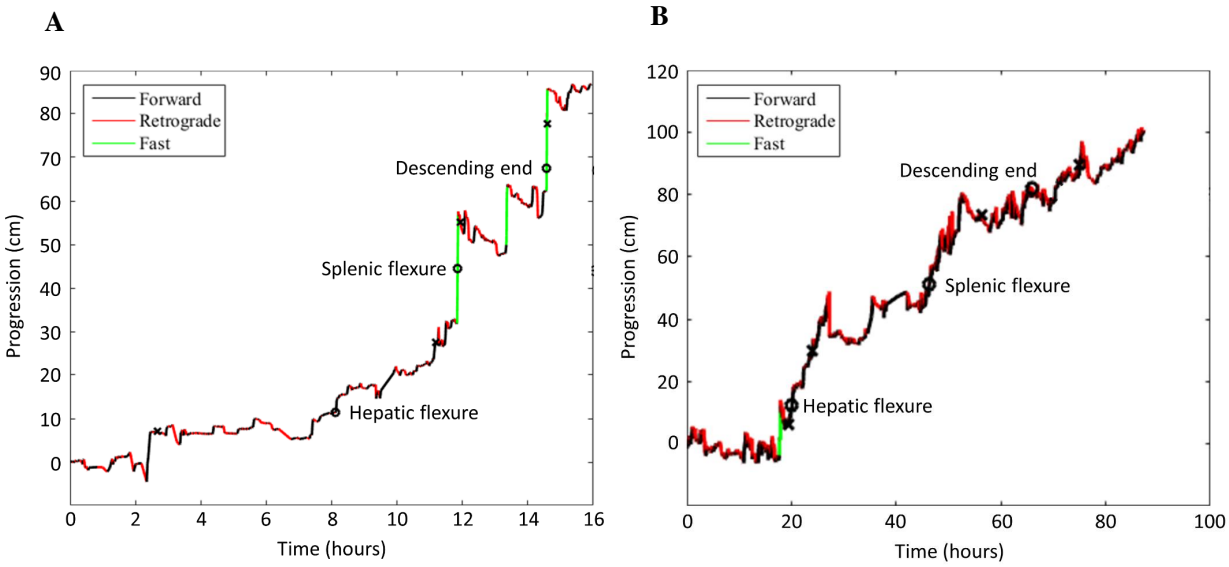

Figure 2.3 Time-progression patterns of the 3D-Transit capsule through the colon of: (A) a healthy volunteer, and (B) a patient with chronic constipation. Anatomical position in the colon is represented by the distance in cm from caecum to the rectum (y-axis). Time in hours is on the x-axis. In the healthy subject, the capsule traverses the entire colon in ~16 hours. There is relatively slow progression through the right colon (caecum to mid-transverse colon in ~12 hours), but rapid progression through the left colon, including three fast movements spanning 10-20 cm each. Little retrograde movement is seen. In the patient with constipation, colonic transit is ~90 hours in total. There is stasis in the both the caecum for ~18 hours, and also the left transverse colon for a further ~20 hours. Throughout, there is considerable retrograde movement of the capsule (images modified from those supplied by Mr Esben Mark, Mech-Sense, Department of Gastroenterology and Hepatology, Aalborg University Hospital, Denmark).

Functionally, the cyclic motor pattern in the distal colon is hypothesised to inhibit transit, thereby acting as an intrinsic colonic "gatekeeper"(116) or "rectosigmoid brake"(116, 176, 316). Several findings support this hypothesis. For example, the propulsive, high-amplitude propagating contractions, which are associated with defaecation, increase in number after a meal (see **2.6.6 Colonic Motor Response to a Meal** & **2.5.2 Pre-Expulsive Phase** & **Figure 2.4**). Despite this, defaecation does not occur after every meal. Concurrently, a meal also results in a significant increase in the cyclic motor pattern, which mostly propagates in a retrograde direction. Therefore, the presence of the cyclic motor pattern may have a functional role in preventing rectal filling. When an urge to defaecate is perceived, defaecation can be voluntarily deferred, after which time the sensation will usually abate. Radiological evidence suggests that, under such circumstances, retrograde motility patterns can return the contents of the

rectum into the sigmoid colon (391). This observation is in keeping with findings of an impaired or absent "rectosigmoid brake" function in patients with conditions characterised by diarrhoea(177-180) and may explain why sacral nerve stimulation can reduce the severity of faecal incontinence by inducing distal colonic motility(173) (181, 392, 393).

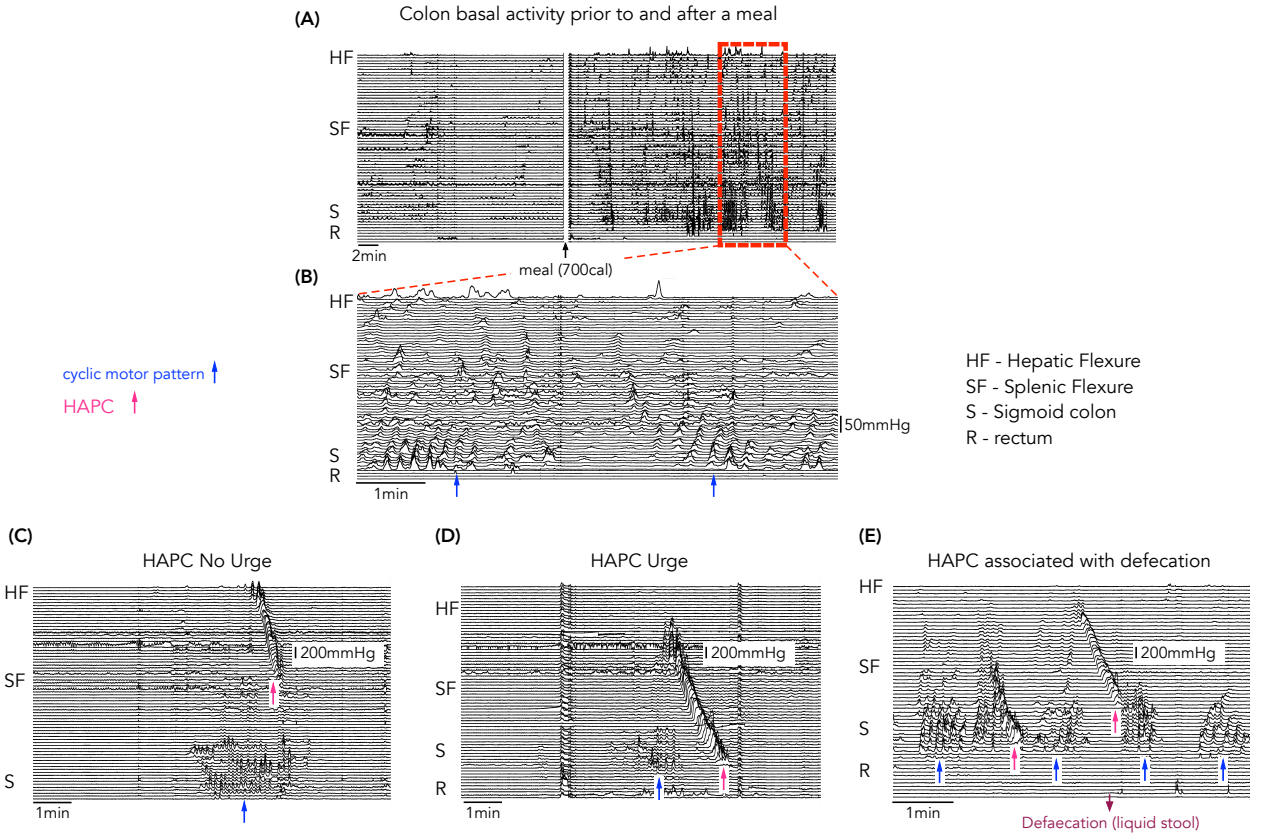

Figure 2.4 High resolution colonic manometry recording capture in 4 healthy volunteers. (A) demonstrates the colonic response to a meal. A section of the trace within the red hatched rectangle is expanded and shown in (B). The cyclic motor pattern can be seen (blue arrow). In (C), the cyclic motor pattern can be seen directly below the high-amplitude propagating contraction (HAPC; magenta arrow). No sensation was reported by the volunteer. In contrast in (D), the HAPC extends into the sigmoid colon, the cyclic motor pattern is inhibited, and the subject reports an urge. In (E), the subject reported the passage of a small amount of liquid stool (manometry performed in a prepared colon with solid faecal content removed). Note that defaecation was associated with an HAPC and inhibition of the cyclic motor pattern.

2.5.2 Pre-Expulsive Phase

2.5.2.1 Defaecatory Urge

The defaecatory urge is a cortical response to rectal distension and anal mucosal sampling(394). This is elicited by propulsive colonic motor patterns, rectal filling and distension, and the rectoanal inhibitory reflex.

2.5.2.2 Colonic Motor Activity

Low-resolution colonic manometry in the unprepared colon (with faecal content present) of healthy adults has demonstrated that both propagating and non-propagating activity begins to increase up to one hour prior to defaecation(224). Importantly, these changes are not associated with any conscious awareness or urge. A series of antegrade propagating contractions sequentially originate at a more distal location(224). These coordinated motor patterns are likely to move intraluminal content towards the rectum in readiness to be evacuated. A recent study using the magnet tracking system demonstrated distal transit of the capsule from the descending colon to the sigmoid colon 30-60 minutes prior to defaecation(98).

The functional significance of increased non-propagating activity prior to defaecation is unknown. As described in the previous section, this activity may assist in slowing antegrade movement. To date, high-resolution colonic manometry studies have only been performed in the prepared colon over short time periods (4-6 hours) and, therefore, episodes of spontaneous defaecation are rarely captured(395) (**Figure 2.4**).

2.5.2.3 Anorectal Sensorimotor Activity

The compliance of the rectal wall allows passive distension, but also adaptive reductions in rectal tone in response to distension, permitting storage of increasing volumes of content with minimal alteration in intraluminal pressure(396, 397). Rectal distension is detected by mechanoreceptors or "rectal intraganglionic laminar endings" (rIGLEs)(19), which transmit this information along $S_{2\text{-}4}$ parasympathetic neurons in the pelvic splanchnic nerves to the spinal cord. There may additionally be mucosal thermoreceptors and chemoreceptors with afferent signalling via spinal nerves, however this has not been established(398). Sensory receptors are also present in the extra-rectal tissues and pelvic floor, as the defaecatory urge can still be perceived in patients following rectal excision with colo- or ileo-anal anastomoses(382). Some authors have suggested that rectal contractions are required to generate a conscious defaecatory urge(399, 400). During balloon inflation in the rectum, the sensation of rectal distension is not consciously perceived until rectal contractions occur(401, 402).

Distension of the rectum beyond a threshold initiates the rectoanal inhibitory reflex (RAIR)(403), causing reflex relaxation of the IAS and contraction of the EAS. The RAIR is an intramural reflex mediated by the myenteric plexus and is characteristically absent in Hirschsprung disease, in which the affected segment of rectum and/or colon lacks myenteric ganglia(404). Intramural mediation is further evidenced by preservation of the RAIR in patients following spinal cord injury(405), or following surgical mobilisation and extrinsic denervation of the rectum(406). In patients with an enlarged or hypercompliant rectum (e.g. megarectum) allied to rectal hyposensitivity(407), the RAIR is still

present; however significantly higher rectal volumes are required to deform the rectal wall and hence elicit the reflex(408, 409).

Transient IAS relaxations occur approximately seven times per hour, and a proportion of these (~40%) may be consciously perceived(410). The upper third of anal canal is the region of greatest compliance(343) and, during these transient relaxations, intraluminal pressures within the proximal anal canal equalise with rectal pressures(411). This allows for luminal content to be "sampled" by the mucosa of the anal canal(367, 394, 410), where specialised sensory receptors are present including; Meissner's corpuscles (touch), Golgi-Mazzoni bodies and Pacinian corpuscles (pressure), Krause end-bulbs (thermal), and genital corpuscles (friction)(190, 412, 413). Sampling of content allows for sensory discrimination between solid, liquid, and/or gas(293, 414, 415).

The sensory information gathered from anal canal sampling is relayed to the lumbosacral defaecation centre in the spinal cord via parasympathetic neurons within the pelvic splanchnic nerves (S_2-S_4)(21). These afferent neurons include both myelinated Aδ fibres and unmyelinated C fibres(416). A spinal cord reflex arc may mediate contraction of the EAS(367), whilst sensory information is additionally relayed to the brainstem and cerebral cortex via the spinothalamic tracts(417).

2.5.2.4 Central Nervous System

In animal studies, there have been two defaecation centres described in the central nervous system; the lumbosacral centre in the spinal cord and supraspinal centre in the brainstem(418, 419). In humans, conscious perception of rectal distension involves multiple cortical areas including the prefrontal cortex, anterior cingulate gyrus, insula, thalamus, and somatosensory cortex(420, 421). The awareness of rectal filling is graded by the extent of distension, from a mild awareness initially to maximum tolerance(402). Cortical input is critical to both voluntary inhibition or initiation of defaecation. This is evidenced by patients with spinal cord injury who lack cortical input and require stimulation via manual digitation to initiate defaecation(422). Brainstem motor control of colonic, rectal, and IAS smooth muscle is located in the pontine micturition centre; *Barrington's nucleus*(17). Motor efferent neurons have cell bodies in *Onuf's nucleus* in the ventral sacral spinal cord. These neurons return to the anal canal via the inferior rectal/haemorrhoidal branches of the pudendal nerves(423, 424), where they elicit inhibition and relaxation of the anal sphincter(423).

2.5.3 Expulsive Phase

2.5.3.1 Colonic Motility

In the 15-minute period preceding defaecation, antegrade propagating contractions in the colon increase in both frequency and amplitude(224). The site of origin of propagating contractions also migrates

during this period, with each subsequent propagating event commencing at a more proximal location(224). Unlike the pre-defaecatory phase, these propagating contractions are associated with the urge to defaecate. Studies using the magnet tracking system have shown that the pill can move from the ascending colon to the rectum during this period(141). Stool expulsion may be associated with high-amplitude propagating contractions which can span the entire length of the colon(100, 224, 425), emptying the colon from caecum to rectum(426, 427). However, stool expulsion may also occur in the absence of high-amplitude propagating contractions, which may require more voluntary effort via abdominal wall contraction.

Manometry studies have also shown synchronous pressurisations across the distal pressure channels at the termination of high-amplitude propagating contractions. This is hypothesised to function as a means to maintain colonic wall tone and facilitate transit as the lumen distal to a propagating contraction dilates to accommodate propulsion of content(152, 183). These events are additionally associated with rectal balloon expulsion(167).

The cyclic motor pattern is inhibited during stool expulsion, presumably to allow intraluminal content to enter the rectum and anal canal. While never specifically studied, the cyclic motor pattern appears to be inhibited during high-amplitude propagating contractions that either terminate in the rectum or are associated with defaecation (**Figure 2.4D & E**).

2.5.3.2 Rectoanal Pressure Gradient

In contrast to the basal and pre-expulsive phases, during which anal canal pressure exceeds rectal pressure, this pressure differential is, in theory, reversed during the expulsive phase. This pressure gradient exceeds the frictional resistance of the anal canal(90) and provides the necessary yield stress to deform solid faeces to enable transit through the anal canal(428). This is facilitated by both voluntary and involuntary processes.

A reduction in anal pressure is elicited via:

- Voluntary relaxation of the EAS.
- A reduction in the acuity of the anorectal angle from 65-108° to 110-155°(336, 337, 429). This can be further enhanced by squatting, hip flexion, and/or posterior pelvic tilt(430, 431), or via use of a 'defaecation postural modification device'(432) (see **2.6.3 Posture**).
- Reflex relaxation of the IAS and pelvic floor musculature. The extent of IAS relaxation is graded by the rectal stool volume, in that greater stool bulk will cause greater rectal distension and elicit more marked IAS relaxation and reduction in anal canal pressure(433).

- Dilation of the anal canal. This may be a combination of passive dilation to accommodate stool, as well as active dilation elicited by perineal descent and contraction of the conjoint longitudinal muscle(428, 434).
- Conjoint longitudinal muscle contraction flattens the anal endovascular cushions(435) and shortens and widens the anal canal(354, 436), an action which lead Shafik et al. to describe the conjoint longitudinal muscle as the "evertor ani"(437).

Simultaneously, an increase in rectal pressure is produced by:

- Performing a Valsalva manoeuvre: contraction of the diaphragm and abdominal wall musculature with a closed glottis to increase intra-abdominopelvic pressure.
- There may additionally be low-amplitude, propulsive rectal contractions(293, 438), however the contribution of rectal wall contractions to increasing intraluminal pressure during the expulsive phase is unclear(382).

Disturbance of the normal reversal of the rectoanal pressure gradient during attempted evacuation is described as "dyssynergic defaecation", a functional disorder of defaecation characterised by failure of relaxation or paradoxical contraction of the anal canal, and/or a failure to increase intrarectal pressure(439). Dyssynergic defaecation is generally described through anorectal manometric investigation of patients who report difficult evacuation. However, in several studies, a majority of healthy volunteers also demonstrate a negative rectoanal pressure gradient (i.e. anal pressure *exceeding* rectal pressure) during simulated defaecation(440-442). Thus, while a reversal of the rectoanal pressure gradient changes may explain stool expulsion conceptually, the investigative tools by which to optimally investigate this process have not yet been determined.

2.5.3.3 Anal Canal Dilation

It has been suggested that reflexive anal canal relaxation alone would be insufficient to permit evacuation of faeces; instead, Petros and Swash proposed an active anorectal "opening mechanism" during defaecation(434). Using defaecography (MRI and video myogram), they demonstrated an increase in anorectal luminal diameter during defaecation. The ratio of rectal to anal luminal diameter decreased from approximately 4:1 at rest to 2:1 during defaecation(90). They proposed that this action was elicited by simultaneous muscle action in three directional vectors (anterior, posterior, inferior)(434), this being achieved by:

- Straightening of the anorectal angle via relaxation of the puborectalis, and contraction of the levator plate (posterior vector) and conjoint longitudinal muscle of the anus (inferior vector)(434).

- Actively increasing the luminal diameter of the anal canal via contraction of pubococcygeus to shift the perineal body (anterior vector), and contraction of the postanal plate to splint the posterior wall of the anal canal (posterior vector)(434).

2.5.3.4 Evacuation

Dynamic assessment of defaecation has been performed using the balloon expulsion test(443), simulated "push" manoeuvres on anorectal manometry(444), fluoroscopic or MRI defaecography(320), artificial stool(445)(**Figure 2.1**), and – most recently – biomechtronics devices such as Fecobionics(446, 447). Preliminary findings from the Fecobionics device have demonstrated that some healthy volunteers expelled the device using a single, sustained pressure effort, whereas others used several abdominopelvic pressure efforts(447). Similar patterns of expulsion have been demonstrated in healthy adults using defaecography(336). When expelling a slurry of barium sulphate, oats, and water, the three patterns observed were(336);

- Type 1: a single, rapid expulsive motion.
- Type 2: frequent, pulsatile expulsion of small volumes.
- Type 3: slow, sustained, steady expulsion.

Using MRI to quantify pre- and post-defaecation colonic volumes in healthy volunteers, the total colonic volume decreased from a mean 892 mL pre-defaecation to 726 mL post-defaecation, with statistically significant volume reductions observed in rectosigmoid volume and total colonic volume (volume reduction of 44% and 19% respectively) during defaecation(448). In a similar MRI study of colonic volume, the fractional clearance of non-gaseous colonic content following a bowel motion was 35-38%(449), with significant volume reductions observed in all colonic segments (separated into right, transverse, left, and pelvic colon)(449). Colonic gas volume also reduced following defaecation, but only in the pelvic colon(449).

2.5.4 End Phase

This phase represents termination of defaecation and the "closing reflex". Following evacuation, a series of changes occur to re-establish the basal rectoanal pressure gradient and restore continence. The "closing reflex" is theorised to be initiated by cessation of traction on the anal sphincter(396). This elicits;

- Contraction of the anal sphincter and pelvic floor.
- Relaxation of the conjoint longitudinal muscle of the anal canal to allow distension of the anal endovascular cushions.
- Contraction of puborectalis to restore the anorectal angle.

- Perineal ascent.

Colonic motility patterns in the immediate post-defaecation period have not been described. In the stomach and small intestine, prolonged quiescent periods occur after "events" such as phase III of the migrating motor complex(450, 451). However, based upon years of colonic manometry studies performed by the authors (data unpublished), there is no clear period of quiescence following defaecation before motility returns to basal activity. This is supported by the consistency of the colonic meal response which; (1) occurs irrespective of its temporal proximity to defaecation, and; (2) occurs in the prepared colon, demonstrating that it is independent of colonic intraluminal volumes and distension.

2.5.5 Knowledge Gaps

- A detailed characterisation of human anal sensory receptors and afferent pathways.
- Understanding the relationship between rectal contraction and rectal sensation, and the contribution of rectal contractions to faecal expulsion.
- Modelling of the colo-recto-anal force vectors generated during evacuation.
- Functional significance of pancolonic pressurisations in luminal transit and defaecation (addressed in **Chapter 7**).
- An in vivo demonstration of the "rectosigmoid brake" and how this relates to rectal filling and continence (addressed in **Chapter 7**).
- High-resolution colonic and anorectal manometry recordings characterising pre-expulsive and expulsive phases, specifically detailing the relationships between colonic, rectal, and anal contractile activity prior to and during defaecation.
- Characterisation of colonic motility immediately following defaecation in returning to basal activity.
- The relative contributions of voluntary and involuntary components to defaecation, and whether these simply reflect behavioral differences (e.g. responding immediately to the call to stool, or following deferral of defaecation).

2.6 Factors Influencing Defaecation

2.6.1 Frequency of Normal Defaecation

Frequency of defaecation varies widely in healthy adults but is most commonly reported as between three bowel motions per day and three bowel motions per week(452-460). The largest study assessing stool frequency in healthy adults included 4,775 participants in the USA, of whom 95% self-reported a

stool frequency within this range(454). These findings are consistent with those from smaller samples in China (n=1,952)(458), UK (n=1,897(459) and n=1,055(452)), USA (n=1,128(460) and n=789(461)), Iran (n=1,045)(457), Singapore (n=271)(462), Italy (n=140)(453), and Sweden (n=124)(455).

Compared with adults, the normal frequency of defaecation is substantially higher in infants, particularly during the first month of life. In 240 healthy infants studied in the UK, the mean stool frequency was four bowel motions/day at two weeks of age, decreasing to two bowel motions/day by 12 weeks of age(463). A higher stool frequency was observed in infants who were breast-fed when compared to those who were fed with a milk formula; however, this appeared to equalise by 12 weeks of age(463). Mean stool frequency gradually decreases during the first few years of life (four weeks of age = three times/daily; six months of age = two times/daily; 18 months of age = 1.8 times/daily, 30 months of age = 1.5 times/daily, 42 months of age = 1.3 times/daily)(464).

2.6.2 Psychobehavioural Factors and Voluntary Suppression of Defaecation

The relationships between cortical activity and gastrointestinal function were described over a century ago when Pavlov demonstrated the classical conditioning of his dogs, whereby the ringing of a bell could induce salivation(465). In human studies in the 1950s, an increase in sigmoid motility was demonstrated during painful and emotionally-stressful stimuli(466). Similar findings describing acute stress responses on sigmoid motility were replicated in the 1980s by Welgan et al.(162, 163) and in the 1990s by Rao et al.(467)

Stress and psychosocial factors alter colonic motility(162, 163, 466, 467) and can contribute to the onset and severity of functional gastrointestinal diseases(468). Contributing factors may include acute and chronic stress, psychiatric diseases, personality disorders, and a history of abuse(469). These factors may lead to local alterations in autonomic function which impact gastrointestinal motility, vascular tone, and gastrointestinal secretions. Symptoms can be compounded by hypervigilance, somatisation, and maladaptive illness behaviours(469). Thus, bidirectional brain-gut interactions have a role in pathogenesis of functional gastrointestinal disorders and psychiatric disorders(470-473). In a prospective longitudinal study, Koloski et al.(470) demonstrated that anxiety reported on an initial survey was predictive of later developing a functional gastrointestinal disorder (n=1,002). Conversely, participants with a functional gastrointestinal disorder on the initial survey were found to have a higher incidence of anxiety and depression 12 years later(470).

Voluntary behaviours also influence defaecation. Toilet training, the process of establishing continence, usually begins between 21-36 months of age(474) and may take over seven months to complete(475). The voluntary suppression and deferral of defaecation, or stool withholding, is seen most commonly in

children and is implicated in the pathophysiology of constipation(476). Stool withholding is thought to be associated with painful or unpleasant defaecation and can result in faecal retention, constipation, and overflow incontinence(306, 477, 478). In adults, learned behaviours via operant conditioning are also the basis for biofeedback therapy in disorders of defaecation(479). Using visual and/or auditory feedback, patients are able to rehearse the activation and coordination of abdominopelvic musculature during defaecation(439, 480, 481).

2.6.3 Posture

Posture has a significant influence on the biomechanics of the anorectum(482). There are cultural differences in the postures assumed during defaecation, with a squatting position more common in African, Asian, and Middle Eastern cultures, and a seated position more common in Western cultures.

Squatting increases hip flexion and posterior pelvic tilt, facilitating straightening of the anorectal angle(431). When assessed with fluoroscopy, the mean anorectal angle in healthy volunteers during defaecation in squatting was 126°, compared with 100° in sitting(431). Squatting is associated with a reduction in the duration of defaecation, as well as an increased sense of complete evacuation(483).

From a seated position, leaning forward to adopt the "Thinker" (i.e. Penseur) position also increases hip flexion(430). This position, when compared with sitting upright, was shown on anorectal manometry to increase intrarectal pressure(484) and, on cinedefaecography, to result in greater puborectalis relaxation, straightening of the anorectal angle, increased perineal plane distance, and improved ease of evacuation of barium neostool(430). However, in another study assessing the anorectal angle with fluoroscopy, there was no significant difference in the anorectal angle when comparing a seated position to a forward-leaning seated position(431).

In a Western population, the use of a foot stool or 'defaecation postural modification device'(432) (e.g. "Squatty Potty", LLC, St. George, UT) to partially replicate the biomechanics of squatting may result in a reduction in straining and the duration of defaecation and an increase in the perceived completeness of evacuation(432). However, the findings from another study showed no differences when comparing defaecation with or without a foot stool in the anorectal angle, puborectalis length, perineal plane distance, duration of defaecation, or volume of stool(484).

2.6.4 Colonic Transit, Stool Volume and Consistency

Faeces is composed predominantly of water (median 75%; mean range 63-86% across studies)(485), in addition to a suspension of bacterial biomass, protein, carbohydrates, and lipids(485, 486). An analysis combining a distribution of means from 116 studies in healthy adults reported a median faecal wet mass of 128g/day (mean range 51-796g/day across studies)(485). Stool volume increases considerably in diarrhoeal illnesses, which is predominantly due to an increase in water volume(487). Enormous increases in stool water volume, in excess of 10L/day, can be seen in severe, secretory diarrhoeal illnesses such as cholera(488).

Stool consistency is determined by the proportion of solid matter to fluid and is commonly described using the Bristol stool form scale(489), which includes a range in consistency from stool type 1 ("separate hard lumps, like nuts") to type 7 ("watery, no solid pieces"). In healthy adults, normal stool consistency has considerable variation, from stool type 2 ("sausage-shaped but lumpy") to type 6 ("soft blobs with clear-cut edges")(454, 490).

Extremes of stool consistency, from hard stools to watery stools, are associated with slow and rapid colonic transit respectively(491). Lewis et al. initially designed the Bristol stool form scale in order to estimate transit time(489). Using a combination of radiopaque marker studies, stool diaries, and stool roentography, it was demonstrated that whole gut transit time was most strongly correlated with stool consistency, followed by stool volume, and stool frequency(489). Jarunvongvanich et al.(492) demonstrated similar findings in a Thai population sample, with the Bristol stool form score independently associated with both colonic transit time and stool frequency. Using simultaneous radiopaque markers and wireless motility capsules, Saad et al.(493) reported that Bristol stool form types 1 and 2 were predictive of delayed whole-gut transit in 46 patients with chronic constipation (sensitivity 85%; specificity 82%). However, in contrast to previous studies, there was no correlation between consistency, transit, or stool frequency in the healthy control group (n=64).

Stool consistency is considerably softer in infancy, with approximately 60-80% of healthy infants demonstrating soft or liquid stools(464, 494, 495). When compared with breast-fed infants, firmer stools can be observed in formula-fed infants (1.1% in breast-fed vs 9.2% in formula-fed)(494). Stool consistency appears to normalise from early childhood onwards, with no difference observed in children between the ages of 4-15 years(496). Similarly to adults, stool consistency is strongly correlated with whole gut transit time in children(496).

Recent work on the rheology of faeces demonstrated that stool consistency alters faecal yield stress, which describes the pressure required to deform the faeces to enable rectoanal transit(428). Bannister et al.(433) used balloons and beads of differing size, volume, and consistency to demonstrate that soft,

large, deformable balloons were more easily evacuated than harder, smaller beads, requiring a shorter time, lower rectal pressure, and with more complete evacuation.

Colonic transit and stool consistency are interrelated with colonic microbiota composition(497). Microbial composition is altered by diet and transit time which may, in turn, alter host physiology(498). Whilst causal associations between the microbiome and bowel dysfunction remain unclear(499), longer colonic transit times can be associated with altered carbohydrate fermentation, short-chain fatty acid production(500), and methanogen composition(497). However, independent of transit time, one study demonstrated that the colonic microbiota profile had a 94% accuracy for discriminating between healthy controls and patients with constipation(501). Further studies have demonstrated that the *Prevotella*-predominant enterotype is associated with softer stool consistency when compared with the *Ruminococcus-Bacteroides* predominant enterotype(497). Despite these findings, a recent systematic review demonstrated no differences in colonic microbiota when comparing patients with diarrhoea-predominant and constipation-predominant irritable bowel syndrome(502).

"Dysbiosis", or a disturbance in the colonic microbiota, has been associated with functional gastrointestinal disorders(503, 504). Therapeutic modulation of the colonic microbiome using pre-, pro-, syn-, and anti-biotics for the treatment of gastrointestinal diseases has been the subject of great interest(505-513)}, and has shown some benefit in treating inflammatory bowel disease(505, 506), irritable bowel syndrome(507-509), chronic constipation(507, 510, 511), acute infectious diarrhoea(512), and traveller's diarrhoea(513). However, the specific mechanisms relating colonic microbiota and colonic function are yet to be determined. As such, the ideal dietary composition of prebiotics, specific probiotic microorganisms, synbiotic combinations, antibiotic regimes, and use for specific disorders are still areas of ongoing research.

2.6.5 Circadian Rhythm

Colonic motility exhibits diurnal variation and, in humans, can be inhibited by sleep and increased following awakening(80, 111, 112, 153, 156). Propulsive high-amplitude propagating contractions (HAPC) can be associated with morning waking and also with the morning call to defaecate(224).

2.6.6 Colonic Motor Response to a Meal

Over 100 years ago, eating a meal was identified as a stimulus for "mass movements" of colonic content(514). This colonic response was labelled the gastrocolonic reflex(515). More recently, the colonic meal response was hypothesised to be a neurohormonal response to gastric distension, causing

the release of neuropeptides including cholecystokinin, serotonin, neurotensin, and gastrin(59). However, the colonic response to a meal can occur independent of gastric stimulation. This is demonstrated by the presence of the colonic meal response following the smell of food or verbal discussion of a meal(61), and preservation of the response post-gastrectomy(60). While still commonly used in current journals and textbooks, the terminology of a "gastrocolonic reflex" is therefore misleading.

The colonic meal response occurs rapidly. Within minutes of starting to eat a 1000Cal meal, Snape et al. demonstrated an increase in contractility of the sigmoid colon and rectum(157). The intensity of the colonic meal response is dependent upon the nutritional content of the meal. For example, a meal of 300Cal has a less marked impact on colonic motor function in comparison to a 1000Cal meal(157) and dietary fats cause a greater increase in colonic contraction than carbohydrates(516, 517). The colonic meal response must be mediated in part by the central nervous system, as this response is absent in patients with spinal injury(62) and can be inhibited by the muscarinic receptor antagonist clidinium bromide(518).

Low-resolution colonic manometry studies (70-150mm spacing between recording sensors) have shown that meals are temporally associated with an increase in "non-propagating" and low-amplitude propagating contractions throughout the colon(80, 111, 112, 519). In addition, HAPC (which may be associated with defaecation(111, 112, 131)) are seen more frequently in the postprandial period(80, 111, 112) (see **2.5.2 Pre-Expulsive Phase**).

With the introduction of high-resolution colonic manometry (10-30mm spacing between recording sensors), the timing and characteristics of the colonic meal response have been described in greater detail. Within 60sec of starting a 700Cal meal, a significant increase in cyclic motor activity, predominantly propagating in a retrograde direction, occurs in the rectosigmoid region(2, 316) (also see **2.5.1 Basal Phase**). Other high-resolution colonic manometry studies have shown that synchronous intraluminal pressure increases (termed "pan-colonic pressurisations")(166) also increase in frequency during a meal(166).

2.6.7 Influence of Dietary Intake

Dietary intake alters the composition of luminal content, colonic microbiota(520), and bowel function(521). Dietary fibre, found in cereals, fruits, vegetables, and legumes, are carbohydrates that are poorly absorbed in the upper gastrointestinal tract. Different fibre sources can be described by their water-solubility (water-soluble or water-insoluble), by their amenability to fermentation by the colonic

microbiota (degradable or non-degradable), or categorised by volume of intake as high- or low-residue (high- or low-fibre diets).

Degradable fibres are fermented in the colon and increase stool volume predominantly via additional bacterial biomass, which can account for over half of the total dry stool volume(486, 522). Degradable fibres include fermentable oligo-, di-, mono-saccharides and polyols (FODMAPs) and resistant starches, and are often synonymous with prebiotics, defined in a recent consensus as compounds within the diet that are selectively utilised by colonic microbiota to confer a health benefit(523). A recent systematic review and meta-analysis demonstrated that an increased dietary intake of resistant starch (22g-45g/day) decreased stool pH, increased stool volume and stool butyrate concentration, but had no effect on stool frequency(524). Using MRI, consumption of kiwifruit(525) increases retention of water in the small bowel and ascending colon and increases the volume of colonic contents, while a high-FODMAP diet(526) is associated with an increase in small bowel luminal water content and colonic gas volume. Implementing a low-FODMAP diet may provide symptom reduction in patients with irritable bowel syndrome(527-529) or faecal incontinence(530), although current evidence is of poor quality.

Fibre sources which are less amenable to colonic fermentation, such as cereal fibres (e.g. wheat fibre, psyllium husk), confer a greater increase in stool volume when compared with fermentable fibres(531, 532). Using MRI to assess the volume of colonic intraluminal content in participants consuming high-residue (35g fibre/day) or low-residue (8g fibre/day) diets, non-gaseous colonic content increased significantly following a high-residue diet(449).

Wheat has been the most extensively studied cereal fibre relating to bowel function(533-536). A systematic review and weighted regression analysis including 65 studies demonstrated that, for every 1g increase in wheat fibre, stool volume increased by 3.7g±0.09g/day(533). Wheat fibre consumption was associated with an increase in stool water content, stool frequency, and an apparent normalisation of delayed whole gut transit time, meaning that increased fibre intake expedited whole gut transit time in those who had a pre-intervention transit of >48 hours, but did not alter transit for those with a pre-intervention transit of 24-48 hours(531, 533). A vegetarian diet, which in most cases contains a high fibre content, is associated with a slight increase in stool frequency when compared with healthy adults on an omnivorous diet, however this difference is minimal (vegetarian = 11.8±4.5 bowel motions/week compared with omnivorous = 11.3±4.7 bowel motions/week(456)).

2.6.8 Age, Gender, Parity, and Body Mass Index

Defaecation is influenced by age, gender, and body mass index (BMI). Most published studies show that ageing is associated with a higher prevalence of constipation(537-541), though a recent population-based survey of nearly 6,000 adults in the USA, Canada and the UK reported a significantly higher prevalence of constipation in the youngest age group studied (in those aged 18-29 years compared to age >70 years(542). This is in contrast to earlier North American epidemiological studies, demonstrating a higher prevalence of constipation in those >65 years of age(540, 541). Both scintigraphic(543, 544) and wireless ingestible electromagnetic pill transit studies(98) demonstrate longer colonic and whole gut transit time with increasing age. An age-related decrease in cholinergic function in the ascending colon has also been demonstrated(545). However, the causation of altered bowel function in older age is difficult to pinpoint, as a multitude of other changes occur with ageing which may contribute, including diet, medications, and decreased physical activity(545, 546).

In childhood, the prevalence of constipation is equally distributed by gender but, in adulthood, constipation is reported more commonly in women(539, 547). Prolonged colonic transit times have been demonstrated in radio-opaque marker studies(548-551), scintigraphic studies(544, 552), and wireless pill studies(98, 138) in women. Women also report less frequent bowel motions(462) and have greater variability in stool consistency (men: Bristol type 3-5, women: Bristol type 2-6)(454), with softer stool consistency during the perimenstrual period(553) and firmer stools during the postpartum period(553). Inter-gender variability in bowel habit has been hypothesised to be due to cyclical fluctuations in sex hormones(554). Exogenous progesterone administered to postmenopausal women has been shown to accelerate colonic transit and result in softer stool consistency(555). However, other studies have demonstrated slower transit during the luteal phase of the menstrual cycle, during which serum progesterone levels reach their peak(491, 556, 557), or no variation in stool consistency or colonic transit during the luteal phase and follicular phase(491) of the menstrual cycle.

In terms of anorectal function, men have a greater functional anal canal length(317, 346, 347) and higher rectal sensory thresholds to mechanical distension compared to women(221, 558). Women are at risk of pelvic floor and anal sphincter injury during pregnancy and childbirth. Anal sphincter injury is reported in approximately one third of primiparous women(212, 213, 559), yet less than one third of women with sphincter injuries report faecal incontinence postpartum(214). In some cases, the onset of symptoms can be delayed, often decades after pregnancy(196, 560, 561).

Parity may be associated with a reduction in anal canal squeeze pressure(317). In women with faecal incontinence or constipation, each successive child is associated with a mean reduction in anal canal resting tone of 4.3 cmH_2O and prolongation of pudendal nerve terminal motor latencies(562). However, there is only a weak correlation between anal sphincter resting and squeeze pressures and faecal incontinence symptom severity(563, 564) (**Chapters 4 & 5**). In the absence of direct anal sphincter

injury, a recent Swedish study demonstrated that both Caesarean section and vaginal delivery were associated with a risk of developing faecal incontinence, suggesting that other pregnancy-related factors are also involved in the pathogenesis of faecal incontinence(561). Vaginal delivery is also a risk factor for developing descending perineum syndrome, which can be associated with evacuation disorders and chronic constipation(381).

The effects of BMI on colonic and anorectal function have been assessed using scintigraphy(565), wireless electromagnetic ingestible capsules(98), as well as anorectal investigations(347). In a study including 72 participants, there was a trend towards more rapid colonic transit on scintigraphy in patients with BMI >30kg/m^2, however this difference was not significant once adjusted for gender(565). When assessed with wireless capsules, a higher BMI was significantly related to shorter whole gut transit time(98). Symptomatically, obesity has been associated with a higher prevalence of patient-reported chronic diarrhoea,(566-572) even when adjusted for demographics, diet, and co-morbidities(573). In a cohort of over 35,000 people in France, the association between chronic diarrhoea and obesity was observed in women only(574). The relationship between obesity and diarrhoea is hypothesised to be the result of a multitude of factors, which may include rapid gastric emptying, a greater luminal osmotic load, higher luminal bile acid concentration, faster colonic transit(98, 565), altered colonic sensorimotor function(565), chronic intestinal inflammation, altered permeability, and/or medication side effects(567).

In a Swedish study including 1,001 people, obesity was additionally associated with stool urgency and the sensation of incomplete rectal evacuation(572). Obesity is also an independent risk factor for faecal incontinence(201). This may be due to the additional weight of visceral adiposity causing chronic stress to the pelvic floor, similar to the pregnancy-related effects on pelvic floor integrity. Among healthy women, an increase in BMI is correlated with a longer balloon expulsion time (reflecting impaired evacuatory efficiency) and a higher threshold volume during rectal sensory testing(347). The mechanisms of symptom causation remain unclear in obese patients and remains an area for ongoing research.

2.6.9 Other Influences

A multitude of other factors influence the physiology of defaecation, some of which include co-morbidities, medications, and physical activity (see **Table 2.1**). It is beyond the scope of this review to describe each in detail.

Many common medications alter bowel function (**Table 2.1**). Opioids, for example, are the most frequently prescribed drug class in the USA(575) and are associated with constipation in >40% of patients with chronic non-cancer pain(289, 576), which is frequently reported as severe and the most bothersome side effect(289) (**1.8.3 Opioid-Induced Constipation** & **Chapter 9**). To reflect this, an additional category was included in the Rome IV criteria for functional bowel disorders; opioid-induced constipation(231). Up to 45% of patients taking regular opioids report bowel motions less than three times weekly(289). It is well acknowledged that opioids delay gut transit, but opioid use is also associated with rectal hyposensitivity and functional evacuation disorders in patients with constipation(577).

A direct relationship between exercise and bowel function is not clear(456). Physical activity is commonly considered to be important for normal bowel function and acute periods of inactivity are generally regarded to result in constipation. Iovino et al.(578) demonstrated decreased stool frequency and new onset of constipation in six of ten healthy volunteers after a strict 35-day period of bed rest(578). Conversely, "runner's diarrhoea" can be induced by high intensity running training (1-2 hours/day), resulting in higher stool frequency, softer stool consistency, and more rapid small bowel and distal colonic transit(579).

2.6.10 Knowledge Gaps

- Pathophysiological mechanisms underlying brain-gut/gut-brain associated disorders.
- Physiology describing interactions between diet, colonic microbiota, and colonic function.
- Physiological mechanisms to explain the inter-gender variation in colonic transit time.
- Explanation for gender differences in constipation prevalence at different ages.
- Pathophysiological mechanisms to account for why slow transit constipation almost exclusively effects women.
- How postural changes alter the biomechanics of the anorectum and the resultant efficiency of evacuation.

2.7 Pathophysiology of Common Disorders of Defaecation

Parameters that constitute a disorder of defaecation are ill-defined. They include the obvious, where defaecation is difficult to initiate or complete (constipation) or control (faecal incontinence), but also include related syndromes such as functional diarrhoea and irritable bowel syndrome (IBS). Some sense of where diagnoses start and end are provided by the Rome IV criteria using specific combinations of symptoms to define syndromes(185, 231). Such syndromes overlap considerably (a point that is

unsurprising when one considers the limited symptom repertoire of the bowel and the large number of currently defined syndromes). For example, the Rome IV criteria allows categorisation of disorders into four subtypes; (a) functional constipation (FC); (b) irritable bowel syndrome with constipation (IBS-C); (c) opioid-induced constipation (OIC), and; (d) functional defaecation disorders (FDD). However, there is considerable overlap between these groups and the accumulating clinical and mechanistic evidence suggests that these subtypes of chronic constipation actually exist on a spectrum rather than being distinct entities(580-582). It is further acknowledged that it is sometimes difficult to distinguish one from another, and that transition from one functional bowel disorder or from one predominant symptom to another is common. Specifically, considerable overlap between IBS-C and FC exists(581-584), and transition from FC to IBS-C, and vice versa, is common(582, 585). Likewise, as noted previously, constipation and faecal incontinence are not distinct conditions, with >40% of patients having significant concurrent symptoms (108, 306-308, 586). Regrettably, pathophysiological findings do not neatly equate with syndromes**.** Rather, it is possible to categorise common abnormalities affecting each of the four phases of defaecation and thence note where these have been documented for various clinical syndromes (**Table 2.2).**

2.8 Summary

Our understanding of the physiology of defaecation and continence (and also the pathophysiology of conditions such as constipation and incontinence) has progressed considerably, although fundamental uncertainties still remain, particularly regarding the actions, interactions, and integration of the myogenic, neural, and hormonal mechanisms involved in colonic and anorectal function. It is through resolution of these uncertainties that more effective assessment and individualised treatment of disorders of defaecation will hopefully be achieved. Our ability to effectively assess and treat disorders of defaecation is unlikely to improve greatly until these uncertainties can be resolved.

2.9 Tables

Table 2.1 Common Medications and Co-morbidities which Affect Bowel Function

Medications which Affect Bowel Function	Comorbidities and Biopsychosocial Factors which Affect Bowel Function
Gastrointestinal	Gastrointestinal
Laxatives	Inflammatory bowel disease
Bile acid sequestrants	Small intestinal malabsorption (e.g. Coeliac disease, pancreatic insufficiency)
Serotonin (5-HT_3) antagonists	Colorectal cancer
Analgesics	Congenital
Opioids	Hirschsprung disease
Non-steroidal anti-inflammatory drugs	Anorectal malformations
Cardiovascular	Neurological
Ca^{2+} channel blockers	Parkinson's disease
Diuretics	Multiple sclerosis
β-adrenergic antagonists	Spinal cord injury
α^2-adrenergic agonists	Stroke
Neurological	Psychiatric
Antiepileptics	Anxiety and psychological stress
Dopaminergics	Depression
Spasmolytics	Sexual abuse
Psychiatric	Eating disorders
Antidepressants	Obsessive compulsive disorder
Antipsychotics	Endocrine
Lithium	Diabetes mellitus
Other	Hyper-/hypothyroidism
Antihistamines	Menopause
Chemotherapeutics	Metabolic

Cation containing agents Sympathomimetics Antibiotics	Dehydration Hypercalcaemia Hypokalaemia Hypomagnesaemia Uraemia Obstetric/gynaecological Pregnancy Endometriosis Systemic Scleroderma Amyloidosis Myotonic dystrophy Myelodysplasia Infective Chagas' disease HIV Bacterial, viral or protozoa pathogens Intestinal Surgery Partial or complete colectomy Ageing Cognitive impairment Reduced physical activity Reduced access to sanitation

Table 2.2 Common Disorders of Defaecation Categorised by the Phases of Defaecation, the Proposed Pathophysiological Mechanisms, and their Pathoaetiologies

Phase of Defaecation	Primary Disorder*	Proposed Mechanisms	Proposed Aetiology
1. Basal Phase	Slow transit constipation (STC)	Prolonged colonic transit time results in increased mucosal fluid absorption, causing firmer and less frequent stools.	Decreased density of interstitial cells of Cajal(238, 587)
			Disordered colonic motility(251)
			Extrinsic peripheral autonomic nervous system dysfunction(256)
	Constipation with normal colonic/ whole-gut transit	Many cases relate to a predominant evacuation disorder (ED) (see 'Expulsive phase' below). There may be a multitude of secondary causes, for which the pathogenesis is not clear; these may alter gastrointestinal function during the basal phase.	Colonic dysbiosis(501)
			Medication side-effects, metabolic disturbances, endocrine diseases, psychiatric diseases(232)
			Reduction in number of enteric glial cells in myenteric & submucosal plexus(233, 588, 589)
			Reduction in number of enteric neurons in the submucosal plexus(233, 588, 589)
	Functional diarrhoea	Higher stool water volume caused by; (a) enteric malabsorption or; (b) increased small bowel fluid section; resulting in more rapid colonic transit, and reduced colonic mucosal water absorption. This results in reduced colonic transit time with stools of looser consistency and higher frequency.	Disordered colonic motility(133)
			Secondary causes: Medication side-effects, malabsorptive diseases, chronic enteric parasitic infections, colitis, endocrine diseases(590)
	Irritable bowel syndrome	Abdominal pain is associated with defaecation (exacerbation or alleviation of pain) as a result of several proposed pathophysiological mechanisms occurring at both a peripheral and cortical level. In addition, alterations in stool form and/or consistency are observed, which may be the result of altered gut transit and/or absorption.	Visceral hypersensitivity(591)
			Altered cortical responses to pain (592)
			Brain-gut/gut-brain axis dysfunction(593)
			Colonic dysbiosis(594, 595)
			Increased intestinal permeability(596, 597)
			Altered motility and transit(598)
			Gastrointestinal mucosal inflammation(599)

			Non-coeliac gluten sensitivity(600)
			Sodium channelopathy (*SCN5A* mutation)(601)
			Disordered bile acid metabolism(602, 603)
2. Pre-Expulsive Phase	Faecal incontinence	An incompetent anal sphincter and weakened pelvic floor musculature can result in urgency and incontinence or passive incontinence during rectal filling.	Anal sphincter injury (most commonly obstetric or iatrogenic following anorectal surgery) (196, 208, 378)
			Puborectalis atrophy(220)
			Pelvic organ prolapse(604)
			Pudendal nerve neuropathy(605, 606)
			Pelvic floor denervation(379, 607)
			Ligamentous injury(376)
		Structural changes in the anorectum can cause stool trapping and leakage, as well as urgency and incontinence during rectal filling.	Rectal prolapse(608)
			Rectal intussusception(609)
			Rectocoele(201, 320)
		Rectal hyposensitivity is associated with an impaired defaecatory urge, and can result in (or be the result of) gross rectal distension and faecal impaction, with overflow incontinence. Rectal hypersensitivity can lead to urgency and incontinence, even at low rectal volumes.	Impaired rectal sensation(194, 227, 610, 611)
		Anal sensory impairment may also be associated with an impaired defaecatory urge, resulting in involuntary stool leakage	Impaired anal sensation(612, 613)
3. Expulsive Phase	Faecal incontinence	Voluntary control of defaecation can be affected by cognitive impairment, neurological diseases, and bowel disturbances such as diarrhoea.	Secondary causes (cognitive impairment, stroke, diarrhoeal illnesses)(614)
	Slow transit constipation (STC)	Firmer and less frequent stools are more difficult to expel.	Impaired evacuation due to harder, smaller stools(428, 433)
		Structural or functional obstructive phenomena impede defaecation (these may overlap).	Rectal prolapse/intussusception(609)

	Constipation with normal colonic/ whole-gut transit (i.e. evacuation disorders [ED])		Rectocoele(320).
			Enterocoele(615)
			Faecal impaction, enlarged rectum, and megarectum(616, 617)
			Functional obstruction via poor coordination of anorectal and pelvic floor musculature or dissipated force vectors(618-620)
			Descent and hypermobility of the rectum(621)
			Reduction in number of enteric glial cells in myenteric & submucosal plexus(233, 588, 589)
			Reduction in number of enteric neurons in the submucosal plexus(233, 588, 589)
4. End Phase	Faecal incontinence	An incompetent anal sphincter and pelvic floor musculature can result in the inability to restore a "seal" following defaecation, resulting in post-defaecation stool leakage.	Anal sphincter injury (most commonly obstetric or iatrogenic following anorectal surgery) (196, 208, 378)
			Puborectalis atrophy(220)
			Pelvic organ prolapse(604)
			Pudendal nerve neuropathy(605, 606, 622)
			Pelvic floor denervation(379, 607)
			Ligamentous injury(376)

		Structural changes in the anorectum can also result in an inadequate closing reflex, causing stool trapping during defaecation and leakage following defaecation.	Rectal prolapse(608)
			Rectal intussusception(609)
			Rectocoele(201)

* may be overlap

Chapter 3: Specific Aims of This Thesis

3.1 Specific Aims of this Thesis

This thesis intended to address research questions pertaining to the physiology of human colonic motility, the functional role of colonic motility in transit and defaecation, as well as the role of colonic dysmotility in faecal incontinence and constipation. A combination of experimental techniques were undertaken to address the thesis aims, including clinical, in vivo, and ex vivo human studies.

These studies were made possible by the unique research opportunities available at Flinders Medical Centre and Flinders University. With the co-location of the hospital and university, long-standing arrangements between surgical and pathology departments, and – most importantly – the willingness of patients to participate in research, there is the unique opportunity to study ex vivo specimens of human colon immediately following surgical excision on-site(39, 256, 623). In addition, there is the capability to perform in vivo human colonic manometry studies on-site and the Flinders gastrointestinal motility laboratory is world-renowned for analysis of colonic manometry data(2, 105, 125). This not only provides an opportunity for analysis of data collected on-site, but also the opportunity to participate in international collaborations with sharing of colonic manometry data recorded in North America and Europe.

Firstly, a literature review on the functional physiology of defaecation and continence was performed (**Chapters 1 & 2**), with specific knowledge gaps in the literature summarised at the end of each section. While it is beyond the scope of this thesis to address all of these knowledge gaps, these were included as a means to highlight the limitations in our current scientific knowledge and pose research questions to be addressed both within this thesis as well as in future research in this field.

Secondly, clinical data was collected and analysed with the aims of;

1. Demonstrating the discordance between faecal incontinence severity and anorectal dysfunction. This is important as a means to highlight the likelihood that there are mechanisms extrinsic to the anorectum which contribute to the pathogenesis of faecal incontinence which are not captured by our current diagnostic techniques. This will also provide insight into the limitations of our current diagnostic approaches which are primarily focused upon the structure and function of the anorectum (**Chapters 4 & 5**).
2. Characterising colonic motility in children with severe, treatment-refractory constipation before and after pharmacological provocation with bisacodyl. In contrast to faecal incontinence, constipation is a symptom where patterns of colonic dysmotility have been demonstrated (79, 115, 249-255). This will provide insight into the relationships between specific motility patterns which have a functional role in defaecation and constipation (**Chapter 6**).

Thirdly, human in vivo experimental studies were conducted with the aims of;

1. Assessing the associations between colonic motility and gas transit using high-resolution impedance

manometry. The intent of this study was to characterise the functional role of distal colonic motility in the regulation of gas transit, continence, and evacuation (**Chapter 7**).

And, finally, human ex vivo laboratory studies were conducted with the aims of;

1. Describing the neuromuscular mechanisms which are responsible for the generation of colonic motor patterns. This will be achieved by recording with high-resolution impedance manometry and mechanical force transducers, using a combination of electrical stimulation and pharmacological stimulation/inhibition techniques (**Chapter 8**).
2. Describing the contractile responses in colonic smooth muscle to excitatory and inhibitory neuromuscular transmission and how these can be altered by opioid receptor agonists. This will be performed as a means to assess how opioids alter human colonic neuromuscular action to provide insight into the pathogenesis of opioid-induced constipation. This was achieved by recording contractile activity in isolated strips of colonic circular muscle using force transducers and a combination of electrical stimulation and pharmacological stimulation/inhibition techniques (**Chapter 9**).

Chapter 4: The Relationships Between the Results of Conventional Anorectal Investigations and Faecal Incontinence Severity

4.1 Statement

The content of this chapter has been published in the *International Journal of Colorectal Disease*.

Heitmann PT, Rabbitt P, Schloithe A, Patton V, Skuza PP, Wattchow DA, Dinning PG (2019). Relationships between the results of anorectal investigations and symptom severity in patients with faecal incontinence. *Int J Colorectal Dis* 34(8);1445-1454. © 2019 Springer Nature (Licence number 4881160740603)

The co-authors have provided permission for the inclusion of the study in this thesis. The percentage contributions of each author to this study were as follows:

- Research design: **PH 50%**, DW 25%, PD 25%.
- Data collection: AS 33%, PR 33%, DW 33%.
- Data entry & analysis: **PH 100%**,
- Writing and editing: **PH 80%,** PR 4%, VP 4%, PS 4%, DW 4%, PD 4%.

4.2 Abstract

Purpose: Anorectal dysfunction is the focus of diagnostic investigations for faecal incontinence. However, severity of incontinence and anorectal investigation results can be discordant. The aim of this study was to define the relationships between anorectal investigation results and incontinence severity to determine which measures, if any, were predictive of incontinence severity. **Methods:** Patients presenting for investigation of faecal incontinence completed a questionnaire, anorectal manometry, rectal sensation, pudendal nerve terminal motor latency, and endoanal ultrasound. Bivariate analyses were conducted between the Jorge-Wexner score and investigation results. Subgroup analyses were performed for gender and symptom subtypes (urge, passive, mixed). A multiple regression analysis included investigation results and the Jorge-Wexner score. **Results:** 538 patients were included. There were weak correlations between the Jorge-Wexner score and maximal squeeze pressure (r=-0.24, 95%CI(-0.31, -0.16), p<0.001), and resting pressure (r=-0.18, 95%CI(-0.26, -0.10), p<0.001). In the male subgroup only, there were significant associations between the Jorge-Wexner score and endoanal sonography (t(113)=-2.26, p=0.03, d=0.58, 95%CI(-4.38, -0.29)), and rectal sensation (r_s=-0.24, 95%CI(-0.41, -0.06), p=0.01). No substantial differences were observed in the urge/passive/mixed subgroup analyses. Multiple regression analysis included three variables; age (β=0.02, p=0.17), maximal resting pressure (β=-0.01, p=0.28), and maximal squeeze pressure (β=-0.01, p<0.01). The variance in the Jorge-Wexner score accounted for by this model was <10%, (R^2=0.07, p=<0.01, adjusted R^2=0.06). **Conclusion:** Anorectal investigations cannot predict the severity of faecal incontinence. This may be due to limitations of diagnostic modalities, the heterogeneity of anorectal dysfunction in these patients, or contributing factors which are extrinsic to the anorectum.

4.3 Introduction

Faecal incontinence is a common symptom with significant implications upon health, socialisation, and quality of life(189, 624-626). The estimated prevalence in the community is 8.3-12.4%(186), with symptoms likely to be under reported given their sensitive nature(189). Faecal incontinence is a major contributor to aged care placement in the elderly(624) and incurs substantial healthcare costs with an estimated total annual expenditure exceeding AUD$1.5 billion(627) in Australia.

Anal sphincter injury and/or anorectal dysfunction are considered to be the predominant causes of faecal incontinence(205-207). Diagnostic investigations include endoanal sonography, anorectal manometry, and pudendal nerve terminal motor latency (PNTML). Collectively, these tests can determine the presence of a structural injury of the anal sphincter, neuromuscular dysfunction of the anal sphincter and pelvic floor, and the presence of pudendal nerve neuropathy. However, objective evidence of anorectal dysfunction in patients with severe incontinence is not always apparent, whereas some patients with a demonstrable anorectal injury are asymptomatic or experience only mild incontinence. For example, anal sphincter injury is reported in 27% of primiparous women(212, 213), yet less than one third of women with sphincter injuries report faecal

incontinence postpartum(214). Conversely, 40% of patients presenting with faecal incontinence have normal anal sphincter morphology on endoanal sonography(215). Whilst lower anal canal resting and squeeze pressures have been associated with faecal incontinence when compared with healthy controls(216-218), there remains a considerable overlap in findings between these groups(219, 220). One study found that 9% of women and 18% of men with faecal incontinence had normal results on all routine anorectal investigations(221).

Using the St Mark's(217) and Jorge-Wexner(205) symptom scores as measures of incontinence severity, Lam et al. demonstrated a significant association between abnormal findings on anorectal manometry and incontinence severity in a cohort of 218 patients(628). However, that correlation was evident only in a subgroup of patients who reported soft stool consistency(628). Studies with smaller sample sizes have failed to demonstrate any association between anorectal investigation results and incontinence severity using the Jorge-Wexner score(629), or the Faecal Incontinence Severity Index and Faecal Incontinence Quality of Life Scale(630). The most important outcome pre- and post-intervention for faecal incontinence is whether the treatment can elicit a reduction in severity or resolution of symptoms. In order to achieve this, it is critical to be informed of which structural or physiological abnormalities are of most benefit to address.

In the anorectal clinic at Flinders Medical Centre, Australia, a clinical database of anorectal investigation results was established for patients with faecal incontinence. Using this database, our aim was to examine the relationships between anorectal investigations and the severity of incontinence to determine which measure or combination of measures, if any, were predictive of faecal incontinence severity.

4.4 Methods

Ethics approval was received from the Southern Adelaide Clinical Human Research Committee. All adult patients who presented to Flinders Medical Centre, South Australia, for investigation of faecal incontinence between 1998-2015 were considered for inclusion.

4.4.1 Symptom Questionnaire

The patient questionnaire included past medical, surgical, and obstetric histories and the Jorge-Wexner faecal incontinence severity score(205). The Jorge-Wexner score is a summative, five-category score, with categories including frequency of; (1) incontinence of solids; (2) incontinence of liquids; (3) incontinence of flatus; (4) use of continence pads, and; (5) lifestyle alteration. A frequency score from 0-4 is assigned for each category (0=never, 1=rarely or <1/monthly, 2=sometimes or <1/weekly, 3=usually or <1/day, 4=always or >1/day), to produce a total score of 0-20. An additional question using the same five-category, summative score was used to elicit features suggestive of urge incontinence with the question; how often do you have to rush to the toilet to open your bowels? Passive incontinence was determined with the question; do you know when you open

your bowels (yes/no)?

4.4.2 Anorectal Investigations

4.4.2.1 Anorectal Manometry

No bowel preparation was performed prior to investigations. A single clinical scientist performed all manometry procedures with the patients in the left lateral decubitus position. Manometry was performed using a water-perfused, three-channel, polyvinyl chloride or silicone, 3.0mm external diameter catheter (Mui Scientific, Mississauga, Ontario, Canada). Each channel had a side hole, spaced at 5mm, orientated circumferentially and at 120° to one another. Sequential pressure measurements were recorded using a station pull-through technique. The catheter was withdrawn 5mm at 60s intervals, with the patient instructed to squeeze maximally for 3s at each station. The peak resting pressure and peak squeeze pressure from each of the three channels was recorded. Normal ranges (maximal resting pressure 54-104cmH_2O, maximal squeeze pressure ≥179cmH_2O) were based upon the results of two series of healthy patients studied with similar water-perfused systems(631, 632).

4.4.2.2 Rectal Sensation

Rectal sensation was recorded using a balloon which was incrementally inflated with air (10, 20, 40, 70, 100, 150, 200mL). The volume at which sensation was first perceived by the patient and the maximum tolerable volume were recorded (normal ranges: first perceived volume 10-80mL, maximum tolerable volume 200mL).

4.4.2.3 Pudendal Nerve Terminal Motor Latency (PNTML)

PNTML was measured using a disposable, glove-mounted St Mark's electrode (13L40 St Mark's Pudendal Electrode™; Medtronic Functional Diagnostics A/S, Skovlunde, Denmark). Square wave stimuli were delivered via transrectal stimulation at the level of the ischial spine (0.05ms duration, 10mA, 1Hz). The time between the onset of the stimulus and depolarisation of the anal sphincter on electromyography was recorded as the PNTML. Normal latency was defined as <2.2ms(633).

4.4.2.4 Endoanal Sonography

A Bruel and Kjaer type 1846/1101 scanner (Njaerum, Denmark) was used with a 7/10 MHz rotating endosonic probe (model 1850). Axial 360° views of the anal sphincter were obtained from the upper, mid, and lower anal canal as the probe was withdrawn. Images were interpreted by a consultant radiologist or a consultant colorectal surgeon. The internal anal sphincter (IAS) and external anal sphincter (EAS) were categorised as (a) intact or, (b) abnormal if a defect was visualised. When present, a defect was further categorised by the circumferential extent of the defect; <90°, >90°, or >180°.

4.4.3 Statistical Analysis

Bivariate analyses (Pearson's correlation coefficient with bootstrapped 95% confidence intervals to accommodate outliers(634)), were performed between the Jorge-Wexner score and anorectal investigation results including; anorectal manometry, rectal sensation, PNTML, and endoanal sonography (independent samples t-test, one way ANOVA). All continuous variables were normally distributed with the exception of rectal sensation (Shapiro-Wilk test $p<0.01$), for which non-parametric analyses were used (Spearman's rho correlation coefficient with bootstrapped 95% confidence intervals). Multiple regression analysis was then performed, using a model incorporating all variables identified from the bivariate analyses with a statistically significant relationship to the Jorge-Wexner score.

Secondly, subgroup analyses were performed with patients grouped by gender and symptom subtype (urge, passive, or mixed symptoms). Within these subgroups, the above bivariate analyses between the Jorge-Wexner score and each investigation result were repeated.

Thirdly, bivariate analyses were conducted between each anorectal investigation result to assess their associations (Pearson correlation coefficient, Independent samples t-test, Pearson Chi-square test of association).

Statistical analysis was performed using IBM SPSS (Version 19.0, Released 2010; IBM Corp., Armonk, New York, USA), in addition to "Psychometric" R package(635) and Cohen's d online calculator(636). A p-value of ≤ 0.05 was considered statistically significant. Effect sizes and confidence intervals of 95% were reported.

4.5 Results

4.5.1 Sample Demographics

Between 1998-2015, 847 patients presented to Flinders Medical Centre for investigation of faecal incontinence. Of those, 309 were excluded due to having an incomplete symptom questionnaire. The remaining 538 patients were included in the analysis. The study group included 423 women (78.6%) and 115 men (21.4%), with a median age of 67 years (range 18-90). The majority of the women were parous (92.3%, n=370), with a median of two children (range 1-10). Fourteen women did not complete the obstetric history section of the questionnaire. Of the 538 patients, 30.3% (n=163) had undergone previous anorectal surgery (anterior resection, rectopexy, haemorrhoidectomy, fistulotomy, sphincterotomy), and 2.6% (n=14) had previously been treated with pelvic radiotherapy.

Mean (±SD) Jorge-Wexner score for the included patients was 11.1±3.8. Those describing urge symptoms of having to rush to the toilet 'usually' or 'always' comprised 41.8% (n=225) of the cohort. Those with passive symptoms, having no knowledge of when their bowels were opened, totaled n=30 (5.6%). The remaining 52.6% (n=283) reported mixed urge/passive symptoms.

Four patients (0.7%) had normal results on all anorectal investigations. All other patients in the database had at least one abnormal result. Frequencies of normal/abnormal findings on anorectal investigations are shown in **Table 4.1**, and associations between anorectal investigation results are shown in **Table 4.2**.

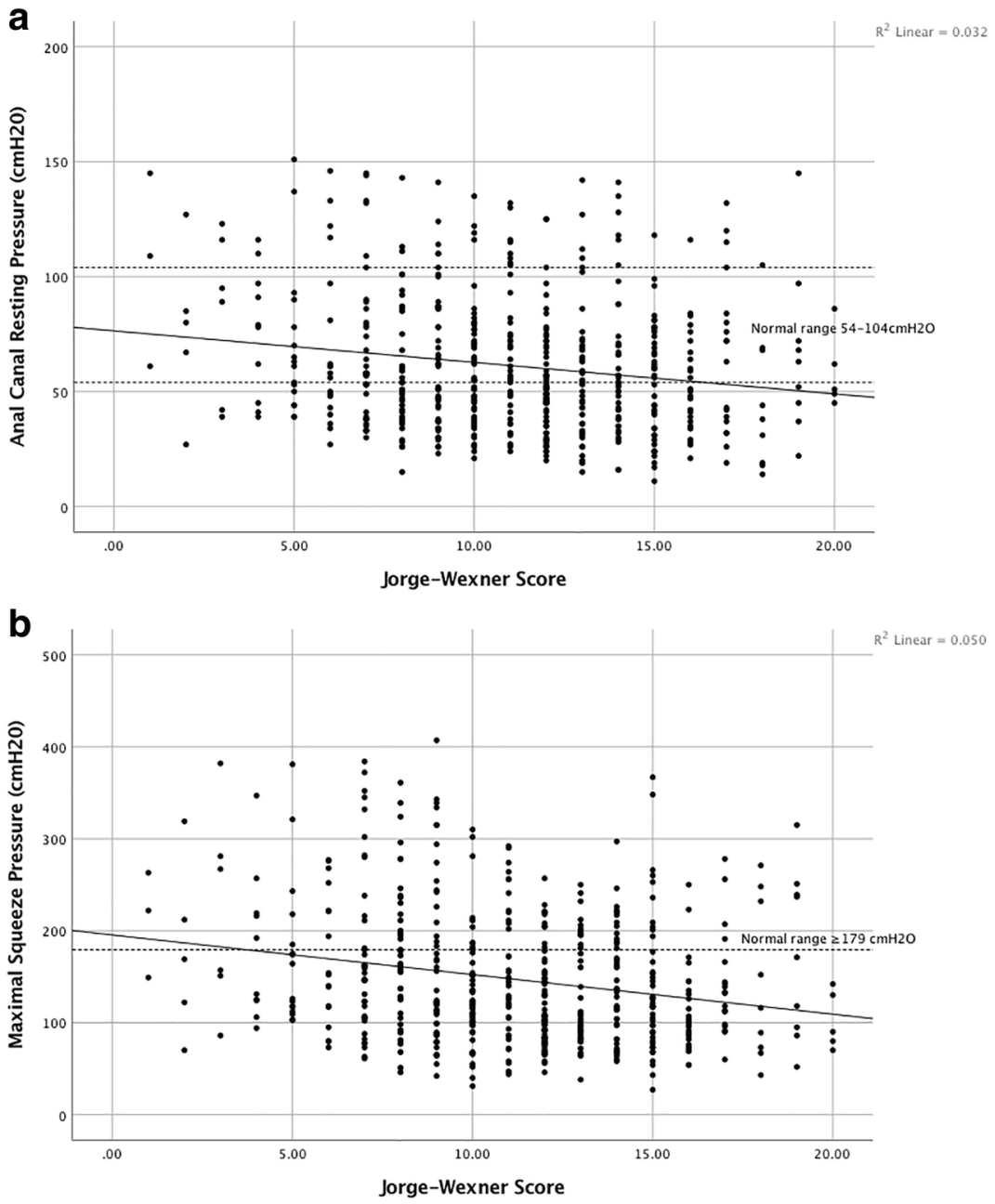

Figure 4.1 Correlations between the Jorge-Wexner score and; (A) anal canal resting pressure, and; (B) squeeze pressure.

4.5.2 Faecal Incontinence Severity and Anorectal Investigation Results

There were weak associations between the Jorge-Wexner score and maximal squeeze pressure (r=-0.24, 95%CI(-0.31, -0.16), p<0.001), resting pressure (r=-0.18, 95%CI(-0.26, -0.10), p<0.001), and age (r=0.12, 95%CI(0.03,0.20), p=<0.01). A group of 161 patients (29.9%) had a Jorge-Wexner score ≥9 and a normal resting pressure, while 98 patients (18.2%) with a Jorge-Wexner score ≥9 had a normal maximal squeeze

pressure. Conversely, 77 patients (14.3%) and 80 patients (14.9%) had a Jorge-Wexner score (<9), and resting pressure and maximal squeeze pressure below the normal ranges, respectively (**Figures 4.1A and 1B**).

There were no statistically significant associations between the Jorge-Wexner score and rectal sensation; (first perceived volume r_s=0.01, 95%CI(-0.07, 0.09), p=0.81, maximum tolerable volume r_s=-0.08, 95%CI(-0.16, 0.00), p=0.06, PNTML; left: r=0.01, 95%CI(-0.09, 0.11), p=0.87, right: r=0.05, 95%CI(-0.05, 0.15), p=0.29, endoanal sonography results (IAS: t(426)=-0.08, p=0.93, d<0.01, 95%CI(-0.85, 0.78), EAS: t(514)=-1.3, p=0.19, d=0.13, 95%CI(-1.21, 0.24)), or gender t(161)=-1.5, p=0.14, d=0.16, 95%CI(-1.54,0.14).

4.5.3 Subgroup Analyses

Gender

When separated by gender, the strength of the correlation between anorectal manometry and the Jorge-Wexner score was marginally stronger in the male subgroup, and weaker in the female subgroup (men; maximal squeeze pressure (r=-0.33, 95%CI(-0.48, -0.16), p<0.01), resting pressure (r=-0.36, 95%CI(-0.51, -0.19), p<0.01); women; maximal squeeze pressure (r=-0.19, 95%CI(-0.28, -0.09), p<0.01), resting pressure (r=-0.10, 95%CI(-0.20, -0.01), p=0.04)).

In men, significant associations were also observed between the Jorge Wexner score and endoanal sonography (IAS only: t(113)=-2.26, p=0.03, d=0.58, 95%CI(-4.38, -0.29)), rectal sensation (tolerance only; r_s=-0.24, 95%CI(-0.41, -0.06), p=0.01), and PNTML (left only; r=0.28, 95%CI(0.04, 0.48), p=0.02). No other statistically significant associations between the Jorge-Wexner score and investigation results were observed in either gender subgroup.

Symptom Subtype: Urge, Passive, or Mixed Incontinence

In patients presenting with urge (n=225) or passive (n=30) symptoms, there were no significant associations between Jorge-Wexner score and any individual anorectal investigation result (**Table 4.3**). In the remaining patients with mixed symptoms (n=283), there were significant relationships between Jorge-Wexner score and anorectal manometry (resting pressure; r=-0.34, (-0.45, -0.22), p=<0.001, maximal squeeze pressure; r=-0.29, (-0.41, -0.16), p=<0.001) and rectal sensation (maximum tolerable volume; r_s=-0.13, 95%CI(-0.24, 0.01), p=0.03).

When comparing the three subgroups, there were statistically significant differences between mean age (F(2,535)=13.14, p=<0.001), resting pressure (F(2,535)=10.78, p=<0.001), maximal squeeze pressure (F(2,535)=4.75, p=<0.01), rectal sensation (tolerance; F(2,529)=5.09, p=<0.01), and Jorge-Wexner score (F(2,535)=33.05, p=<0.001). Those in the passive subgroup were more elderly (mean ages; passive subgroup=74±11 years, urge subgroup=62±14 years, mixed subgroup=67±14 years) and had the lowest pressures recorded on anorectal manometry (mean resting pressures; passive subgroup = 40.0±23.5cmH_2O,

urge subgroup = 66.1±30.7cmH_2O, mixed subgroup = 60.0±30.0cmH_2O; mean maximal squeeze pressures; passive subgroup = 122.1±60.2cmH_2O, urge subgroup = 140.9±63.6cmH_2O, mixed subgroup = 157.1±86.1cmH_2O). Those with urge incontinence reported the most severe Jorge-Wexner scores (mean score; urge subgroup = 12.6±3.4, passive subgroup = 12.3±3.6, mixed subgroup = 10.0±3.8). There were no significant differences between groups in endoanal sonography or PNTML results.

4.5.4 Multiple Regression Analysis

The multiple regression analysis included three variables; age (β=0.02, p=0.17), maximal resting pressure (β=-0.01, p=0.28), and maximal squeeze pressure (β=-0.01, p<0.001). The variance in the Jorge-Wexner score accounted for by this model was <10%, (R^2=0.07, p=<0.001, adjusted R^2=0.06), and therefore cannot explain the variability in the Jorge-Wexner score in >90% of patients with faecal incontinence.

4.6 Discussion

These data demonstrate a weak correlation between anorectal manometry results and faecal incontinence severity, supporting the findings of previous smaller studies(220, 628). However, there are a substantial proportion of patients with severe incontinence despite normal anorectal manometry results and, conversely, patients with mild incontinence yet significantly abnormal manometry results (**Figures 4.1A & 1B**). Significant relationships were demonstrated between faecal incontinence severity and results of rectal sensation and endoanal sonography in men only. The strongest single predictor of severity was an IAS defect detected by sonography in men, with a moderate effect size. The findings from the multiple regression analysis suggest that the results of anorectal investigations do not predict faecal incontinence severity in the majority of patients. While these findings may cast doubt upon the usefulness of anorectal investigations, there are several factors the need to be considered in relation to the discord between test results and faecal incontinence severity.

Firstly, diagnostic modalities used in this study may simply have been inadequate to detect relevant features of anorectal dysfunction. The manometry data was derived from low-resolution, water-perfused anorectal manometry. In many tertiary hospitals and research centres, this equipment has been superseded by high-resolution, solid state anorectal manometry(637, 638), and it is possible that high-resolution manometry may improve diagnostic accuracy(120, 121). However, low-resolution, water-perfused manometry is still in common use, with approximately half of institutions still using this technology in a recent international survey of >100 specialist centres(638).

Our study also recorded anal sphincter integrity using two-dimensional endoanal sonography. As with anorectal manometry, this technology has been updated to three-dimensional endoanal ultrasound and/or high-

frequency ultrasound in many centres(639). Using two-dimensional sonography, we identified anal sphincter defects in 41.7% of our cohort. In those patients, there were strong associations between an anal sphincter defect and reduced anal canal resting or squeeze pressures (**Table 4.2**). This would suggest that our findings from two-dimensional endoanal sonography correspond with functional impairment, demonstrated by reduced manometric pressures.

A previous study by Bharucha et al.(220) using the same sonography equipment, also reported anal sphincter defects in a similar proportion of patients with faecal incontinence (21/53 women; 39.6%). In that study, Bharucha et al. reported a significant association between IAS and EAS defects and the presence, but not severity, of faecal incontinence. In addition to sonography, Bharucha et al.(220) also assessed the musculature of the pelvic floor using dynamic magnetic resonance imaging (MRI), and found that puborectalis atrophy (present in 8/51 women) was the only anorectal investigation finding that had a significant association with faecal incontinence severity. Our two-dimensional probe did not allow for assessment of puborectalis integrity. Therefore, it is possible that a proportion of patients with severe incontinence may be explained by puborectalis injury or atrophy. However, there is no strong evidence from other studies for puborectalis injury being a primary cause of increased faecal incontinence severity.

Assessment of neurological integrity via PNTML and rectal sensation are used to determine whether disruption to the motor innervation of the external anal sphincter and pelvic floor, or altered sensation, are contributing to symptoms(293, 379, 401, 605, 640, 641). Previous studies have demonstrated conflicting findings, with PNTML having no correlation with symptom severity scores in one study(642) but a significant relationship in another study(643). PNTML has been demonstrated to correlate with the results of other anorectal investigations including manometry(215, 644), which is consistent with our findings. The utility of PNTML remains the subject of debate(645), with particular criticisms including; (1) low sensitivity/specificity for detecting EAS weakness(646-648); (2) considerable variability in range seen in healthy controls(211), and; (3) operator-dependency(648). Rectal hypersensitivity and reduced rectal compliance are considered to be associated with urge symptoms(220), whereas hyposensitivity is related to passive symptoms(401). In our subgroup analysis, there were no significant associations between rectal sensation and symptom severity in either urge or passive subgroups.

In addition to these equipment considerations, there are also potential limitations in using a quantitative symptom score to determine faecal incontinence severity. While many other symptom severity scores are available(209, 649-651), the Jorge-Wexner score remains the most widely used validated symptom score for faecal incontinence(652). Criticisms regarding the use of the Jorge-Wexner score include; (1) the equivalent weighting of the nature of the per rectal loss (gas, liquid, and/or solid); (2) not including symptoms of urgency(209, 653); (3) the inclusion of continence pad use, which is influenced by personal behavior and concurrent urinary incontinence(209, 654), and; (4) the day-to-day variability of symptoms in any individual patient(629). While it is difficult to encapsulate the subjective nature of symptomatology in a quantitative

score, the correlation of symptom scores to validated quality of life scores(625, 655-657) would suggest that such measures do bear a reflection of the holistic impact of symptoms upon the patient. Additionally, the correlation between symptom scores and quality of life scores have been replicated and cross-validated in multiple cultures and languages(656-658). Whilst a quality of life score was not included in our symptom questionnaire, this would be beneficial as an additional outcome measure to assess the overall burden of symptoms on the patient pre- and post-intervention.

Relating symptom scores to investigation results may also be problematic given the heterogeneity of our sample population. The pathophysiology of faecal incontinence is complex, manifesting in varied symptom subsets and severity(205, 207, 614). For example, some patients may report symptoms of predominantly urge or passive incontinence, or incontinence only to flatus but not solids, among other patterns of symptoms. Women are at considerable risk of incontinence following obstetric injury, which of course does not affect men. In an attempt to homogenise the sample and address these differences, we performed subgroup analyses with patients separated by gender and symptom subtype. In the gender subgroup analysis, stronger correlations were observed in men between symptoms and anorectal manometry, however the strength of the correlation remained weak to moderate. No anorectal investigation had any bearing on the severity of incontinence in those with predominantly urge or passive incontinence (**Table 4.3**). This would suggest that both gender and common symptom subtypes are unlikely to account for the discord that remains between our anorectal investigation results and the faecal incontinence severity.

A third possible explanation for the results of our study is to consider that some contributing factors to faecal incontinence severity are extrinsic to the anorectum, and therefore not recorded by routine anorectal investigations. Continence and defaecation require coordinated motility between the colon and anorectum(131, 224, 425). Previous studies have demonstrated that rectal contents can be shifted to the sigmoid colon when defaecation is inappropriate(391) and that motor patterns in the sigmoid colon may assist in slowing or preventing premature rectal filling(2, 116, 133, 173, 177). As a result, dysmotility in the distal colon may contribute to faecal incontinence. Examination of colonic motor patterns in patients with faecal incontinence is rarely performed. Two previous studies comparing colonic motility between patients with faecal incontinence and healthy controls in small cohorts report conflicting results; Herbst et al.(131) demonstrated no substantial change in colonic motility between patients and healthy adults, whereas Rodger et al. demonstrated increased colonic motility in the patient group whilst fasting, but a similar meal response to healthy controls(228) (see **1.7.1.4 The Functional Role of Colonic Motility in Continence**). However, both of those studies used low-resolution colonic manometry which would overlook much of the propagating activity that may be of importance in the distal colon(2, 114, 173, 316).

Given the discord between symptom severity and anorectal investigation results, there has been much debate on the utility of anorectal investigations. Some authors suggest that history and examination alone are sufficient(659, 660) or that anorectal investigations should be used selectively rather than routinely in this

patient group(628). Other studies have demonstrated that anorectal investigations provide more diagnostic and prognostic information than clinical examination alone, which alters patient management(661-664). At our anorectal clinic, we continue to use anorectal investigations in the diagnostic investigation of patients presenting with faecal incontinence. In our experience, the information provided by anorectal investigations complements our history and examination findings, and assists in both planning treatment and re-assessment post-intervention.

4.7 Conclusion

The presence or extent of anatomical and/or physiological anorectal dysfunction cannot predict the severity of faecal incontinence. Furthermore, no single diagnostic investigation, or combination of investigation results, can reliably identify or predict faecal incontinence severity. Further studies with a more detailed assessment of symptomatology, and utilisation of three-dimensional, high-resolution anorectal manometry and three-dimensional endoanal sonography are needed (**Chapter 5**).

4.8 Tables

Table 4.1 Frequencies of Normal/Abnormal Anorectal Investigation Results

	Normal results **Women** Count, % of valid	Abnormal results **Women** Count, % of valid	Missing results **Women** Count, % of total	Normal results **Men** Count, % of valid	Abnormal results **Men** Count, % of valid	Missing results **Men** Count, % of total
Resting pressure	n=173, 40.9%	n=250, 59.1%	-	n=48, 41.7%	n=67, 58.3%	-
Maximal squeeze pressure	n=79, 18.7%	n=344, 81.3%	-	n=76, 66.1%	n=39, 33.9%	-
Rectal sensation Combined	n=205, 49.2%	n=212, 50.8%	n=6, 1.4%	n=68, 59.1%	n=47, 40.9%	-
FPV	n=400, 95.5%	n=19, 4.5%	n=4, 0.9%	n=105, 91.3%	n=10, 8.7%	-
MTV	n=223, 53.5%	n=194, 46.5%	n=6, 1.4%	n=78, 67.8%	n=37, 32.2%	-
PNTML	n=123, 32.7%	n=216, 57.4%	n=84, 19.9%	n=36, 50.7%	n=35, 49.3%	n=44, 38.3%
Unable to obtain trace			n=37, 8.7%			n=20, 22.0%
Not performed			n=47, 11.1%			n=24, 20.9%
EAUS Combined	n=223, 53.7%	n=192, 46.3%	n=8, 1.9%	n=82, 75.9%	n=26, 24.1%	n=7, 6.1%
IAS	n=324, 76.6%	n=99, 23.4%	-	n=94, 81.7%	n=21, 18.3%	-
EAS	n=258, 61%	n=151, 35.7%	n=14, 3.3%	n=98, 90.7%	n=10, 9.3%	n=7, 6.1%

*FPV = First perceived volume, MTV = Maximal tolerable volume, PNTML = Pudendal nerve terminal motor latency, EAUS = Endoanal sonography, IAS = Internal anal sphincter, EAS = External anal sphincter

Table 4.2 Associations Between Anorectal Investigation Results

		Anorectal manometry		Rectal Sensation		PNTML		Endoanal Sonography
		Resting pressure	Maximal squeeze pressure	First perceived volume	Maximum tolerable volume	Left	Right	IAS
ARM	MSP	r=0.52 p=<0.01						
Rectal sensation	FPV	r=0.10 p=0.06	r=0.05 p=0.31					
	MTV	r=0.03 p=0.58	r=-0.17 p=<0.01	r=0.42, p=<0.01				
PNTML	Left	r=-0.29 p=0.64	r=-0.07 p=0.23	r=-0.06, p=0.32	r=0.42, p=<0.01			
	Right	r=-0.20 p=0.01	r=-0.22 p=<0.01	r=0.05, p=0.44	r=0.42, p=<0.01	r=0.26 p=<0.01		
EAUS	IAS	t(348)=4.75p=< 0.01 d=0.59	t(348)=1.9 p=0.05 d=0.30	t(347)=0.27 p=0.79 d=0.04	t(346)=1.98 p=0.05 d=0.28	t(265)=0.25 p=0.80 d=0.04	t(255)=-2.04 p=0.04 d=0.34	
	EAS	t(203)=3.2 p=<0.01 d=0.37	t(217)=3.77 p=<0.01 d=0.43	t(338)=1.19 p=0.24 d=0.16	t(149)=2.47 p=0.02 d=0.31	t(258)=0.85 p=0.40 d=0.12	t(248)=0.85 p=0.40 d=0.12	$x^2(1)=29.47$ p=<0.01 Phi=0.3

*ARM = Anorectal manometry, RP = Resting pressure, MSP = Maximal squeeze pressure, FPV = First perceived volume, MTV = Maximal tolerable volume, PNTML = Pudendal nerve terminal motor latency, EAUS = Endoanal sonography, IAS = Internal anal sphincter, EAS = External anal sphincter

Table 4.3 Subgroup Analyses: Associations Between the Jorge-Wexner Score and Anorectal Investigation Results in Subgroups Separated by Gender, Urge, Passive, or Mixed Faecal Incontinence Symptoms

		Women n=423	Men n=115	Urge incontinence n=225	Passive incontinence n=30	Mixed symptoms n=283
ARM	RP	r=-0.10 95%CI(-0.20, -0.01) p=0.04	r=-0.36 95%CI(-0.51, -0.19) p<0.01	r=-0.05 95%CI(-0.18, 0.08) p=0.45	r=-0.20 95%CI(-0.52, 0.18) p=0.30	r=-0.34 95%CI(-0.45, -0.22) p=<0.01
	MSP	r=-0.19 95%CI(-0.28, -0.09) p<0.01	r=-0.33 95%CI(-0.48, -0.16) p<0.01	r=-0.08 95%CI(-0.21, 0.05) p=0.24	r=-0.05 95%CI(-0.40, 0.31) p=0.78	r=-0.29 95%CI(-0.41, -0.16) p=<0.01
Rectal sensation	FPV	r_s=0.07 95%CI(-0.03, -0.05) p=0.15	r_s=-0.14 95%CI(-0.32, 0.04) p=0.14	r_s=0.09 95%CI(-0.05, 0.21) p=0.21	r_s=-0.01 95%CI(-0.38, 0.36) p=0.96	r_s=-0.39 95%CI(-0.15, 0.08) p=0.51
	MTV	r_s=-0.03 95%CI(-0.13, 0.06) p=0.59	r_s=-0.24 95%CI(-0.40, -0.06) p=0.01	r_s=0.08 95%CI(-0.05, 0.21) p=0.24	r_s=-0.01 95%CI(-0.37, 0.37) p=0.99	r_s=-0.13 95%CI(-0.24, 0.01) p=0.03
PNTML	Left	r=-0.04 95%CI(-0.15, 0.07) p=0.46	r=0.28 95%CI(0.04, 0.48) p=0.02	r=-0.05 95%CI(-0.20, 0.10) p=0.53	r=0.18 95%CI(-0.32, 0.60) p=0.48	r=0.04 95%CI(-0.09, 0.17) p=0.56
	Right	r=0.01 95%CI(-0.11, 0.12) p=0.93	r=0.11 95%CI(-0.14, 0.34) p=0.40	r=0.06 95%CI(-0.10, 0.21) p=0.50	r=0.42 95%CI(-0.03, 0.72) p=0.07	r=0.08 95%CI(-0.06, 0.21) p=0.27
EAUS	IAS	t(421)=1.04 d=0.12 95%CI(-0.39, 1.28) p=0.30	t(113)=-2.26 d=0.58 95%CI(-4.38, -0.29) p=0.03	t(188)=0.92 d=0.16 95%CI(-0.59, 1.62) p=0.36	t(27)=-1.25 d=0.45 95%CI(-4.68, 1.15) p=0.22	t(207)=-0.39 d=0.06 95%CI(-1.42, 0.95) p=0.70

	EAS	t(407)=-1.11 d=0.11 95%CI(-1.17, 0.33) p=0.27	t(15.36)=0.14 d=0.05 95%CI(-1.83, 2.23) p=0.84	t(216)=-0.43 d=0.06 95%CI(-1.17, 0.76) p=0.67	t(27)=0.50 d=0.19 95%CI(-2.14, 3.55) p=0.62	t(267)=-0.92 d=0.13 95%CI(-1.51, 0.55) p=0.63

*ARM = Anorectal manometry, RP = Resting pressure, MSP = Maximal squeeze pressure, FPV = First perceived volume, MTV = Maximal tolerable volume, PNTML = Pudendal nerve terminal motor latency, EAUS = Endoanal sonography, IAS = Internal anal sphincter, EAS = External anal sphincter

Chapter 5: The Relationships Between the Results of Contemporary Anorectal Investigations and Faecal Incontinence Severity

5.1 Statement

The content of this chapter has been published in *Neurogastroenterology & Motility*.

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The co-authors have provided permission for the inclusion of the study in this thesis. The percentage contributions of each author to this study were as follows:

- Research design: **PH 100%**.
- Data collection: AS 33%, PR 33%, DW 33%.
- Data entry & analysis: **PH 100%**,
- Writing and editing: **PH 80%**, PR 5%, DW 5%, MS 5%, PD 5%.

5.2 Abstract

Background: Diagnostic investigations for faecal incontinence (FI) assess the structure and sensorimotor function of the anorectum. Investigations include anorectal manometry, anorectal sensory testing, pudendal nerve terminal motor latencies (PNTML), and endoanal sonography. The severity of FI and results of investigations are often discordant, and the rate of symptom resolution following treatment remains <40%. High-resolution anorectal manometry (HRAM) and three-dimensional endoanal ultrasound (3D-US) have been introduced during the last decade. This study aims to assess the strength of relationships between contemporary investigation results and FI severity. **Methods:** Adults presenting for investigation of FI were assessed using the St Mark's FI severity score (SMIS), HRAM, anorectal sensory testing, PNTML, and 3D-US. **Key results:** 246 patients were included. There were significant relationships between the SMIS and HRAM (resting pressure r_s=-0.23, 95%CI=(-0.34, -0.11), p<0.001; squeeze pressure r_s=-0.26, 95%CI=(-0.37, -0.14), p<0.001) and 3D-US (anterior EAS length r_s=-0.22, 95%CI=(-0.34, -0.09), p=0.001). The relationships between SMIS and HRAM had a greater effect size in those with urge-predominant symptoms (resting pressure: r_s=-0.40, 95%CI=(-0.57, -0.20), p<0.001; squeeze pressure: r_s=-0.34, 95%CI=(-0.52, -0.12), p=0.003). Overall, the variance in SMIS accounted for by anorectal investigations was 8.6% (R^2=0.098, adjusted R^2=0.086, p<0.001). **Conclusions & Inferences:** Anorectal investigation results are not strongly predictive of FI severity. These findings may reflect the multifactorial, heterogeneous pathophysiology of FI, the limitations of the SMIS and anorectal investigations, and contributing factors extrinsic to the anorectum.

5.3 Introduction

Faecal incontinence is a common symptom, affecting 6-15% of the community(186-188), incurring a substantial biopsychosocial burden, and considerable healthcare expenditure(627). The pathophysiology of faecal incontinence is varied and often multifactorial(190-192), which presents diagnostic and therapeutic challenges for treating clinicians(627). Loose stool consistency and faecal urgency are recognised risk factors(201). Common causes also include anal sphincter and/or pelvic floor injury(190, 193), altered rectal and anal canal sensation and compliance (hyper/hyposensitivity)(194), and rectal/perianal diseases and surgery(196, 197).

Concurrent symptoms of faecal incontinence and constipation may be a confounding feature when attempting to correlate symptoms and results of anorectal investigations. In paediatric and geriatric populations, the coexistence of both symptoms is well recognised(306, 665-669). This is now also increasingly recognised in adulthood, with recent studies suggesting that >40% of patients referred with either isolated faecal incontinence or constipation actually have concurrent symptoms of both(108, 306, 307, 586); an issue not recognised by the referring clinician in 80% of cases(670). As observed in children(306), rectal evacuation disorders in adults may contribute to worsening symptoms of faecal incontinence.

Diagnostic investigations assess the structure and sensorimotor function of the anorectum. These can include anorectal manometry, rectal/anal canal sensory testing, pudendal nerve terminal motor latencies (PNTML), and endoanal sonography(211). In many cases, the severity of incontinence and results of diagnostic anorectal investigations are discordant(220, 563, 628-630) and, despite detailed diagnostic work up, symptom resolution following treatment remains <40%(187). We demonstrated weak correlation (Pearson's r<0.3) between a symptom severity score and results from anorectal manometry (**Chapter 4**). Overall, anorectal investigation results accounted for <10% variance in symptom severity(563). Based upon these findings, we concluded that faecal incontinence severity is not a strong predictor of anorectal dysfunction.

A number of reasons were proposed to account for these findings. These included; the limitations of the Jorge-Wexner score; the heterogeneity of anorectal dysfunction; and contributing factors extrinsic to the anorectum. Our study also utilised low-resolution, water-perfused manometry and two-dimensional endoanal sonography, both of which have undergone significant technological advances during the last decade. High-resolution, solid-state manometry has increased the diagnostic yield in oesophageal motility disorders(117-119) and improved the accuracy of detecting motor patterns in colonic manometry recording(114). In anorectal studies, there is some evidence to suggest that high-resolution anorectal manometry (HRAM) may also provide improved sensitivity for detecting anorectal dysfunction(121, 318, 643).

Similarly, three-dimensional endoanal ultrasound (3D-US) has superseded two-dimensional sonography(671-673). 3D-US provides a multiplanar reconstruction of anal sphincter morphology, an assessment of sphincter length(674), and improved imaging of puborectalis(675). When compared with two-dimensional sonography, 3D-US has higher inter-observer agreement(672). Importantly, 3D-US provides an appreciation of the radial or longitudinal orientation of a sphincter defect(672, 673), which can assist in pre-operative planning.

Since completing data collection for the previous study(563), our anorectal clinic has upgraded to HRAM, 3D-US, and now use the St Mark's faecal incontinence severity score (SMIS)(209). The SMIS includes all five variables featured in the Jorge-Wexner score, while also including a measure of urgency and the use of anti-diarrhoeal medications. The SMIS is sensitive in detecting change when comparing pre- and post-intervention(676) and, due to its widespread use, provides consistency with other clinical research centres and collaborators(120).

The primary endpoint of this study was to determine the relationships between diagnostic anorectal investigations and faecal incontinence severity. The goals of treatment in faecal incontinence are, at best, to achieve full curative resolution of symptoms or, failing that, to aim for a significant reduction in symptom severity. Given that anorectal investigations are often performed both pre- and post-intervention, it is important to ascertain how these results relate to symptom severity as a means to inform clinicians, who may use these results in guiding management decisions and assessing management outcomes. In addition, we included

constipation severity in our assessment, to determine whether concurrent constipation was related to an increase in faecal incontinence severity.

5.4 Methods

Ethics approval was received from the Southern Adelaide Clinical Human Research Committee. A database was established for all adult patients who presented to Flinders Medical Centre, Australia, for investigation of faecal incontinence between January 2016-March 2019. Investigation included a symptom questionnaire, HRAM, rectal and anal canal sensation, pudendal nerve terminal motor latency (PNTML), and 3D-US.

5.4.1 Symptom Questionnaire

A SMIS(209) and Cleveland Clinic constipation score(257) were derived from questionnaire responses. The SMIS is a summative, seven-category score, including frequency of; (1) incontinence of solids; (2) incontinence of liquids; (3) incontinence of flatus; (4) lifestyle alteration; (5) use of continence pads or anal plugs; (6) use of anti-diarrhoeal medications, and; (7) ability to defer defaecation for 15 minutes. A frequency score from 0-4 is assigned for each of the first four categories (0=never, 1=rarely or <1/monthly, 2=sometimes or <1/weekly, 3=usually or <1/day, 4=always or >1/day), yes/no responses to the remaining three categories (weighted yes=2, no=0 for (5) and (6), and yes=4, no=0 for (7)) to produce a total score of 0-24. A score of ≥5/24 defines the presence of significant faecal incontinence(209).

An additional question was used to elicit features of urge incontinence, with the question: how often do you have to rush to the toilet to open your bowels (never, rarely or <1/monthly, sometimes or <1/weekly, usually or <1/day, always or >1/day). Passive incontinence was assessed with the question; do you know when you open your bowels (yes/no)?

The Cleveland Clinic constipation score(257) is a summative, eight-category score, including frequency of; (1) bowel movements; (2) painful evacuation; (3) incomplete evacuation; (4) abdominal pain; (5) minutes in lavatory per attempt; (6) type of assistance; (7) unsuccessful attempts, and; (8) duration of symptoms. A frequency score from 0-4 is assigned for all categories, with the exceptions of (6) which is scored 0-2 to produce a total score of 0-30. A score ≥9/30 defines the presence of constipation(257).

5.4.2 Anorectal Investigations

5.4.2.1 High-Resolution Anorectal Manometry (HRAM)

Bowel preparation was not routinely performed prior to clinical investigations. If significant faecal loading was identified during digital per rectal examination which would obstruct manometry catheter insertion, a

Microlax® enema was administered. A single clinical scientist performed all anorectal manometry studies. Patients were positioned in the left lateral decubitus position. A solid-state, high-resolution catheter was used, featuring five circumferential sensors spaced at 10mm (UniTip catheter K122359-L5-1323-D, Medical Measurement Systems, The Netherlands). The mean resting pressure and maximal squeeze pressure were recorded (normal ranges(317); resting pressure 33-101mmHg women, 38-114mmHg men; maximal squeeze pressure 90-397mmHg women, 94-590mmHg men).

5.4.2.2 Rectal Sensation

Rectal sensation was recorded using an intrarectal balloon which was inflated manually with air by increments of 10mL up to a total volume of 360mL. The volumes at which sensation was first perceived by the patient, first urge to defaecate, and the maximum tolerable volume were recorded (normal ranges(221, 317); first perceived volume 20mL-110mL women, 15mL-150mL men; first urge volume 40mL-200mL women, 40mL-190mL men; maximum tolerable volume 75mL-290mL women, 75mL-325mL men).

5.4.2.3 Anal Canal Sensation & Pudendal Nerve Terminal Motor Latency (PNTML)

Anal canal sensation and PNTML were recorded using a disposable, glove-mounted electrode(677) (13L40 St Mark's Pudendal Electrode™; Medtronic Functional Diagnostics A/S, Denmark). The stimulating electrode was positioned in the high, mid, and low anal canal, with electrical stimulation delivered at 0.1s duration, 5Hz, increasing in 1mA increments from 0-20mA. The patient was asked to identify the lowest current that induced a perceivable sensation at each station(678). Normal ranges were based upon those described using electrodes mounted on a Foley catheter; (1) high anal canal: 3.3-7.3mA; (2) mid anal canal: 2.0-6.0mA; (3) low anal canal: 3.0-7.0mA)(678).

To record PNTML, square wave stimuli were delivered via transrectal stimulation at the level of the ischial spine (0.05ms duration, 10mA, 1Hz). The time between the onset of the stimulus and compound muscle action potential response of the external anal sphincter was recorded as the PNTML. Normal latency was defined as <2.3ms for age <30, <2.4ms for age 30-60, and <2.5 for age >60(679).

5.4.2.4 Three-Dimensional Endoanal Sonography (3D-US)

3D-US images were recorded with a rotating endosonic probe (type 2052, BK Medical (Peabody, USA). High-frequency imaging (16MHz) was used to visualise the anal sphincter. In women, transvaginal 9Mhz imaging was used to assess for puborectalis avulsion. Images were interpreted by a consultant radiologist or a consultant colorectal surgeon. The internal anal sphincter (IAS), external anal sphincter (EAS), and puborectalis were categorized as normal, abnormal if a defect was visualised, or not identified. Where present, the circumferential extent of an anal sphincter defect was reported. IAS thickness (mm) and EAS length (anterior and posterior; mm) were also recorded (normal ranges(680); IAS thickness 30-49 years 11-22mm, ≥50 years 12-26mm; anterior EAS length 14-17mm women, 21-29mm men; posterior EAS length 23-28mm women, 26-30mm men).

5.4.3 Statistical Analysis

Bivariate analyses were performed between the SMIS and; (1) HRAM; resting pressure and maximal squeeze pressures; (2) anal canal sensation; low-, mid- , and high; (3) rectal sensation; first perceived volume, first urge volume, and maximum tolerable volume; (4) PNTML; left and right; (5) 3D-US; IAS/EAS/PR integrity, circumferential extent of defect to IAS/EAS, IAS thickness, and EAS length anterior/posterior. The SMIS results were not normally distributed (Shapiro-Wilk test $p<0.01$), so non-parametric analyses were used (Spearman rank-order correlation coefficient). The Mann-Whitney U test was used to determine whether patients with anal sphincter or puborectalis injuries visualised on 3D-US had a more severe SMIS when compared to patients who had intact musculature.

A multiple regression analysis was performed to assess the variation in the SMIS which could be attributed to the results of anorectal investigations. Anorectal investigations included in the model were those that had a statistically significant association to the SMIS on the bivariate analyses.

Bivariate analyses were also conducted between; (1) the SMIS and Cleveland Clinic constipation score in order to assess the relationship between faecal incontinence and constipation severity (Spearman rank-order correlation coefficient), and; (2) investigation results to assess their inter-relatedness, as a means to identify patterns of anorectal dysfunction. The associations between anorectal investigations were assessed using; (a) Spearman rank-order correlation coefficient for associations between the quantitative variables (HRAM, anal canal sensation, rectal sensation, PNTML, IAS thickness, EAS length); (b) Mann-Whitney U tests for the associations between the quantitative variables listed above and the categorical variables (3D-US results: EAS/IAS/puborectalis integrity), and; (c) Pearson Chi-square tests of association for associations between the categorical variables (3D-US results: EAS/IAS/puborectalis integrity).

Secondly, subgroup analyses were performed with patients grouped by; (1) gender, and; (2) symptom subtype (urge, passive, or mixed symptoms). This was conducted in an attempt to identify more homogenous subgroups within the sample, to ascertain whether particular phenotypes of anorectal dysfunction were present within these groups which were more strongly associated with the overall severity of symptoms.

Whilst the SMIS variable was not normally distributed (Shapiro-Wilk test $p<0.01$), the assumption of normality of residuals was not violated. This was determined by inspection of a histogram of residuals, of which the mean and standard deviation values were approximately 0 and 1 respectively (mean=2.39×10^{-16}, SD=0.993). This was further supported by inspection of the P-P plot of regression, where the residuals were closely aligned with the regression line. As a result, no transformations were performed to the variables included in the model.

Statistical analysis was performed using IBM SPSS (Version 19.0, Released 2010; IBM Corp., Armonk, New York, USA), in addition to "Psychometric" R package(635) and Cohen's d online calculator(636). A p-value of ≤0.05 was considered statistically significant. Bonferroni correction was applied to multiple statistical comparisons. Effect sizes and confidence intervals of 95% are reported.

5.5 Results

5.5.1 Sample Demographics and Anorectal Investigation Results

Two-hundred and fifty-three consecutive patients presented for investigation of primary symptoms of faecal incontinence. Seven patients (2.8%) recorded a SMIS of <5/24 (range 2-4/24), and were hence excluded from analysis. The overall study cohort therefore comprised 246 patients, including 210 women (85.4%) and 36 men (14.6%) with a median age of 65 years (range 19-91). The majority of the women were parous (90.5%, n=190), with a median of two children (range 1-6). Those describing urgency in isolation, having to rush to the toilet 'usually' or 'always', comprised 31.7% (n=78) of the cohort. Those with passive symptoms in isolation, having no knowledge of when involuntary leakage occurred, totaled n=26 (10.6%). The remaining 57.7% (n=142) reported mixed urge/passive symptoms.

The mean (±SD) symptom scores were: SMIS = 14.8±4.8, and Cleveland Clinic constipation score = 8.1±5.0. The majority of patients (n=209, 85.0%) reported stool frequency of 1-2 times per 1-2 days. Nevertheless, 115 patients (46.7%) reported a sensation of incomplete evacuation "usually" or "always". One hundred and six patients (43.1%) had concurrent faecal incontinence and constipation, with a Cleveland Clinic constipation score ≥9/30.

Table 5.1 details the frequencies of normal/abnormal results on anorectal investigations. **Table 5.2** details the inter-relatedness of the anorectal investigation results. Associations were consistent with recognised patterns of anorectal dysfunction in faecal incontinence, including;

1. External anal sphincter injury and reduced anal canal resting and squeeze pressures; sphincter injury resulting in functional sphincter weakness.
2. Reduced anal canal resting and maximal squeeze pressures; global anal sphincter weakness.
3. Reduced anal canal sensation (low, mid, and high); global anal canal hyposensitivity.
4. Reduced rectal sensation (first perceived volume, first urge volume, maximum tolerable volumes); global rectal hyposensitivity.
5. Delayed PNTML with reduced anal canal resting and squeeze pressures; pudendal motor neuropathy and anal sphincter weakness.

5.5.2 Bivariate Analyses: St Mark's Faecal Incontinence Severity Score (SMIS) and Anorectal Investigation Results

Correlations between SMIS and anorectal investigation results are detailed in **Table 5.3**. There were significant relationships (Bonferroni-adjusted $\alpha = 0.0025$ (0.05 / 20)) between the SMIS and HRAM (resting pressure and maximal squeeze pressure), and 3D-US (anterior EAS length).

No significant relationships (**Table 5.3**) were observed between the SMIS and rectal sensation, anal canal sensation, PNTML, 3D-US (IAS defect, EAS defect, puborectalis defect, circumferential extent of IAS/EAS defects, posterior EAS length, and IAS thickness).

There was no significant relationship between the SMIS and the Cleveland Clinic constipation score (r_s=0.14, 95%CI=(0.02, 0.26), p=0.025), or stool frequency (r_s=-0.02, 95%CI=(-0.15, 0.11), p=0.74). However, patients reporting the feeling of incomplete evacuation "usually" or "always" demonstrated more severe SMIS than those without this symptom (*U*=5685.00, r=-0.21, p=0.001). When selecting this group only, reporting incomplete evacuation "usually" or "always" (n=115), there was no correlation between the SMIS and Cleveland Clinic score (r_s=0.05, 95%CI=(-0.13, 0.23), p=0.58).

5.5.3 Multiple Regression Analysis: Variance in the St Mark's Faecal Incontinence Severity Score (SMIS) Accounted for by the Results of Anorectal Investigations

The multiple regression model included the three variables with significant associations to the SMIS on bivariate analyses; anal canal resting pressure, anal canal squeeze pressure, and anterior EAS length. This model significantly predicted SMIS ($F(3,213)=7.74$, $p<0.001$). The variance in the SMIS accounted for by this model was 8.6% (R^2=0.098, adjusted R^2=0.086). Regression coefficients and standard errors are displayed in **Table 5.4**.

Anal canal squeeze pressure was the only variable in the model with a statistically significant slope coefficient (**Table 5.4**). For every 0.02mmHg reduction in squeeze pressure, the SMIS score increased by 1. The physiological significance of this finding is uncertain. It seems unlikely that such a subtle reduction in pressure could confer such a substantial change in symptom severity (eg. a reduction in squeeze pressure by 1mmHg would therefore cause a five-point increase in the SMIS). With the 95% confidence interval approaching zero, this may instead reflect a type I error.

Anal canal resting pressure and anterior EAS length did not demonstrate statistically significant slope coefficients. No model building was performed to remove these variables, particularly given that both variables have theoretical importance to the severity of symptoms.

The cumulative effect of abnormal anorectal investigation results on the SMIS was also assessed by tallying the number of anorectal investigation results outside the normal range for each patient, and correlating with the SMIS (r_s=0.24, 95%CI=(0.12, 0.36), p<0.001). Notably, 11/246 (4.5%) returned results within the normal range on all investigations, whereas 59.8% (147/246) had ≥3 abnormal results (**Table 5.5**).

5.5.4 Subgroup Analyses

Bivariate analyses between the SMIS and anorectal investigation results were repeated for subgroups separated by gender and symptom subtype (urge/passive/mixed symptoms). Full results of the subgroup analyses are detailed in **Table 5.6**.

5.5.4.1 Women

When compared with the whole cohort analysis, significant associations (Bonferroni-adjusted $\alpha = 0.0038$ (0.05 / 13)) were additionally observed between the SMIS and rectal sensation (first perceived volume; r_s=0.25, 95%CI=(0.11, 0.37), p<0.001), and anal canal sensation (low r_s=0.27, 95%CI=(0.10, 0.44), p=0.003).

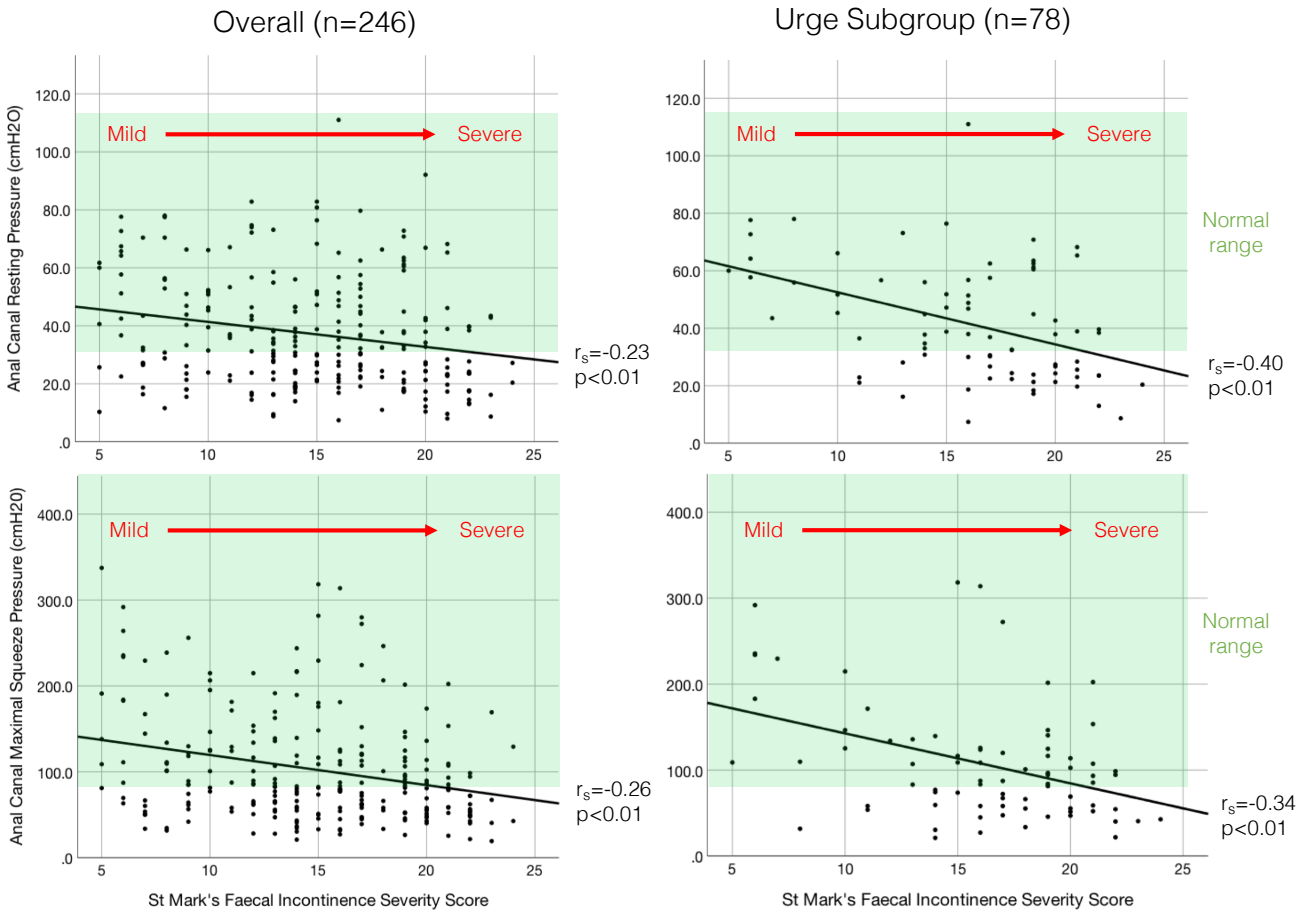

Figure 5.1 Scatterplots depicting the correlations between the St Mark's symptom severity score and anorectal manometry results (resting tone and maximal squeeze pressures).

5.5.4.2 Men

No significant relationships (Bonferroni-adjusted $\alpha = 0.0038$ (0.05 / 13)) were identified between the SMIS and any anorectal investigation result. There was a high incidence of abnormal anal canal sensation in men (**Table 5.1**). All men studied (100.0%) demonstrated abnormal anal canal sensation in the high and mid anal canal, with the majority demonstrating hypersensitivity (mid anal canal: hypersensitivity n=17/19, 89.5%; hyposensitivity 2/19, 10.5%; high anal canal: hypersensitivity n=12/19, 63.2%; hyposensitivity, n=7/19, 36.8%).

5.5.4.3 Urge Incontinence

Moderate effect sizes (Bonferroni-adjusted $\alpha = 0.0038$ (0.05 / 13)) were observed between HRAM and SMIS (resting pressure: r_s=-0.40, 95%CI=(-0.57, -0.20), p<0.001, maximal squeeze pressure: r_s=-0.34, 95%CI=(-0.52, -0.12), p=0.003; **Figure 5.1**).

5.5.4.4 Passive Incontinence

There were no significant associations between the SMIS and anorectal investigations in the passive incontinence subgroup (**Table 5.6**).

5.6 Discussion

In patients attending our clinic with a primary complaint of faecal incontinence, the strongest predictors of symptom severity were reduced anal canal resting and squeeze pressures in patients with urge incontinence. Overall, however, the relationships between anorectal investigations and faecal incontinence severity were generally weak, predicting <10% of the variance in severity. The majority of patients returned multiple abnormal results on different tests, supporting the contemporary view of the multifactorial pathophysiology of faecal incontinence.

In **Chapter 4**, we proposed a number of reasons for the discord between anorectal investigation results and symptom severity(563). One of our primary hypotheses was the potential technical and diagnostic limitations of low-resolution water perfused manometry and two dimensional endoanal sonography. To address this, we upgraded our equipment to high-resolution, solid-state anorectal manometry (HRAM) and three-dimensional endoanal ultrasound (3D-US). While the multiple regression analysis accounted for a slightly greater proportion of the variance in symptom severity in this study when compared with our previous study (8.6% versus 6%(563)), the updated techniques were still poor predictors of symptom severity. This finding is of interest because low resolution anorectal manometry and two-dimensional endoanal sonography are still in use in approximately half of all specialist anorectal centres(638).

Despite the improved sensor resolution on the HRAM equipment, we recorded and analysed the same variables in this study as we had with conventional manometry: resting and maximal squeeze pressures. Other studies have reported improved sensitivity for detecting anorectal dysfunction via the calculation of functional metrics using HRAM results(121, 318, 643). HRAM functional metrics may have a stronger correlation with symptom severity that the standard analysis techniques used in this study, however this is yet to be determined.

Using two-dimensional sonography, we had previously demonstrated a relationship between anal sphincter defects and symptom severity in men only(563). The results of 3D-US, used in this study, had a significant correlation with symptom severity in the cohort overall. Of particular interest was the correlation between external anal sphincter length measurements and symptom severity – an added utility of 3D-US. In our clinical experience, some patients have 3D-US imaging which does not appear completely normal, but it is difficult to define a specific defect. The measure of short sphincter length may indicate a significant injury (obstetric or otherwise) which increases the risk of developing faecal incontinence. In our data, an EAS defect was associated with shorter anterior and posterior EAS length measurements. Whilst internal anal sphincter atrophy and degeneration is increasingly recognised to be of pathological importance, *increased* IAS thickness is observed in rectal intussusception and/or rectal prolapse, which can also contribute to faecal incontinence(608, 681, 682). A greater Oxford rectal prolapse grade(683) is associated with increased faecal incontinence severity(609).

The subgroup analysis indicated that anal canal hyposensitivity was associated with more severe incontinence scores in women. There was a high incidence of anal canal sensory abnormalities overall (**Table 5.1**), most notably in men (100% abnormal results in men in high and mid anal canal). This is consistent with recent studies which suggest that afferent anal canal sensory dysfunction contributes to the pathophysiology of faecal incontinence(684). In a 2019 study by Mundet et al.(613), approximately half of women with faecal incontinence demonstrated anal sensory evoked potentials outside of the normal range. In our study, the proportion of women with abnormal anal canal sensation was even higher (56.8%-89.4%, **Table 5.1**). However, it is important to note that techniques used for measuring anal canal sensation differ amongst groups. In our study we used a glove-mounted St Mark's electrode(677), whereas other studies have used electrodes mounted on a probe(613) or a Foley catheter(678). Therefore, differences in results may reflect the differing recording techniques.

Bharucha et al.(220) demonstrated no relationships between faecal incontinence severity and anorectal manometry, endoanal sonography, or rectal sensory testing in a cohort of 52 women. In that study, the only investigation correlated with symptom severity was dynamic magnetic resonance imaging (MRI). Dynamic MRI was used to assess puborectalis function, with dysfunction defined as a reduction of <11% in the anorectal angle between rest and squeeze(220). Notably, however, only one third of their cohort had puborectalis dysfunction. Our findings did not demonstrate any relationship between puborectalis injury and faecal incontinence severity. Whilst endoanal sonography remains the gold standard for assessing the integrity of the

internal anal sphincter (685), MRI provides more detailed imaging of the external anal sphincter and puborectalis(686, 687). When present however, puborectalis defects are most commonly associated with anal sphincter injury, with isolated puborectalis defects accounting for <10% of all puborectalis injuries in faecal incontinence(688). In our cohort, the incidence of an isolated puborectalis defect was substantially higher (57.7%). MRI imaging may have therefore provided additional diagnostic information in this study.

Another potential confounder when attempting to correlate symptom severity with anorectal investigation results is the heterogeneity of our patient population. In an attempt to address this, we performed subgroup analyses based upon gender and symptom subtype (urge/passive/mixed symptoms). The subgroup analysis demonstrated clearer patterns of association and phenotypes in some areas. Most notably, reduced anal canal manometry pressures in patients with urge incontinence were associated with a higher SMIS; a result which supports the findings of previous studies(216). The absence of any significant findings in the subgroup with passive incontinence is of uncertain significance. While some may suggest that a complete discord between symptom severity and investigation results provides an argument not to investigate this subgroup, it should be noted that this subgroup was small (n=26) and may have been underpowered. Passive incontinence is often associated with an evacuation disorder(190). A limitation of this study is the omission of routine testing of evacuatory function, which may have been particularly salient in those with passive incontinence. Defaecography is performed selectively in our institution, when there is a clinical suspicion of evacuation disorder based upon history and/or examination findings.

The use of a symptom score to quantify severity is a limitation in the interpretation of these findings. Another potential criticism is our use of the Jorge-Wexner score in **Chapter 4** and the SMIS in this study. Irrespective of the score used, our findings were similar. Both the Jorge-Wexner and SMIS scores receive criticism for similar features; (1) equivalent weighting of incontinence to solids, liquids, and flatus(651); (2) inclusion of continence pad use, which may be confounded by patient fastidiousness and concurrent urinary incontinence(209, 654); (3) use of vague quantifiers (e.g. “sometimes”) which are subject to patient interpretation(651), and; (4) inclusion of coping mechanisms such as continence pad use or quality of life measures such as lifestyle alteration, which may infer a greater severity of symptoms but are not a direct measure of symptoms(651). Additionally, four of the seven question categories produce a score based upon frequency of symptoms. While more frequent symptoms may be considered more severe in some instances, frequency of symptoms does not necessarily correspond with severity. Also, urgency is heavily weighted, and is assessed using the respondent’s ability to defer defaecation for 15 minutes. This may be viewed as an ability to delay evacuation, rather than describing the experience of urgency.

An additional confounder not addressed in **Chapter 4** is the potential overlap between the symptoms of constipation and faecal incontinence. While all of our patients had faecal incontinence, they were not routinely assessed for constipation as per Rome IV criteria(184). Yet, almost half of the cohort (46.7%) reported the sensation of incomplete evacuation “usually” or “always”, which was associated with a more severe SMIS.

Furthermore, 43.1% of patients had a Cleveland Clinic constipation score ≥9/30, which defines the presence of constipation (257). Our analysis demonstrated no significant correlation between symptom severity scores of constipation and faecal incontinence. We hypothesised that this result may have been diminished by those without constipation, but severe incontinence, in the cohort. We also performed a subgroup analysis including only those reporting the sensation of incomplete evacuation "usually" or "always" (n=115), which again demonstrated no significant relationship between the SMIS and Cleveland Clinic constipation scores. However, this would have only captured those with constipation due to obstructed defaecation, but not necessarily those with slow transit constipation.

Our cohort had a higher median age (65 years) than most other series. Few normative datasets will include many participants in this age group and, as a result, we must question the applicability of normal ranges to our patient population.

Extrinsic "supra-sphincteric"(185, 346) contributing factors are of importance in faecal incontinence, which are not recorded by routine anorectal investigations. The structural and functional integrity of the pelvic floor is not assessed beyond the anal sphincter and puborectalis. While efferent pudendal nerve integrity is evaluated, the pelvic floor is innervated by a dense network of sacral nerves branching from $S_{2\text{-}4}$ nerve roots. Full afferent and efferent neurophysiological assessment has been described in a number of studies, using local, trans-lumbar, trans-sacral, or trans-cranial stimulation, and assessment of cortical responses and evoked potentials(613, 689-691). Colonic motility is also of critical importance in the normal physiology of transit and defaecation(136, 137, 224). In the control of continence, the cyclic motor pattern in the distal colon is theorised to act as an "intrinsic colonic gatekeeper"(116), functioning as a "rectosigmoid brake"(116, 176) (**1.6.3 The Cyclic Motor Pattern**). A reduction or absence of retrograde activity in the rectosigmoid has been hypothesised to be contributory to faecal incontinence(173).

5.7 Conclusion

Anorectal dysfunction is not a strong predictor of faecal incontinence severity, as measured by the St Mark's faecal incontinence severity score. Contemporary tests of anorectal structure and sensorimotor function relate similarly with severity of symptoms when compared with conventional anorectal investigations (**Chapter 4**). These findings are likely to reflect the multifactorial, heterogeneous pathophysiology of faecal incontinence, the limitations of anorectal investigations and symptom scoring, and factors contributing to symptoms which are extrinsic to the anorectum.

5.8 Tables

Table 5.1 Frequencies of Normal/Abnormal Anorectal Investigation Results Separated by Gender

		Women			Men		
		Normal Count, % of valid	Abnormal Count, % of valid	Missing Count, % of total	Normal Count, % of valid	Abnormal Count, % of valid	Missing Count, % of total
HRAM	RP	n=92, 43.8%	n=118, 56.2%	-	n=27, 75.0%	n=9, 25.0%	-
	MSP	n=74, 35.2%	n=136, 64.8%	-	n=32, 88.9%	n=4, 11.1%	-
Anal canal sensation	Low	n=51, 43.2%	n=67, 56.8%	n=92, 43.8%	n=6, 31.6%	n=13, 68.5%	n=17, 47.2%
	Mid	n=18, 15.0%	n=102, 85.0%	n=90, 42.9%	-	n=19, 100.0%	n=17, 47.2%
	High	n=12, 10.6%	n=101, 89.4%	n=97, 46.2%	-	n=19, 100.0%	n=17, 47.2%
Rectal sensation	FPV	n=175, 84.1%	n=33, 15.9%	n=2, 1.0%	n=31, 86.1%	n=5, 13.9%	-
	FUV	n=184, 88.9%	n=23, 11.1%	n=3, 1.4%	n=27, 75.0%	n=9, 25.0%	-
	MTV	n=173, 86.1%	n=28, 13.9%	n=9, 4.3%	n=32, 91.4%	n=3, 8.6%	n=1, 2.6%

PNTML	Left	n=117, 57.1%	n=88, 42.9%	n=5, 2.4%	n=19, 57.6%	n=14, 42.4%	n=3, 8.3%
	Right	n=101, 49.5%	n=103, 50.5%	n=6, 2.9%	n=24, 72.7%	n=9, 27.3%	n=3, 8.3%
3D-US	IAS intact/defect	n=165, 78.9%	n=44, 21.1%	n=1, 0.5%	n=31, 86.1%	n=5, 13.9%	-
	IAS thickness	n=131, 70.1%	n=56, 29.9%	n=23, 11.0%	n=31, 88.6%	n=4, 11.4%	n=1, 2.8%
	EAS intact/defect	n=162, 77.5%	n=47, 22.5%	n=1, 0.5%	n=35, 97.2%	n=1, 2.8%	-
	EAS length anterior	n=71, 38.4%	n=114, 61.6%	n=25, 11.9%	n=11, 34.4%	n=2, 65.6%	n=4, 11.1%
	EAS length posterior	n=35, 18.6%	n=153, 81.4%	n=22, 10.5%	-	n=32, 100.0%	n=4, 11.1%

	PR intact/defect	n=164, 78.5%	n=45, 21.5%	n=1, 0.5%	n=36, 100.0%	-	-

Abbreviations: HRAM (high-resolution anorectal manometry), PNTML (Pudendal nerve terminal motor latency), 3D-US (three-dimensional endoanal sonography), RP (resting pressure), MSP (maximal squeeze pressure, FPV (first perceived volume), FUV (first urge volume), MTV (maximum tolerable volume), IAS (internal anal sphincter), EAS (external anal sphincter), PR (puborectalis)

Table 5.2 Associations Between Anorectal Investigation Results

Legend: (Bonferroni-adjusted $\alpha = 0.0033$ (0.05 / 15)). Significant correlations are highlighted in bold text.

		HRAM		Anal Canal Sensation			Rectal Sensation			PNTML		3D-US				
		RP	MSP	Low	Mid	High	FPV	FUV	MTV	Left	Right	IAS intact/defect	IAS thickness	EAS intact/defect	EAS length (anterior)	EAS length (posterior)
HRAM	MSP	**r_s=0.52 p<0.001**														
Anal canal sensation	Low	r_s=-0.16 p=0.07	r_s=-0.09 p=0.31													
	Mid	r_s=-0.19 p=0.03	r_s=0.07 p=0.45	**r_s=0.59 p<0.001**												
	High	r_s=-0.11 p=0.29	r_s=-0.03 p=0.81	**r_s=0.39 p<0.001**	**r_s=0.55 p<0.001**											

Rectal sensation	FPV	r_s=0.01 p=0.91	r_s=0.01 p=0.87	**r_s=0.37 p<0.001**	r_s=0.21 p=0.02	**r_s=0.35 p<0.001**										
	FUV	r_s=0.03 p=0.66	r_s=0.07 p=0.26	**r_s=0.33 p<0.001**	r_s=0.21 p=0.02	r_s=0.21 p=0.04	**r_s=0.88 p<0.001**									
	MTV	r_s=0.04 p=0.58	r_s=0.18 p=0.007	r_s=0.23 p=0.009	r_s=0.21 p=0.02	r_s=0.11 p=0.24	**r_s=0.63 p<0.001**	**r_s=0.79 p<0.001**								
PNTML	Left	r_s=-0.12 p=0.07	**r_s=-0.28 p<0.001**	r_s=0.10 p=0.28	r_s=0.10 p=0.30	r_s=0.14 p=0.18	r_s=-0.07 p=0.28	r_s=-0.06 p=0.41	r_s=-0.06 p=0.39							
	Right	**r_s=-0.22 p=0.001**	**r_s=-0.27 p<0.001**	r_s=-0.10 p=0.30	r_s=-0.03 p=0.79	r_s=-0.07 p=0.48	r_s=-0.07 p=0.30	r_s=-0.09 p=0.21	r_s=-0.13 p=0.06	**r_s=0.40 p<0.001**						
3D-US	IAS infact/defect	*U*=3940 p=0.05	*U*=4298 p=0.26	*U*=1200 p=0.80	*U*=826 p=0.16	*U*=565 p=0.22	*U*=4343 p=0.35	*U*=3886 p=0.05	*U*=3604 p=0.05	*U*=3543 p=0.41	*U*=3666 p=0.79					
	IAS thickness	r_s=0.02 p=0.78	r_s=-0.16 p=0.02	r_s=0.14 p=0.14	r_s=0.10 p=0.31	r_s=0.23 p=0.03	**r_s=0.22 p=0.001**	**r_s=0.20 p=0.003**	**r_s=0.21 p=0.002**	r_s=0.20 p=0.006	r_s=0.16 p=0.02	*U*=2663 p=0.51				
	EAS intact/defect	***U*=2953 p<0.001**	***U*=2975 p<0.001**	*U*=1257 p=0.87	*U*=1021 p=0.55	*U*=559 p=0.12	*U*=4643 p=0.93	*U*=4176 p=0.27	*U*=3361 p=0.01	*U*=3596 p=0.40	*U*=2859 p=0.01	**χ^2(1)=49.0 p<0.001**	*U*=3180 p=0.96			

	EAS length (anterior)	**r_s=0.46 p<0.001**	**r_s=0.58 p<0.001**	r_s<0.01 p=0.93	r_s=-0.07 p=0.43	r_s=-0.11 p=0.27	r_s=0.07 p=0.32	r_s=0.14 p=0.05	r_s=0.14 p=0.05	r_s=-0.12 p=0.09	**r_s=-0.25 p<0.001**	*U*=2150 p=0.008	r_s=-0.17 p=0.02	***U*=827 p<0.001**		
	EAS length (posterior)	**r_s=0.35 p<0.001**	**r_s=0.36 p<0.001**	r_s=0.02 p=0.81	r_s=-0.09 p=0.32	r_s=-0.19 p=0.07	r_s=0.14 p=0.03	r_s=0.18 p=0.008	r_s=0.18 p=0.009	r_s=-0.11 p=0.11	r_s=-0.18 p=0.01	*U*=3217 p=0.63	r_s<0.01 p=0.91	***U*=2089 p<0.001**	**r_s=0.53 p<0.001**	
	PR	*U*=4354 p=0.73	*U*=4078 p=0.33	*U*=1170 p=0.26	*U*=1138 p=0.75	*U*=872 p=0.99	*U*=3891 p=0.18	*U*=3622 p=0.06	*U*=4034 p=0.68	*U*=3383 p=0.58	*U*=3258 p=0.31	$\chi^2(1)$=4.3 p=0.04	***U*=2589 p=0.002**	$\chi^2(1)$=1.8 p=0.19	*U*=2752 p=0.02	*U*=3010 p=0.11

Abbreviations: HRAM (high-resolution anorectal manometry), PNTML (Pudendal nerve terminal motor latency), 3D-US (three-dimensional endoanal sonography), RP (resting pressure), MSP (maximal squeeze pressure, FPV (first perceived volume), FUV (first urge volume), MTV (maximum tolerable volume), IAS (internal anal sphincter), EAS (external anal sphincter), PR (puborectalis)

Table 5.3 Associations Between the St Mark's Incontinence Severity Score and Anorectal Investigation Results

Legend: (Bonferroni-adjusted $\alpha = 0.0027$ (0.05 / 18)). Significant correlations are highlighted in bold text.

		St Mark's Incontinence Severity Score
HRAM	RP	**r_s=-0.23, 95%CI=(-0.34, -0.11), p<0.001**
	MSP	**r_s=-0.26, 95%CI=(-0.37, -0.14), p<0.001**
Anal canal sensation	Low	r=0.24, 95%CI=(0.07, 0.39, p=0.007
	Mid	r_s=0.18, 95%CI=(0.01, 0.35), p=0.04
	High	r_s=0.14, 95%CI=(-0.06, 0.33), p=0.16
Rectal sensation	FPV	r_s=0.15, 95%CI=(0.02, 0.27), p=0.021
	FUV	r_s=0.11, 95%CI=(-0.02, 0.23), p=0.10
	MTV	r_s=0.02, 95%CI=(-0.11, 0.15), p=0.76
PNTML	Left	r_s=0.17, 95%CI=(0.04, 0.30), p=0.01
	Right	r_s=0.17, 95%CI=(0.04, 0.30), p=0.01
3D-US	IAS intact/defect	*U*=4175.00, r=-0.04, p=0.58

	IAS circumferential extent of defect	r_s=0.05, 95%CI=(-0.29, 0.38), p=0.79
	IAS thickness	r_s=0.17, 95%CI=(0.04, 0.30), p=0.009)
	EAS intact/defect	*U*=3621.50, r=-0.12, p=0.06
	EAS circumferential extent of defect	r_s=-0.23, 95%CI=(-0.54, 0.15), p=0.23
	EAS length (anterior)	**r_s=-0.22, 95%CI=(-0.34, -0.09), p=0.001**
	EAS length (posterior)	r_s=-0.16, 95%CI=(-0.29, -0.03), p=0.016
	PR intact/defect	*U*=3863.00, r=-0.02, p=0.73

Abbreviations: HRAM (high-resolution anorectal manometry), PNTML (Pudendal nerve terminal motor latency), 3D-US (three-dimensional endoanal sonography), RP (resting pressure), MSP (maximal squeeze pressure, FPV (first perceived volume), FUV (first urge volume), MTV (maximum tolerable volume), IAS (internal anal sphincter), EAS (external anal sphincter), PR (puborectalis)

Table 5.4 Results of the Multiple Regression Analysis on the St Mark's Faecal Incontinence Severity Score

	Unstandardized regression coefficients (β)	Standard error of the coefficients	Standardized coefficients	95% Confidence intervals	Significance
Constant	18.17	0.92		16.36, 19.97	**p<0.001**
Anal canal resting pressure	-0.02	0.02	-0.08	-0.06, 0.02	p=0.32
Squeeze pressure	-0.02	0.01	-0.20	-0.03, -0.02	**p=0.02**
EAS length: anterior	-0.06	0.06	-0.09	-0.17, 0.06	0.33

Table 5.5 Frequency Table Displaying the Number of Anorectal Investigation Results Outside of the Normal Range for Each Patient

There were eight summarised anorectal investigation metrics including anorectal manometry: (1) resting pressure, (2) maximal squeeze pressure, (3) rectal sensation (≥1 abnormal result recorded from first perceived volume, first urge volume, maximal tolerable volume), (4) PNTML (unilateral and/or bilateral abnormality), (5) anal canal sensation (≥1 abnormal result recorded from low, mid, and high anal canal), and 3D-US findings of: (6) puborectalis, (7) IAS, and (8) EAS.

Number of abnormal results	Frequency (n=)	Cumulative frequency (%)
0	11	4.5
1	34	18.3
2	54	40.2
3	51	61.0
4	46	79.7
5	31	92.3
6	14	98.0
7	5	100.0
8	0	100.0

Table 5.6 Subgroup Analyses: Associations Between the St Mark's Faecal Incontinence Severity Score and Anorectal Investigation Results in Subgroups Separated by Gender and Symptom Subtype

Legend: (Bonferroni-adjusted $\alpha = 0.0038$ (0.05 / 13)). Significant correlations are highlighted in bold text.

		Women n=210	Men n=36	Urge incontinence n=78	Passive incontinence n=26	Mixed symptoms n=142
HRAM	RP	r_s=-0.19 p=0.006	r_s=-0.18 p=0.29	**r_s=-0.40 p<0.001**	r_s=-0.09 p=0.65	r_s=-0.21 p=0.01
	MSP	**r_s=-0.22 p=0.002**	r_s=-0.03 p=0.85	**r_s=-0.34 p=0.003**	r_s=-0.36 p=0.07	r_s=-0.22 p=0.01
Anal canal	Low	**r_s=0.27 p=0.003**	r_s=0.08 p=0.75	r_s=0.21 p=0.17	r_s=0.54 p=0.07	r_s=0.25 p=0.03
	Mid	r_s=0.15 p=0.13	r_s=0.50 p=0.04	r_s=0.24 p=0.12	r_s=0.29 p=0.39	r_s=0.20 p=0.11
	High	r_s=0.20 p=0.07	r_s=-0.36 p=0.24	r_s=-0.02 p=0.93	r_s=-0.59 p=0.12	r_s=0.29 p=0.03
Rectal sensation	FPV	**r_s=0.25 p<0.001**	r_s=-0.25 p=0.15	r_s=0.12 p=0.29	r_s=-0.03 p=0.87	r_s=0.24 p=0.005
	FUV	r_s=0.20 p=0.004	r_s=-0.13 p=0.44	r_s=0.11 p=0.33	r_s=-0.06 p=0.78	r_s=0.16 p=0.06
	MTV	r_s=0.10 p=0.15	r_s=-0.21 p=0.22	r_s=0.10 p=0.39	r_s=-0.02 p=0.93	r_s=0.05 p=0.56
PNT	Left	**r_s=0.22 p=0.003**	r_s=-0.23 p=0.23	r_s=0.19 p=0.13	r_s=-0.02 p=0.94	r_s=0.19 p=0.03

	Right	r_s=0.16 p=0.025	r_s=0.02 p=0.93	r_s=0.28 p=0.02	r_s=0.26 p=0.21	r_s=0.14 p=0.12
3D-US	IAS	*U*=3275.50 r=-0.07 p=0.32	*U*=66.50 r=-0.08 p=0.61	*U*=326.00 r=-0.06 p=0.59	*U*=37.50 r=-0.27 p=0.17	*U*=1478.00 r=-0.12 p=0.17
	EAS	*U*=3174.50 r=-0.12 p=0.08	*U*=9.50 r=-0.13 p=0.44	*U*=347.00 r=-0.17 p=0.13	*U*=53.50 r=-0.08 p=0.69	*U*=1223.00 r=-0.14 p=0.09
	PR	*U*=3484.00 r=-0.04 p=0.57	No PR injuries identified in men	*U*=462.00 r<-0.01 p=0.97	*U*=12.00 r=-0.01 p=0.95	*U*=1572.50 r=-0.03 p=0.74

Abbreviations: HRAM (high-resolution anorectal manometry), PNTML (Pudendal nerve terminal motor latency), 3D-US (three-dimensional endoanal sonography), RP (resting pressure), MSP (maximal squeeze pressure, FPV (first perceived volume), FUV (first urge volume), MTV (maximum tolerable volume), IAS (internal anal sphincter), EAS (external anal sphincter), PR (puborectalis)

Chapter 6: An Assessment of Colonic Motor Function During Stimulated Defaecation in Children with Treatment-Refractory Constipation

6.1 Statement

The content of this chapter has been published in *Neurogastroenterology & Motility*.

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The co-authors have provided permission for the inclusion of the study in this thesis. The percentage contributions of each author to this study were as follows:

- Research design: **PH 80%**. CDL 5%, SN 5%, MB 5%, PD 5%.
- Data collection : NT 9.1%, OB 9.1%, CDL 9.1%, DY 9.1%, DB 9.1%, MV 9.1%, SN 9.1%, KEC 9.1%, AK 9.1%, MB 9.1%, IK 9.1%.
- Data management and analysis: **PH 95%**, LW 5%.
- Writing and editing: **PH 80%**, NT 2.2%, OB 2.2%, CDL 2.2%, SN 2.2%, MB 2.2%, IK 2.2%, DW 2.2%, SB 2.2%, PD 2.2%.

6.2 Abstract

Background: Colonic manometry with intraluminal bisacodyl infusion can be used to assess colonic neuromuscular function in children with treatment-refractory constipation. If bisacodyl does not induce high-amplitude propagating contractions (HAPCs), this can be an indication for surgical intervention. A detailed characterisation of the colonic response to intraluminal bisacodyl in children with constipation may help to inform clinical interpretation of colonic manometry studies. **Methods:** Studies were performed in five paediatric hospitals. Analysis included identification of HAPCs, reporting HAPCs characteristics, and an area under the curve (AUC) analysis. Comparisons were performed between hospitals, catheter type, placement techniques, and site of bisacodyl infusion. **Results:** 165 children were included (median age 10 years, range 1-17 years; n=96 girls). 1893 HAPCs were identified in 154 children (12.3±8.8 HAPCs/child, 0.32±0.21 HAPCs/min; amplitude 113.6±31.5mmHg; velocity 8.6±3.8mm/s, propagation length 368mm±175mm). The mean time to first HAPC following bisacodyl was 553s±669s. Prior to the first HAPC, there was no change in AUC when comparing pre- vs post-bisacodyl (Z=-0.53, p=0.60). The majority of HAPCs terminated in a synchronous pressurisation in the rectosigmoid. Defaecation was associated with HAPCs ($\chi^2(1)=7.04$, $p<0.01$). Site of bisacodyl administration, catheter type, and hospital location did not alter the response. **Conclusions & Inferences:** Intraluminal bisacodyl induced HAPCs in 93% of children with treatment-refractory constipation. The bisacodyl response is characterised by ≥1 HAPC within 12 minutes of infusion. The majority of HAPCs terminate in a synchronous pressurisation in the rectosigmoid. Optimal clinical management based upon colonic manometry findings is yet to be determined.

6.3 Introduction

Constipation is one of the most common gastrointestinal symptoms(692, 693). In both adults and children with constipation, colonic manometry studies have recorded abnormal colonic responses to meals and morning awakening(115, 249, 250). However, these responses remain poorly defined, even in healthy adults. Chemical stimuli can induce distinctive, rapid colonic responses that allow researchers and clinicians to assess colonic neuromuscular function. Of particular diagnostic interest is the colonic response to intraluminal infusion of bisacodyl.

Bisacodyl is a stimulant laxative, belonging to the group of diphenylmethanes. In the colon, diphenylmethanes are hydrolysed to their active metabolites, which are hypothesised to exert a local prokinetic effect(277-279) and stimulate intestinal secretion(277, 280, 281). Application of bisacodyl to the colonic mucosa induces rapid, distinctive propagating motor patterns(110), which have been labelled 'high-amplitude propagating contractions' (HAPCs)a(100).

In some paediatric hospitals, colonic manometry with intraluminal bisacodyl infusion is used as a diagnostic modality to assess colonic neuromuscular function in children with treatment-unresponsive (refractory)

constipation(79, 122, 123, 261-263). There are some site-specific differences in equipment and study protocol, including; (1) water-perfused or solid-state catheters; (2) variable sensor count and resolution/spacing, and; (3) catheter placement techniques, all of which may influence results. Initiation of HAPCs following bisacodyl is considered to be a "normal" colonic response(264-269). A diagnosis of an "abnormal" bisacodyl response has a significant bearing on the clinical course of the child, as it can be an indication for surgical intervention(262, 264, 270-274). Surgical management can include anal sphincter botulinum toxin injection(694), caecostomy/appendicostomy for administration of antegrade continence enemas(271, 695), ileostomy or colostomy formation(273, 696), and/or partial/total colectomy(697, 698).

Given the importance of the colonic response to intraluminal bisacodyl in children, a detailed characterisation of the induced motor patterns is critical to inform the interpretation of these findings. Previous smaller studies have reported the time interval between bisacodyl administration and the first HAPC, as well as the amplitude and frequency of HAPCs (699, 700). The "completeness" of the HAPCs is also described, with previous studies demonstrating that HAPCs terminating >15cm from the anal verge may be pathological(701). The colonic bisacodyl response is comparable when bisacodyl is infused into the left(169, 702) or right hemicolon(250), and is dose-dependent with higher doses of bisacodyl being associated with an increase in length of HAPC propagation and total HAPC count(703).

In this study, we have collated colonic manometry data from five quaternary paediatric hospitals in the United States of America (USA), the United Kingdom (UK), and the Netherlands (NL), with the aim of characterising the colonic response to intraluminal infusion of bisacodyl in children with treatment-refractory constipation. In addition, we assessed whether hospital site, catheter type, placement technique, or site of drug administration had any influence upon the recorded colonic response.

6.4 Methods

This study involved a retrospective analysis of de-identified clinical data. Local institution review board approval was granted. A data sharing agreement between the participating hospitals was enacted in accordance with US legislation (HIPAA).

Children referred to a quaternary paediatric hospital for investigation of constipation refractory to intensive medical treatment were included in the study. Medical treatment included a variable combination of per oral osmotic and stimulant laxatives including one, or a combination of, the following; bisacodyl, polyethylene glycol with electrolytes, sodium picosulfate, prucalopride, lubiprostone, senna, lactulose, magnesium oxide, and/or magnesium citrate. Regular per rectal enemas and/or rectal irrigation were additionally included in the treatment regimens for some children.

Colonic manometry studies were performed at five hospitals; Great Ormond Street Hospital, UK; Nationwide Children's Hospital, USA; Boston Children's Hospital, USA; Cincinnati Children's Hospital Medical Centre, USA; and the Emma Children's Hospital, Amsterdam UMC, NL. Data analysis was performed at Flinders University, Australia.

Catheter placement was achieved using either;

1. Colonoscopy, performed under general anaesthesia with propofol. A snare was passed through the biopsy channel of the colonoscope to grasp a suture loop attached to the tip of the catheter. The catheter was then placed under direct vision and, in some instances, fixed to the colonic mucosa using a haemostatic clip. Alternatively, a guide wire was advanced through the colonoscope and an exchange performed under fluoroscopy, with advancement of the catheter over the wire until it reached the caecum.
2. Fluoroscopy(704), performed under general anaesthesia with propofol. A guidewire was used to position the catheter under radiological guidance without colonoscopy. No haemostatic clips were used for fixation via this approach.

An abdominal x-ray was performed pre- and post-study to confirm catheter position. The high-resolution manometry catheters were manufactured by Unisensor, Switzerland (solid state) or Mui Scientific, Canada (water-perfused). All five hospitals used the same signal conditioning and recording equipment (MMS, The Netherlands/Laborie, Canada). All recordings were made at 10Hz and prior to placement all catheters were calibrated in a pressure chamber between 0-100mmHg. The specific details of total sensor count, sensor spacing, placement techniques, and study protocol are detailed for each hospital in **Table 6.1**.

6.4.1 Data analysis

Analysis of colonic manometry data was performed using software (PlotHRM) developed by one of the authors (LW). In-house software PlotHRM has been described in previous publications(2, 114). PlotHRM produces a visual display of the manometry tracing, allowing for manual identification and labeling of motor patterns. Once a motor pattern is labeled, PlotHRM captures the amplitude of pressure waves, length of propagation, the recording sensor at which the contractions commenced and terminated, and the velocity of propagation.

6.4.2 High-Amplitude Propagating Contractions

Manual analysis of the manometry tracings was performed in PlotHRM. All HAPCs that occurred in the pre- and post-prandial and post-bisacodyl periods were identified and labelled. Pressure waves which propagated ≥30cm with an amplitude ≥75mmHg were classified as HAPCs(11).

6.4.3 Colonic Motor Patterns

In children with an absence of HAPCs, visual identification and labelling of other recognised colonic motility patterns was performed to assess the presence of any contractile activity. This included identification of low-amplitude propagating contractions(2). Low-amplitude propagating contractions were classified as pressure waves which propagated ≥30cm with an amplitude ≤75mmHg, thereby not meeting the amplitude criteria for HAPCs.

6.4.4 Statistics

Descriptive statistics were reported for patient demographics, catheter type, catheter placement approach, site of bisacodyl infusion, frequency of positive tests, time from bisacodyl infusion to first HAPC (mean±SD), characteristics of HAPCs (count, frequency, velocity, amplitude, and length of propagation), and whether or not the child defaecated during the study. As there were differences in catheter length and the location of the most proximal sensor (caecum, ascending, transverse, descending, or sigmoid colon), the recorded length of propagation may not be a true representation of the full length of propagation. As such we refer to the minimum propagation distance.

A Pearson's chi-squared test was used to determine the relationship of HAPCs to defaecation. The site of catheter position/bisacodyl infusion (caecum/ascending colon, transverse colon, descending/sigmoid colon) was used as a comparison for mean time between bisacodyl infusion and first HAPC. These data were not normally distributed (Shapiro-Wilk test $p<0.01$), so non-parametric analyses (Kruskall-Wallis H test) were performed.

Subgroup analyses were performed to compare results from; (1) the five hospital sites; (2) catheter type (water-perfused/solid state); (3) catheter placement approach (retrograde per anal/per stomal or antegrade per appendicostomy/caecostomy); (4) inclusion of sodium picosulfate in bowel preparation, and; (5) children with Hirschsprung's disease following surgical resection of the affected colonic segment with the rest of the cohort. Of specific interest was whether these variables altered the frequency of positive tests (Pearson's chi-squared test), HAPC amplitude (independent t-test/one-way ANOVA), and the total HAPC count (Shapiro-Wilk test $p<0.01$, Mann-Whitney U test/Kruskal-Wallis H test).

Statistical analysis was performed using IBM SPSS (Version 19.0, Released 2010; IBM Corp., Armonk, New York, USA). A p-value of ≤ 0.05 was considered statistically significant. Bonferroni correction was applied to multiple statistical comparisons.

6.4.5 Area Under the Curve Analysis

In addition to the characterisation of HAPCs, an area under the curve analysis was used to determine whether bisacodyl induced any change in colonic phasic activity prior to the first HAPC. The manometry trace was

divided into ten 60s epochs pre-bisacodyl and compared to the 60s epochs post-bisacodyl, prior to the first HAPC (up to 10 minutes if no HAPCs occurred; Shapiro-Wilk test $p<0.01$, Wilcoxon signed-rank test).

6.5 Results

6.5.1 Demographics

A total of 165 paediatric colonic manometry studies were included (Great Ormond Street Hospital n=43, Nationwide Children's Hospital n=38, Boston Children's Hospital n=36, Cincinnati Children's Hospital Medical Center n=25, Emma Children's Hospital, AUMC n=23). All children underwent investigation for constipation refractory to intensive medical treatment. Eighteen children had a confirmed previous diagnosis of Hirschsprung's disease on colonic/rectal biopsy, with the affected segment surgically resected prior to this study (pull-through procedure n=12, colectomy with end colostomy formation n=4, not specified n=2). Complete medication history data was available for 47 children, of which 22 children were receiving regular bisacodyl prior to their study.

Median age was 10 years (range 1-17 years), with 96 girls (58.2%) and 69 boys (41.8%). Sixty-six studies (40.0%) were performed with water-perfused catheters and 99 (60.0%) with solid-state catheters. Catheter placement was via either a retrograde approach (per rectal n=144 or per colostomy n=5) or antegrade approach (per appendicostomy/caecostomy n=16). With catheters placed either through the appendicostomy/caecostomy, and those placed via a retrograde approach to the caecum, bisacodyl was infused into the caecum (n=49, 29.7%). In the remaining studies, bisacodyl was infused through the catheter tip into the ascending colon (n=53, 32.1%), transverse colon (n=36, 21.8%), descending colon (n=22, 13.3%), or sigmoid colon (n=5, 3.0%).

6.5.2 High-Amplitude Propagating Contractions

Prior to bisacodyl infusion, spontaneous HAPCs were identified in 59 children (35.8%) during the postprandial period. In 28 of these children (17.0% overall), spontaneous HAPCs were additionally observed whilst fasting. HAPCs were induced following bisacodyl in 154 children (93.3%, population estimate Wilson's 95% CI 88.5%, 96.2%) at a mean time interval of 553s±669s (range=10s-4343s) post-infusion. In the majority of children (122/154, 79.2%), the first HAPCs were recorded within 12 minutes of bisacodyl infusion (**Figure 6.1**). Of the eleven children that did not generate a HAPC pre- or post-bisacodyl (**Figure 6.2**), 10/11 demonstrated low-amplitude propagating sequences(250, 267, 693). Two of these eleven children were those with a previous colonic resection for Hirschsprung's disease.

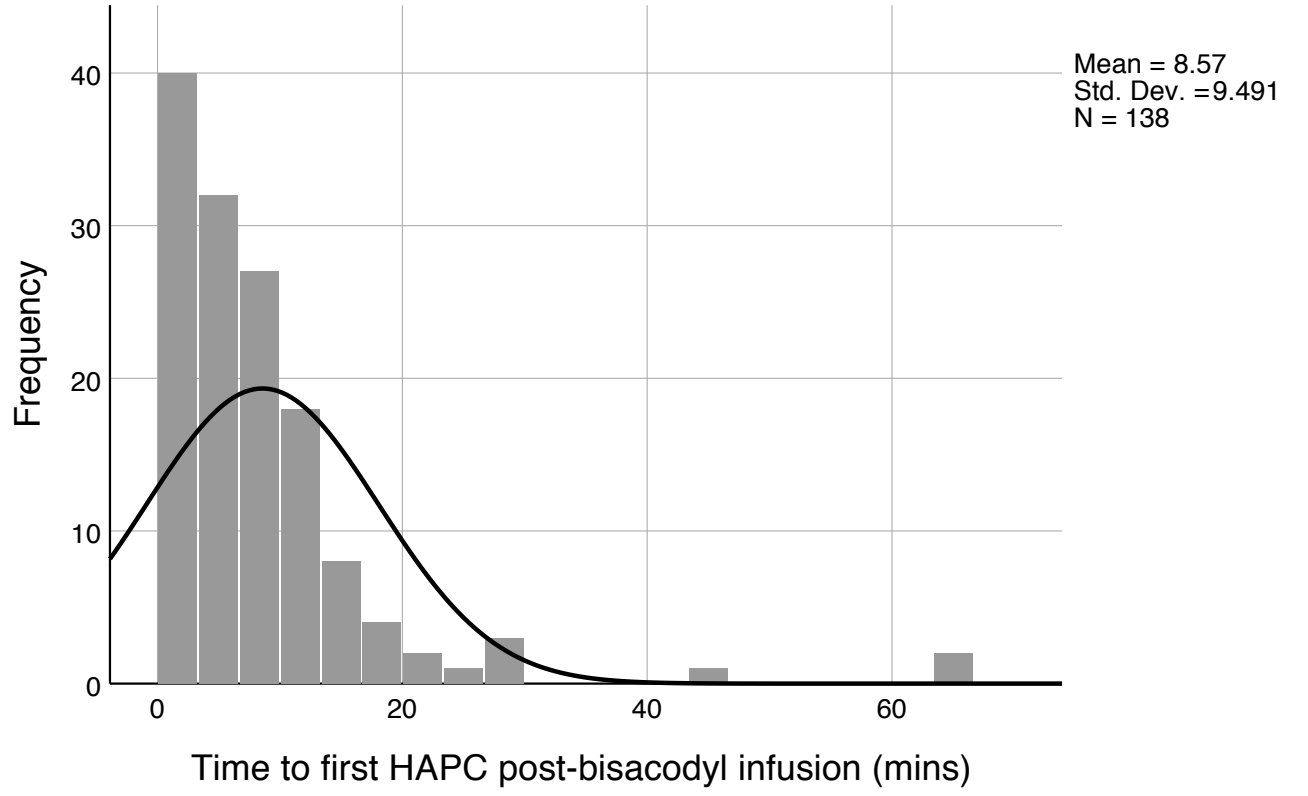

Figure 6.1 A histogram displaying the time interval between bisacodyl infusion and the first high-amplitude propagating contraction.

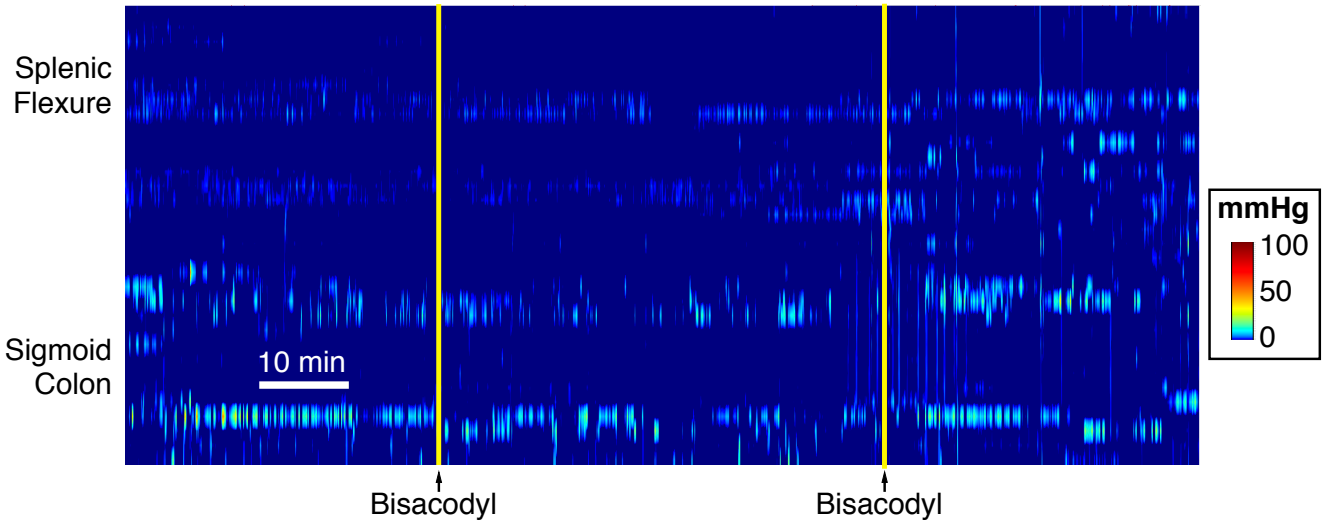

Figure 6.2 A pressure map depicting data from a colonic manometry study (y-axis: sensor position from splenic flexure → sigmoid colon; x-axis: time). No response is observed following two subsequent doses of bisacodyl.

A total count of 1893 post-bisacodyl HAPCs were identified, all propagating in an antegrade direction. The mean count per patient was 12.3±8.8 HAPCs, at a frequency of 0.32±0.21/min. Mean amplitude was 113.6±31.5mmHg, at a velocity of 8.6±3.8mm/sec, with a minimum propagated distance of 368mm±175mm.

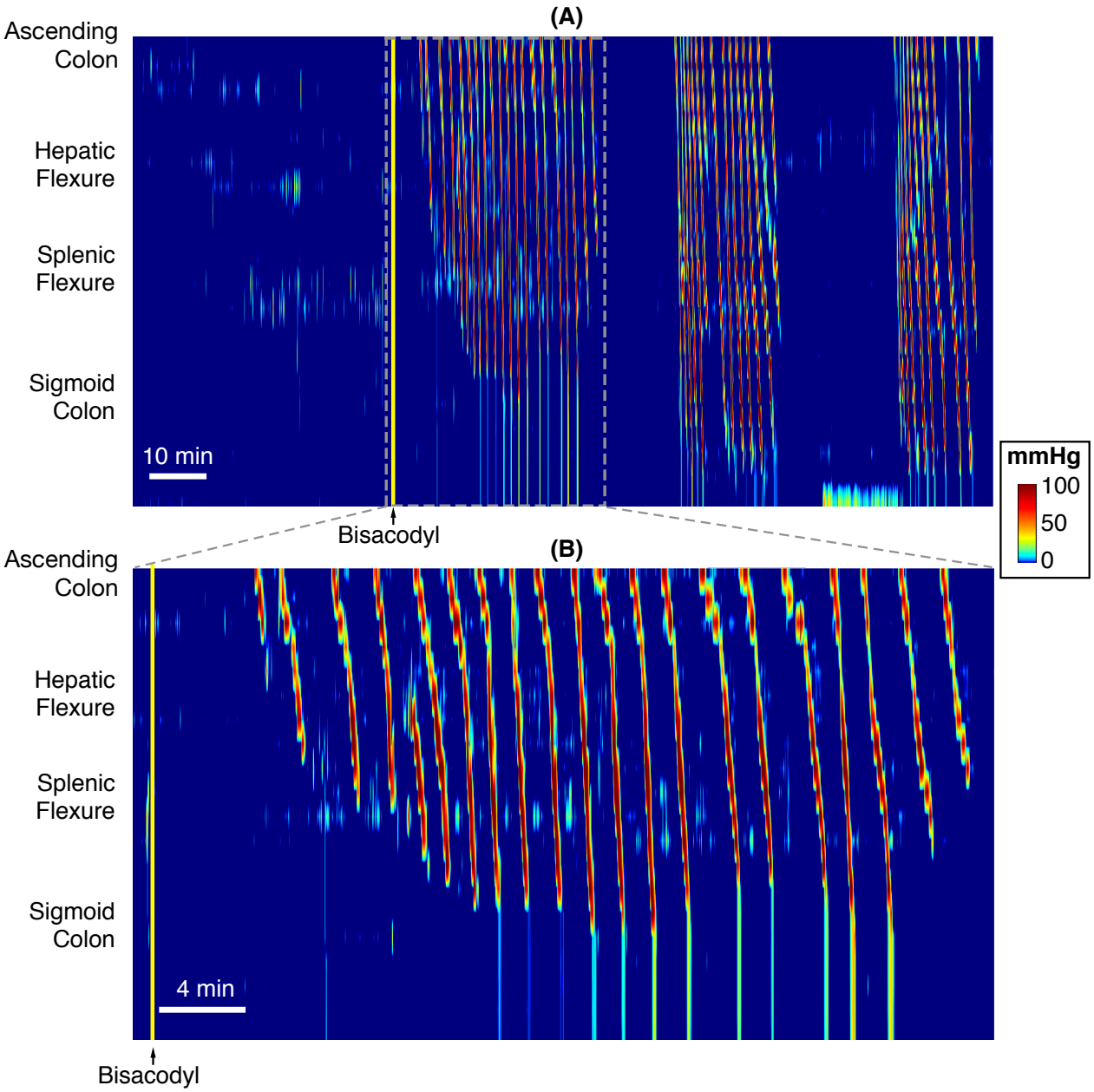

Figure 6.3 Two pressure maps, (A) and (B), depicting data from a colonic manometry study (y-axis: sensor position from ascending colon → sigmoid colon; x-axis: time). The amplitude of phasic pressure changes are differentiated by colour. Multiple consecutive high-amplitude (red) contractions propagate in an antegrade direction from the ascending colon to sigmoid colon. The propagation ceases in the sigmoid colon, terminating in a synchronous pressurisation across the distal sensors (yellow/pale blue).

Of the 1893 HAPCs, 337 (17.8%) propagated along the entire length of the catheter. The majority of HAPCs (82.2%, 1556/1893 total count) terminated prior to the distal recording sensors (**Figure 6.3**). Of the partially-propagating HAPCs, 69.5% (n=1082) terminated in a synchronous pressurisation across the distal channels

(mean distance=288±165mm) (**Figure 6.3**). These appeared to be analogous to the subtype of pancolonic pressurisations associated with propagating sequences described by Corsetti et al.(166) (**1.6.5 Pancolonic Pressurisations**). Using pre- and post-study x-ray imaging to localise sensor location(701), the termination of these HAPCs occurred most commonly in the sigmoid colon (n=46, 59.7%; transverse colon n=14, 18.2%; descending colon n=14, 18.2%; rectum n=3, 3.9%).

6.5.3 Comparison Between Pre-Bisacodyl and Post-Bisacodyl High-Amplitude Propagating Contractions

When comparing the spontaneous pre-bisacodyl HAPCs and post-bisacodyl HAPCs, there were significant differences observed in several characteristics including higher amplitude, length of propagation, and frequency in the post-bisacodyl group (see **Table 6.2**). There were no significant differences in HAPC velocity (**Table 6.2**).

6.5.4 Defaecation

During the post-bisacodyl infusion period, defaecation was induced in 80.6% of children (n=58/72). Defaecation was significantly associated with the presence of HAPCs ($\chi^2(1)=7.04$ p=0.008, Bonferroni-adjusted $\alpha = 0.0083$ (0.05 / 6)). There were no differences in characteristics of HAPCs when comparing those associated with defaecation with those not associated with defaecation (frequency U=163, p=0.04; amplitude t(13.76)=2.32, p=0.04; count U=226, p=0.37; velocity U=189, p=0.12; length of propagation U=244, p=0.57; Bonferroni-adjusted $\alpha = 0.0083$ (0.05 / 6)).

6.5.5 Anatomical Site of Bisacodyl Administration

The mean time to the first HAPC appeared to be more rapid when bisacodyl was administered more distally, however this difference did not reach significance ($\chi^2(3)=3.66$, p=0.30; mean time to first HAPC following bisacodyl infusion into cecum/ascending colon=632s±790s, n=99; transverse colon=456s±341s, n=30; descending colon=375s±323s, n=21; sigmoid colon 282±114s, n=4) (**Figure 6.4**).

6.5.6 Area Under the Curve Analysis

The area under the curve analysis (**Figure 6.5**) demonstrated no significant change in phasic activity in the 10 minutes pre- and post-infusion, prior to the first HAPC (Z=-0.53, p=0.60).

6.5.7 Subgroup Analyses

6.5.7.1 Hospital Site

When comparing the five hospital sites, there were no significant differences in the number of tests with bisacodyl-induced HAPCs ($\chi^2(4)=7.56$, p=0.11) or HAPC amplitude ($F(4,149) = 1.09$, p=0.36). There was a significant difference in total HAPC count ($\chi^2(4)=26.45$, p<0.001; Bonferroni-adjusted $\alpha = 0.0125$ (0.05 / 4)) between the five hospital sites. However, this is likely to have been influenced by the duration of recording following bisacodyl infusion, which also differed significantly between hospital sites ($\chi^2(4)=78.66$, p<0.001; Bonferroni-adjusted $\alpha = 0.0125$ (0.05 / 4); Great Ormond Street Hospital = 129±46 mins; Boston Children's Hospital = 121±47 mins; Emma Children's Hospital = 42±17 mins; Nationwide Children's Hospital = 75±26 mins; Cincinnati Children's Hospital Medical Center = 86±26 mins).

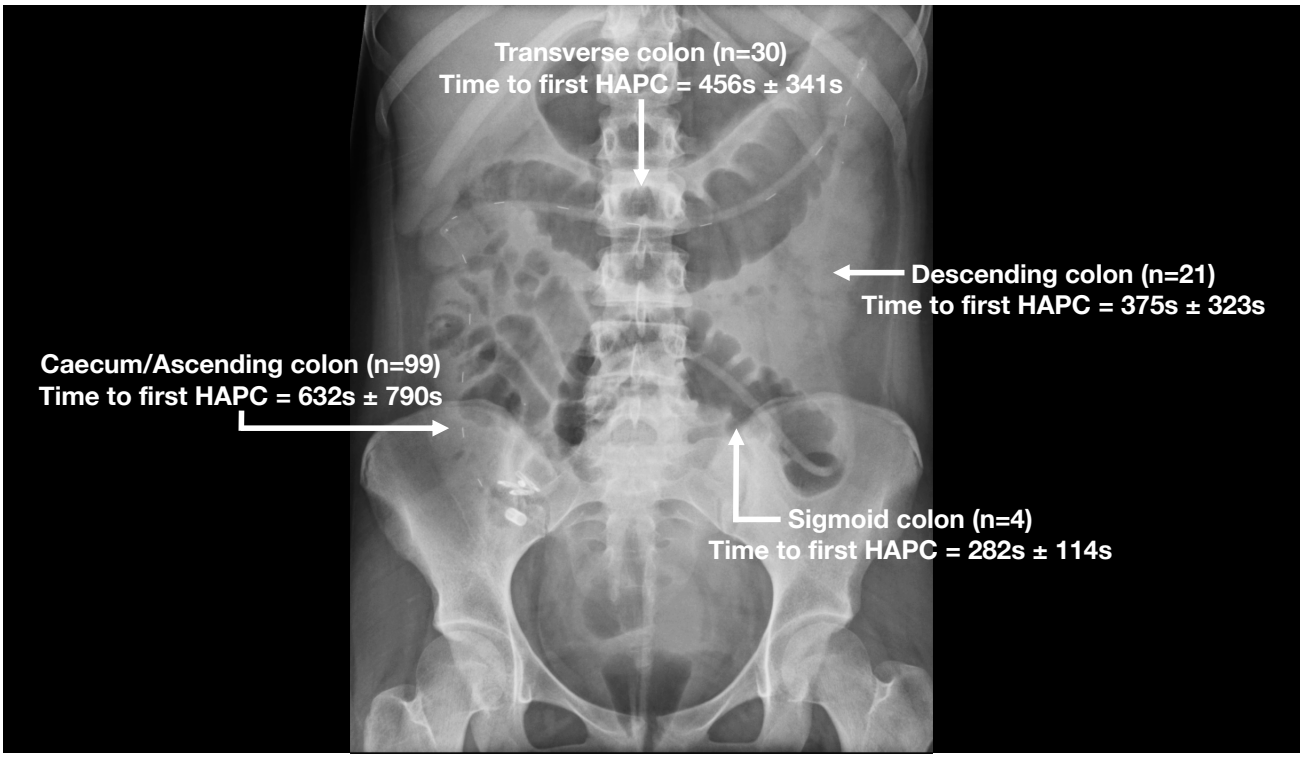

Figure 6.4 An abdominal X-ray demonstrating a manometry catheter in situ. Mean±SD time interval between bisacodyl infusion and first high-amplitude propagating contraction is detailed based upon the location of bisacodyl infusion. The mean time to first HAPC appeared to be more rapid when bisacodyl was administered distally; however, this difference did not reach significance ($\chi^2(3) = 3.66$, p=0.30).

6.5.7.2 Solid State/Water-Perfused Catheters

When comparing solid state and water-perfused catheters, there were no significant differences in the number of tests with bisacodyl-induced HAPCs ($\chi^2(1)=0.26$, p=0.61), HAPC amplitude t(152)=-1.73, p=0.09, or total HAPC count (U=2790, p=0.91).

6.5.7.3 Catheter Placement Approach

When comparing antegrade and retrograde catheter placement, there was a significant difference in the number of tests without post-bisacodyl HAPCs ($\chi^2(1)=9.57$, p=0.002, Bonferroni-adjusted $\alpha = 0.017$ (0.05 / 3)). One

quarter of tests performed in children with antegrade catheter placement (4/16) demonstrated no post-bisacodyl HAPCs, compared with 4.7% (7/149) in the children with retrograde catheter placement. There were no significant differences in HAPC amplitude (t(152)=1.36, p=0.18), or total HAPC count (*U*=625, p=0.13) when comparing catheter placement approach.

6.5.7.4 Hirschsprung's Disease

When comparing the children who had previously undergone surgical resection for Hirschsprung's disease (n=18) with the rest of the cohort (n=147), there were no significant differences in the number of tests with bisacodyl-induced HAPCs ($\chi^2(1)=0.64$, p=0.42), HAPC amplitude t(152)=1.37, p=0.17, or total HAPC count (*U* = 741, p=0.03, Bonferroni-adjusted $\alpha = 0.017$ (0.05 / 3)).

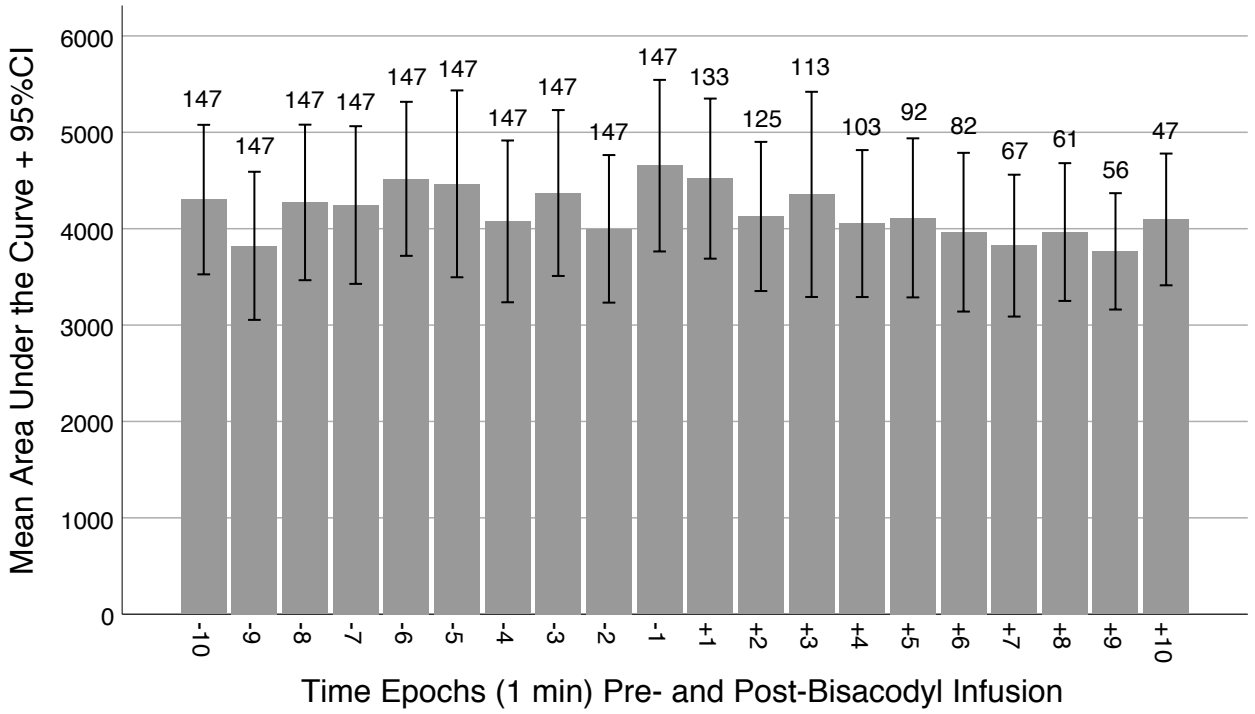

Figure 6.5 An area under the curve analysis of colonic manometry data in ten 60-s epochs pre-and post-bisacodyl, prior to the first high-amplitude propagating contraction. The numbers above each error bar indicate the number of children included in each epoch.

6.5.7.5 Sodium Picosulfate

Five children (3.0%) received sodium picosulfate as a component of bowel preparation prior to their colonic manometry study. Sodium picosulfate, similar to bisacodyl, is a pro-drug and metabolises to the same active chemical within the colon (*bis*-(p-hydroxyphenyl)-pyridyl-2-methane). As such, an additional analysis was performed on this subgroup to assess whether administration of sodium picosulfate prior to investigation diminished the bisacodyl response. All five of these children demonstrated HAPCs post bisacodyl, commencing at 31-344s post infusion (compared with mean time 553s overall).

6.6 Discussion

Following bisacodyl infusion, the majority of children (93.3%) with severe, intractable constipation are capable of generating HAPCs. In 79% of these children, the first HAPC occurs within 12 minutes of bisacodyl infusion. The majority of HAPCs propagate for a partial length of the manometry catheter only, with most terminating in a synchronous pressurisation in the rectosigmoid. A total absence of HAPCs was only observed in 11 children (6.7%). Bisacodyl also appears to induce an all-or-nothing colonic response, with no change in phasic activity prior to the first HAPC. These findings also demonstrate that catheter type, site of bisacodyl infusion, and hospital site do not influence the recorded colonic response.

The colonic response was independent of the anatomical site of bisacodyl administration. While there was a trend towards a more rapid response with more distal administration, this did not reach significance (**Figure 6.4**). The mechanisms by which bisacodyl induces HAPCs is incompletely understood. It has been suggested that bisacodyl may increase luminal secretion(277, 280, 281), and/or decrease water absorption(282). These effects may, in turn, play a role in inducing HAPCs. However, given the brief interval between drug administration and HAPCs, these mechanisms are unlikely to account for the response seen in these data. A more direct pathway has been proposed, whereby bisacodyl acts via excitation of colonic smooth muscle via tetrodoxin-insensitive, nifedipine-sensitive pathways(277-279). Bisacodyl is converted into an active metabolite *bis*-(p-hydroxyphenyl)-pyridyl-2-methane (BHPM) via esterase enzymes in the colonic mucosa(275) and there is minimal systemic absorption(276). The action of bisacodyl can be blocked by a prior application of lignocaine to the colonic mucosa(110). Collectively, these findings may suggest that bisacodyl causes excitation of extrinsic sensory nerves and the subsequent activation of parasympathetic innervation to the proximal colon.

Our data included a high prevalence of partially-propagating HAPCs which terminated in a synchronous pressurisation. It has been suggested that, in children, partial propagation of HAPCs is associated with slow colonic transit(705) and segmental colonic dilation(701). However, synchronous pressurisations at the termination of HAPCs are also hypothesised to function as a means to maintain colonic wall tone to facilitate transit in health(132, 166). Corsetti et al.(166) identified pressurisations at the termination of 74% of propagating contractions in the prandial period in healthy adults. This is a similar frequency to the 82% observed in the post-bisacodyl period in our sample. The significance of this finding remains uncertain and further studies with functional correlation are required.

Colonic pressurisations can also be induced with intraluminal bisacodyl in both adult and paediatric patients with severe constipation(166, 169, 267). In these data, pancolonic pressurisations were not present after intraluminal bisacodyl. During the bisacodyl-evoked HAPCs, all other motor patterns appeared to be suppressed. Therefore, in these children, the presence or absence of HAPCs alone appeared to be sufficient to define the colonic response to bisacodyl.

Whilst pharmacological induction of HAPCs using bisacodyl does not replicate normal colonic physiology, previous smaller studies have reported that no significant differences in the characteristics (frequency, amplitude, velocity, length of propagation) of spontaneous and bisacodyl-induced HAPCs(706). Having pooled data from 165 paediatric studies, our findings demonstrate that bisacodyl-induced HAPCs occur with a greater frequency, are of greater amplitude, and propagate a greater distance than spontaneous HAPCs. Clinically, these findings may suggest that HAPC provocation with bisacodyl demonstrates the integrity of the enteric nervous system. This is supported by the findings in children following surgical resection for Hirschsprung's disease, who demonstrated a colonic response to bisacodyl, confirming the presence of enteric ganglia and enteric nervous system integrity in their remnant colon. Of the children without HAPCs following bisacodyl, 10/11 still demonstrated normal basal motor activity, including low-amplitude propagating sequences both pre- and post-meal, which have been hypothesised to be myogenic in origin(2). Given the presence of myogenic activity, but absence of HAPCs, this may support the hypothesis of colonic inertia being a neurogenic disease, assuming a neural origin of HAPCs.

Clinical decision-making regarding surgical intervention in children with constipation tends to be driven by expert opinion and varies widely between centres, with no established evidence-based guidelines(262, 695). As such, the decision to proceed with surgery is fraught with difficulties, particularly when considering the associated operative risks and irreversibility of certain procedures. In some paediatric hospitals, colonic manometry is used to inform surgical decision-making(263, 273, 707). For example, the presence of bisacodyl-provoked HAPCs on colonic manometry has been described to be predictive of a positive therapeutic response to caecostomy formation with administration of antegrade continence enemas(270). A study performed in adults with constipation also demonstrated that spontaneous and/or bisacodyl-induced HAPCs were associated with positive clinical outcomes to intensive medical treatment(708).

Colonic manometry findings may provide more than dichotomous feedback on the presence/absence of HAPCs. Despite demonstrating an ability to generate HAPCs, children with treatment-refractory constipation may still benefit from surgical intervention due to the refractory and disabling nature of their symptoms. For example, if HAPCs do not propagate to the distal colon, and if the distal colon is dilated, these children could benefit from colonic resection in combination with a caecostomy(701, 709). In addition, surgical intervention may result in improved colonic motility. Following antegrade continence enemas or colonic diversion, some children with no HAPCs observed on their initial study later demonstrate HAPCs on subsequent colonic manometry studies(273, 696). Despite these findings, it should also be acknowledged that – at present – paediatric colonic manometry is performed in relatively few hospitals around the world. The diagnostic value of colonic manometry is yet to be determined, and this paper is not suggesting that surgical procedures should be performed solely upon the basis of colonic manometry findings.

Our approach to clinical investigation and analysis could not identify the causation of symptoms in the majority of these children, with a high proportion of normal results in a severely-affected patient population. It is possible that anorectal dysfunction contributes to symptom causation. While manometry sensors were positioned in the rectum in some children, this data was not available for all children and a separate analysis was not performed using this data. Concurrent anorectal manometry was also not performed, which would have been valuable for the assessment of anorectal function and coordinated colonic/anorectal motor function.

Collating data from several countries using different catheters and placement techniques does raise several potential limitations. However, our subgroup analyses demonstrated no differences between hospital site, catheter type, or site of bisacodyl administration on the recorded colonic response. Therefore, we can infer that these variables had minimal impact on our overall findings. While there were less frequent post-bisacodyl HAPCs detected when comparing antegrade to retrograde catheter placement, this is likely to be influenced by selection bias as those who had previously undergone appendicostomy/caecostomy formation for administration of antegrade continence enemas are also likely to have greater disease severity. The sensor spacing of recording sites is also critical in colonic motor pattern detection(114). In this study, catheter sensor spacing varied between 15mm-40mm. However, a previous study demonstrated that HAPCs are the only motor pattern consistently recognised regardless of the sensor spacing(114). Finally, as with all paediatric manometry studies, there is an absence of healthy age-matched control data. Despite this, we do know that bisacodyl induces HAPCs in healthy adults(700) and it is unlikely that healthy children would have a substantially different response.

6.7 Conclusion

The bisacodyl response in children with treatment-refractory constipation is characterised by an all-or-nothing initiation of HAPCs. For the majority of children, the first HAPC occurs within 12 minutes of bisacodyl infusion and terminates in a synchronous pressurisation in the rectosigmoid. Catheter types, site of bisacodyl infusion, and hospital site have no significant impact upon the recording of this response.

6.8 Tables

Table 6.1 Details of the Manometry Catheters, Placement Techniques, and Study Protocol at Each Hospital Site

	Great Ormond Street Hospital, UK	Nationwide Children's Hospital, USA	Boston Children's Hospital, USA	Cincinnati Children's Hospital Medical Center, USA	Emma Children's Hospital, NL
Diet for 24 hours prior to study	Liquid, low-residue diet	Clear fluids	Clear fluids	Clear fluids	Clear fluids
Bowel preparation	PEG-3350 with electrolytes	PEG-3350 with electrolytes +/- mineral oil enema	PEG-3350 with electrolytes	PEG-3350 with electrolytes +/- rectal lavage	PEG-3350 with electrolytes +/- sodium picosulfate
Manometry sensor count	20 sensors	36 sensors	36 sensors	36 sensors	36 sensors
Manometry sensor spacing	25mm	30mm	30mm	20mm-40mm (proximal sensors 1-20 = 40mm, distal sensors 21-36 = 20mm)	15mm
Solid-solid or water-perfused catheter	Water-perfused	Solid-state	Solid-state	Solid-state	Water-perfused
Meal included in manometry study protocol	Y	Y	Y	Y	Y
Bisacodyl dose	5mg-10mg	0.2mg/kg (max 10 mg)	0.25mg/kg (max 10mg)	0.25mg/kg (max 10mg)	5mg-10mg
Bisacodyl administration route	Central lumen of the catheter	Central lumen of the catheter for retrograde catheter placement, or into the proximal colon via a tube placed through the stoma for antegrade catheter placement	Central lumen of the catheter	Central lumen of the catheter	Central lumen of the catheter

Table 6.2 Comparisons Between the Characteristics of Pre- and Post-Bisacodyl High-Amplitude Propagating Contractions (HAPCs)

	Pre-bisacodyl n=333 (mean±SD)	Post-bisacodyl n=1893 (mean±SD)	Statistical comparison Bonferroni corrected α = 0.0125 (0.05 / 4)
HAPC amplitude (mmHg)	101.2±29.2	119.5±29.3	Paired samples t-test; $t(59)=-5.06$, $p<0.01$
Length of HAPC propagation (mm)	326±136	407±189	Wilcoxon signed-rank test; $Z=-3.75$, $p<0.01$
HAPC frequency (/min)	0.23±0.42	0.30±0.18	Wilcoxon signed-rank test; $Z=-4.61$, $p<0.01$
HAPC velocity (mm/s)	11±9	9±4	Wilcoxon signed-rank test; $Z=-1.39$, $p=0.16$

Chapter 7: The Functional Role of Distal Colonic Motility in Gas Transit and Continence

7.1 Statement

The content of this chapter has been published in *Neurogastroenterology & Motility*.

Heitmann PT, Mohd Rosli R, Maslen L, Wiklendt L, Kumar R, Omari TI, Wattchow DA, Costa M, Brookes SJH, Dinning PG (2021). High-resolution impedance manometry characterizes the functional role of distal colonic motility in gas transit. *Neurogastro Motil* 2021; e14178.

The co-authors have provided permission for the inclusion of the study in this thesis. The percentage contributions of each author to this study were as follows:

- Research design: **PH 80%**, RMR 6.67%, MC 6.67%, PD 6.67%.
- Data collection and analysis: **PH 75%**, RMR 8.33%, LW 8.33%, PD 8.33%.
- Writing and editing: **PH 90%**, TO 2%, DW 2%, MC 2%, SB 2%, PD 2%.

7.2 Abstract

Background: The colonic motor patterns associated with gas transit are poorly understood. This study describes the application of high-resolution impedance manometry (HRiM) in the human colon in vivo. Our aims were to characterise distal colonic motility and gas transit; (1) prior to and after a meal, and; (2) in response to intraluminal gas insufflation in the sigmoid colon. **Methods:** HRiM recordings were performed in 19 healthy volunteers, with sensors positioned from the distal descending colon to the proximal rectum. Protocol 1 (n=10) compared pressure and impedance prior to and after a meal. Protocol 2 (n=9) compared pressure and impedance before and after gas insufflation in the sigmoid colon (60mL total volume). **Key Results:** Both interventions resulted in an increase in the prevalence of the 2-8/minute "cyclic motor pattern" (meal: ($t(9)$=-6.42, $p<0.001$; gas insufflation ($t(8)$=-3.13, p=0.01), and an increase in the number of antegrade and retrograde propagating impedance events (meal: Z=-2.80, p=0.005; gas insufflation Z=-2.67, p=0.008). Propagating impedance events temporally preceded antegrade and retrograde propagating contractions, representing a column of luminal gas being displaced ahead of a propagating contraction. Three participants reported an urge to pass flatus and/or flatus during the studies. **Conclusions & Inferences:** Initiation of the 2-8/minute cyclic motor pattern in the distal colon can occur following a meal and/or as a localised sensorimotor response to gas. The absence of a flatal urge and the temporal association between propagating contractions and gas transit supports the hypothesis that the 2-8/minute cyclic motor pattern acts as a physiological "brake" which modulates rectal filling.

7.3 Introduction

The transit of intraluminal contents within the colon is governed by the action of colonic smooth muscle. Using concurrent manometry and imaging techniques, a number of previous studies have attempted to relate the movements of liquid and solid luminal contents with specific patterns of motor activity(130, 132-137) (see **1.6 Colonic Motor Patterns** & **2.5.1.2 Colonic Motility**). However, the colonic motor patterns associated with gas transit have not been described. Recent studies using high-resolution colonic manometry demonstrated that colonic pressurisations, defined as a synchronous increase in pressure across all sensors of the manometry catheter, had a temporal association with flatus(166, 167). These authors hypothesised that colonic pressurisations may be involved in colonic gas transit.

Colonic gas transit can occur rapidly and efficiently. Exogenous gas infusion at 1-30mL/min into the proximal jejunum(87, 710, 711), ileum, or caecum(88) results in anal gas expulsion at comparable rate within 30 minutes(88, 710, 711). Under normal circumstances, flatus occurs intermittently and is under voluntary control(315). When there is an urge to pass flatus at an inappropriate time, it can be

voluntarily deferred by contraction of the external anal sphincter. The reduction of this urge following voluntary with-holding suggests that gas does not remain pooled in the rectum. A study using computed tomography imaging demonstrated the accumulation of gas in the distal colon following a meal(384). Meals are also associated with an increase in the "cyclic motor pattern" in the distal colon(2) (see **1.6.3 The Cyclic Motor Pattern**). This pattern consists of repetitive contractions, at a frequency of 2-8 per minute, that propagate over short distance, predominantly in the retrograde direction. It has been hypothesised that stool or gas arriving in the distal colon activates the cyclic motor pattern, which may act as a physiological "brake" to regulate rectal filling (111, 116, 171, 173, 176, 316).

To investigate the functional role of motility patterns in gas transit, both contractile activity and transit must be recorded concurrently. This has previously been demonstrated in the esophagus and small intestine using multichannel intraluminal impedance manometry catheters (142-145). We have also used this method to relate gas and liquid transit with colonic contractions in the ex vivo rabbit colon(152). In this present study, we describe the application of impedance manometry in the human colon in healthy volunteers. Our aims were to characterise distal colonic motility and gas transit; (1) prior to and after a meal, and; (2) in response to intraluminal gas insufflation in the sigmoid colon.

7.4 Methods

7.4.1 Participant Recruitment

Ethics approval was received from the Southern Adelaide Clinical Human Research Ethics Committee (Project number 145.18). Volunteers were recruited via flyers posted within the hospital or by word of mouth. Inclusion criteria included healthy adults between the age of 18-79 years, a normal bowel habit (defined as stool frequency between three bowel motions/day and three bowel motions/week(454, 455)), and no chronic gastrointestinal symptoms to suggest the presence of constipation, faecal incontinence, or irritable bowel syndrome. Exclusion criteria included previous abdominal/pelvic surgery or radiotherapy, regular use of laxatives or antidiarrheal medications, or a history of gastrointestinal, metabolic, neurological, and/or endocrine disease.

7.4.2 Colonoscopy and Catheter Placement

On the day prior to the study, participants underwent bowel preparation (PEG-3350 with electrolytes & sodium picosulfate) followed by an overnight fast. Colonoscopy was performed at 08:30 hours under sedation (intravenous fentanyl & midazolam) for catheter placement. The catheter used for all studies was a high-resolution, impedance manometry, solid-state, 12Fr catheter with 32 circumferential pressure sensors spaced at 10mm and 16 impedance channels spaced at 20mm (Sandhill Scientific,

Diversatek Healthcare, Milwaukee, WI, USA). A snare passed through the biopsy channel of the colonoscope was used to grasp a silk suture loop tied to the tip of the catheter. The catheter was then be advanced with the colonoscope and positioned under direct vision. Sensors were positioned in the distal descending colon, sigmoid colon, and proximal rectum. An endoclip was used to fix the catheter tip to the colonic mucosa to prevent displacement (Protocol 1 only). For the shorter, two-hour duration studies (Protocol 2), no endoclips were used. In all studies, the catheter location was assessed with an abdominal x-ray at the conclusion of the study.

7.4.3 Study Protocols

Following catheter placement, participants were relocated from the endoscopy suite to an adjacent room for the duration of the study. Prior to the commencement of recording, a 60-minute recovery period was allowed, as per our previous study protocols(2, 256). All participants were positioned in a semi-recumbent position with the head of the bed elevated to 45°. Participants were recruited sequentially to one of two study protocols, with the first 10 volunteers undergoing protocol 1, and the subsequent 9 volunteers undergoing protocol 2.

1. Protocol 1: Characterisation of the associations between colonic motility and gas transit prior to and following a meal. Using our meal protocol detailed in previous studies(2, 256), a two-hour pre-prandial control period was followed by a 700Cal meal, followed by a further two-hour postprandial period.
2. Protocol 2: Characterisation of distal colonic motility in response to intraluminal gas insufflation in the sigmoid colon. A one-hour control period was followed by a one-hour intervention period. During the intervention period, insufflation of room air was administered into the sigmoid colon at three 20-minute intervals. Air was infused via a 1.67mm diameter neonatal feeding tube which was attached to the catheter prior to insertion, at a rate of 30ml/2mins. The tip of the feeding tube was positioned at the mid-point of the catheter between pressure sensors 18 and 19.

7.4.3.1 Symptom Record

In protocol 1, it was not anticipated that healthy volunteers would experience gastrointestinal symptoms in response to a meal. Participants were asked to report any episodes of flatus, urge to pass flatus or defaecate, or abdominal discomfort. In protocol 2, we assessed whether gas insufflation would result in any conscious awareness or symptoms. Participants were encouraged to report symptoms in real-time and were additionally questioned at ten-minute intervals regarding the presence, duration, and resolution of symptoms. Symptoms were marked on the manometry/impedance trace so that any association with colonic motility or gas movement could be assessed. Symptom information included specifying location on a pictorial body chart, radiation, severity (graded from 0-10 on a visual analogue

scale), duration, and descriptive characterisation (e.g. pain, bloating, pressure). Specific attention was also given to whether or not the urge to pass flatus was present.

7.4.4 Data Analysis

7.4.4.1 Manual Analysis

Analysis of colonic manometry and impedance data was performed using in-house software (PlotHRM) developed by one of the authors (LW). PlotHRM has been described in previous publications(2, 114). Pressure and impedance data were analysed separately in a blinded manner by two observers (PH & PD), with each observer spending between two to six hours analysing each study. The identified pressure events included propagating contractions and synchronous pressure increases (detailed below). Impedance events included positive monophasic waveforms above baseline occurring across multiple channels (detailed below).

Propagating contractions were defined as described previously(2, 166, 712). This included monophasic pressure peaks occurring across three or more adjacent channels (20mm), where the waveforms in each individual channel displayed some overlap with the adjacent channel with a temporal offset (i.e. antegrade: proximal → distal sequences; retrograde: distal → proximal sequences). Where a pressure excursion returned from peak to baseline prior to the commencement of the waveform in the adjacent channel, the pressure peaks were considered to be discrete events. Propagating contractions were characterised by direction (antegrade/retrograde), number (total event count), frequency (cpm), amplitude (mmHg), length of propagation (cm), and pace (s/cm).

Three distinct patterns of colonic propagating contractions have previously been described(11) (see **1.6 Colonic Motor Patterns**);

1. The cyclic motor pattern; repetitive propagating pressure events with a cyclic frequency of 2–8/min in either a retrograde or antegrade direction, or aligned synchronously across ≥3 sensors.
2. High-amplitude propagating contractions (HAPCs); an array of propagating pressure events with the majority having a trough-to-peak amplitude of >116mmHg.
3. Single motor patterns; Propagating contractions that occurred in isolation separated from other propagating motor patterns by intervals of >1 min.

Propagating contractions were classified as the *cyclic motor pattern* if they met the following criteria; repetitive propagating pressure events with a cyclic frequency of 2–8/min in either a retrograde or antegrade direction, or aligned synchronously across ≥3 sensors(2).

Propagating contractions were also assessed as being consistent with descriptions of high-amplitude propagating contractions or single motor patterns(11). HAPCs can be differentiated from other colonic motor patterns using a discriminant analysis(2). A total count of five HAPCs were identified in the studies (two in protocol 1; three in protocol 2). Given their low numbers, no further analysis was performed.

The only method described to date to discriminate between the cyclic motor pattern and single motor patterns is manual analysis. Single motor patterns (short and long) are defined as propagating contractions which do not meet the criteria for a HAPC, which are temporally separated from other propagating contractions by >1min(11). Single motor patterns are otherwise indistinguishable from the cyclic motor pattern using a discriminant analysis(2). Given the high counts of propagating contractions in our recordings (9033 propagating contractions identified in a total recording time of 58 hours = average 2.6 events/minute), events separated by >1min were rarely observed, and therefore no further analysis on single motor patterns was performed. The most prevalent propagating motor pattern was the cyclic motor pattern, which was therefore the focus of the analysis.

Pace (s/cm) was preferred to velocity (cm/s) to quantify the apparent speed of propagation. A pace close to zero is often observed, which corresponds to distributed events with almost no temporal delay between their occurrence in each channel. Such synchrony is unlikely to reflect neural or myogenic transmission occurring at near-infinite velocity. Rather, it is more likely to represent several mutually entrained colonic muscle slow waves(713).

Colonic pressurisations, as described by Corsetti et al.(166), were defined as a synchronous increase in pressure across all sensors of the manometry catheter. In their study, the duration of these events was 24±4s, with an amplitude of 15±3mmHg. Using these values, we defined colonic pressurisations as pressure events occurring in all manometry sensors with a duration between 16–32s (mean±2SD) and with an amplitude between 9-21mmHg (mean±2SD).

Each of our studies also contained numerous examples of the synchronous pressure increases across all channels that did not meet the duration criteria for pressurisations. Each of these was also identified and labelled as "*synchronous pressure increases"*. Pressurisations and synchronous pressure increases were analysed by frequency (cpm), amplitude (mmHg), and duration (s).

Corsetti et al.(166) additionally described pressurisations occurring at the termination of propagating contractions. In our manual analysis, all propagating contractions (as described above) were assessed to determine whether a pressurisation occurred at their termination. For retrograde propagating contractions, this phenomenon was observed as a pressurisation in the channels proximal to where the

contraction ended. Conversely, for antegrade propagating contractions, this was observed in the distal channels to where the contraction ended.

Impedance events were identified using the methods described in our previous impedance manometry study in ex vivo rabbit colon(152). In that study, admittance values - the inverse of impedance - decreased in the presence of gas, represented by a nadir on the tracing. In this study, we used the impedance values rather than admittance and hence an impedance *increase* above baseline was considered to reflect the presence of gas. *Propagating impedance events* were identified as a positive monophasic waveform above baseline occurring across two or more adjacent channels (20mm), where the waveforms in each individual channel displayed some overlap with the adjacent channel with a temporal offset (i.e. antegrade: proximal $\rightarrow$ distal sequences; retrograde: distal $\rightarrow$ proximal sequences). Where an impedance excursion returned to baseline prior to the commencement of the waveform in the adjacent channel, the two events were considered discrete. We did not observe any synchronous impedance events across all sensors. Impedance events were classified by direction (antegrade/retrograde), frequency (cpm), amplitude (Ω), length of propagation (cm), and pace (s/cm).

Comparisons between study periods (protocol 1; pre- and post-prandial; protocol 2; control and post-gas insufflation) were made for the prevalence and characteristics of pressure and impedance data. The same comparisons were performed between asymptomatic periods and time periods during which the volunteers reported symptoms.

7.4.4.2 Time Occupied by the Cyclic Motor Pattern Pre- and Post-Intervention

The prevalence of the cyclic motor pattern was compared pre- and post-intervention (meal or gas insufflation). Using the PlotHRM software(2, 114), each pressure trace was converted to a spatiotemporal colour map. Sample smoothing (30s) was applied to remove low frequency (<2cpm) activity. The colour map was then divided into 60 x 1-minute time epochs pre- and post-intervention. In each channel, for every 1-minute time epoch, the trace was scored with 0 if phasic pressure activity was absent, or 1 if it was present. These values were then averaged over all channels for all time epochs to provide an approximation of the overall time occupied by the cyclic motor pattern.

7.4.4.3 Inter-Event Interval Distribution Analysis

To assess the temporal associations between manually-labelled pressure and impedance data, the time intervals between all impedance events and propagating contractions was calculated. The "true" standard deviation of intervals from each propagating contraction to the next impedance event and from each impedance event to the next propagating contraction was calculated for each participant, study period (control, post-meal, post-gas insufflation), and sensor location. The times for each variable were then randomly shuffled by re-sampling times of the same length. This random re-sampling was

performed 10,000 times, generating a null distribution of standard deviations. The "true" standard deviations were compared to the distribution of the null standard deviations to ascertain if the true standard deviations occurred in the lower tail (representing an association), or within the main body of the null distribution (representing no association).

7.4.4.4 Timing of the Colonic Meal Response and Distal Colonic Gas Accumulation: Hierarchical Change-Point Model

A hierarchical change-point model was used to determine the moment that the colonic meal response commenced based upon pressure data, compared with the moment at which gas volume increased in the distal colon based upon impedance data. This was performed in an attempt to elucidate whether the arrival of gas precedes or occurs simultaneously with the colonic meal response (suggesting that the meal response is primarily a localised response to gas), or whether the colonic meal response commences prior to the arrival of gas (suggesting that the meal response occurs as a result of stimuli more proximally in the gastrointestinal tract and therefore may be primarily driven by extrinsic neural inputs). The mean change-point is reported as a probability based upon the distribution of means.

7.4.4.5 Automated Analysis

Automated continuous wavelet transform analyses were additionally performed. This approach includes a mixed-effects model with Gaussian process responses, as described in previous publications(714, 715). The intent of these analyses was to; (a) describe temporal associations between pressure and impedance data at co-located sensors, as well as; (b) to ascertain the predominant propagation direction of waves across a range of frequencies by using pressure or impedance data separated by time and sensor location.

7.4.4.6 Statistical Analysis

Statistical analysis was performed using IBM SPSS (Version 19.0, Released 2010; IBM Corp., Armonk, New York, USA) and PyStan (v2.19.1.1, Stan Development Team) and MatLab (R2018a, The MathWorks, Inc., Natick, Massachusetts, USA). The characteristics of pressure and impedance events were not normally distributed (Shapiro-Wilk test $p<0.01$), so non-parametric analyses were used for comparisons of characteristics between study periods (Wilcoxon signed-rank test & Mann-Whitney U test). To compare the time occupied by slow wave activity pre- and post-intervention, paired samples t-tests were used. The Holm-Bonferroni correction was applied to multiple comparisons(716).

7.5 Results

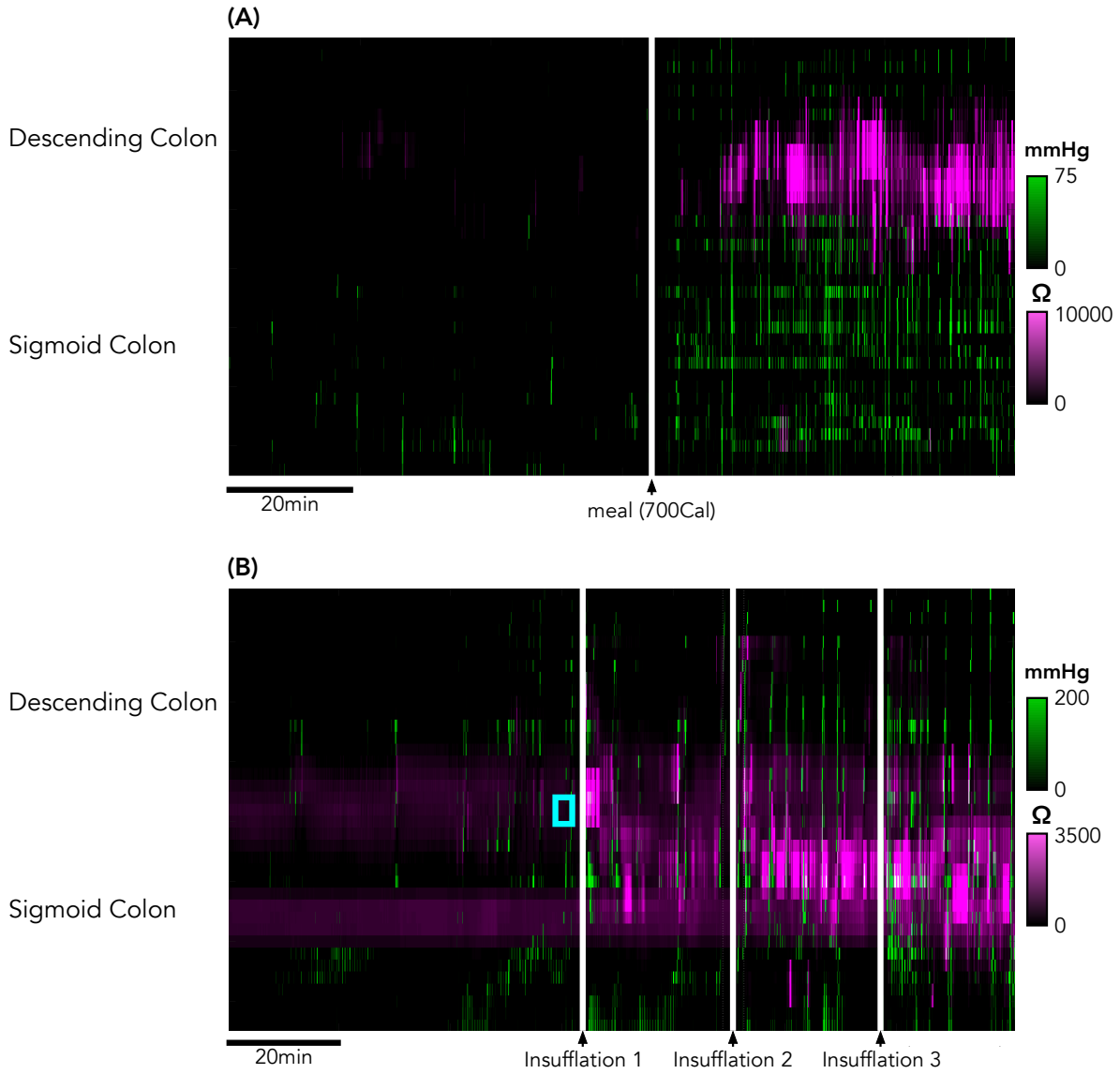

Figure 7.1 A composite colour map displaying overlaid pressure (green) and impedance (gas; magenta) data for volunteers undergoing the meal protocol (A) or gas insufflation protocol (B). The y-axis displays sensor location within the descending and sigmoid colon and the x-axis displays time. During the pre-prandial period in (A), pressure contractions occur infrequently and minimal gas is present. Following the meal, propagating contractions markedly increase and gas enters the proximal sigmoid colon. In (B), three sequential gas insufflations were performed at 20-minute intervals. Gas is introduced at the location of the aqua rectangle. Following gas insufflation, there is an increased volume of gas in the distal colon and the gas insufflation is associated with an increase in propagating contractions.

Nineteen healthy adults participated in the study (10 women, 9 men; median age 52, range 21-65 years). The majority of the women were parous (80.0%, n=8) with a median of two children (range 1-4). Ten participants underwent protocol 1 (meal), and nine participants underwent protocol 2 (gas insufflation). One female participant underwent both protocol 1 and 2 on separate visits. Composite colour maps displaying overlaid pressure and impedance data from volunteers undergoing each protocol are depicted

in **Figure 7.1**. In total, we identified 9033 propagating contractions in 58 hours of recording. The average frequency was 2.6 propagating contractions/minute. As a result, 99% of all propagating contractions were classified as the cyclic motor pattern. Only five HAPCs were identified (two in protocol 1; three in protocol 2).

7.5.1 Protocol 1: Characterisation of the Associations Between Colonic Motility and Gas Transit Prior To and Following a Meal

7.5.1.1 Characteristics of Pressure and Impedance Events

The characteristics of propagating contractions/synchronous pressure increases and impedance events in the pre- and post-prandial periods are detailed in **Table 7.1**.

Propagating Contractions

Following the meal, propagating contractions increased in number (antegrade: Z=-2.80, p=0.005; retrograde: Z=-2.80, p=0.005), frequency (antegrade: Z=-2.80, p=0.005; retrograde: Z=-2.80, p=0.005), length of propagation (antegrade: Z=-2.80, p=0.005; retrograde: Z=-2.80, p=0.005), and amplitude (retrograde only: Z=-2.40, p=0.017). Following the meal, there was also a reduction in pace of propagating contractions (i.e.: an increase in velocity; antegrade: Z=-2.80, p=0.005; retrograde: Z=-2.80, p=0.005; **Table 7.1**).

Of the propagating contractions, n=34 terminated in a pressurisation across the distal (n=26, antegrade) or proximal (n=8 retrograde) pressure channels (examples depicted in **Figure 7.2**; **Table 7.1**). These events all occurred during the post-prandial period. Propagating contractions which were not associated with pressurisations were of lower amplitude than those that were followed by pressurisations (antegrade: Z=-2.80, p=0.005; retrograde: Z=-2.80, p=0.005), had shorter propagation length (antegrade only: Z=-2.70, p=0.007), and were of higher frequency (antegrade: Z=-2.80, p=0.005; retrograde: Z=-2.80, p=0.005). There were no significant differences in pace.

Synchronous Pressure Increases

There were no events recorded that were consistent with the description of colonic pressurisations described by Corsetti et al.(166) The synchronous pressure increases that we observed were of similar amplitude to those described by Corsetti et al.(166) (mean 10.03±3.30mmHg), but had a much shorter duration (mean 3.30±1.40s post-prandial period, 2.83±1.32s post gas-insufflation period), which is more than five standard deviations below the duration of the events described by Corsetti et al.(166). The maximum duration of any synchronous pressure increase in our data was 12s. The characteristics of these events were similar for pre- and post-prandial periods, with no significant differences in number, frequency, amplitude, or duration.

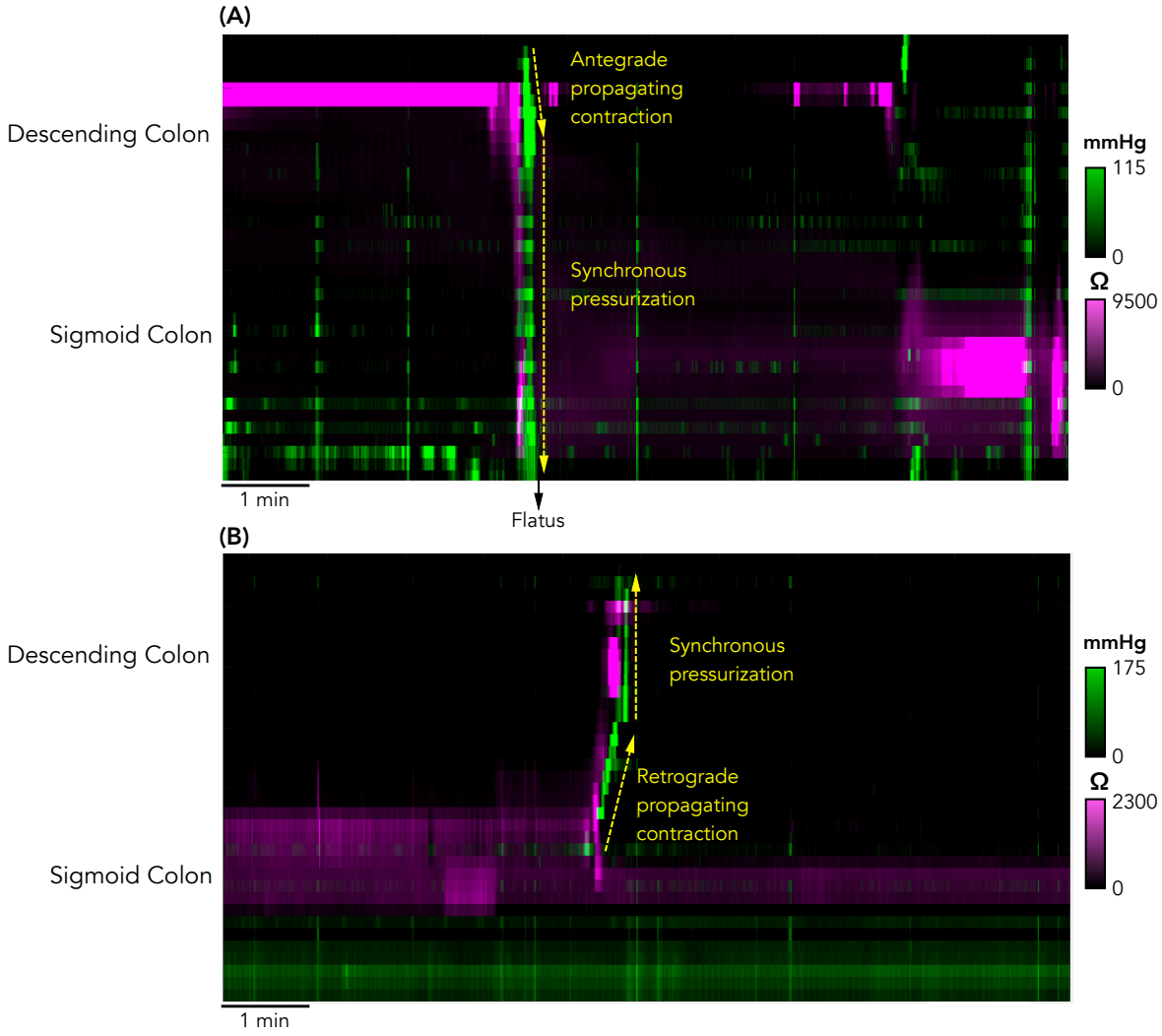

Figure 7.2 (A) A composite colour map displaying overlaid pressure (green) and impedance (magenta) data during flatus. At the top left of the figure, a magenta band represents increased impedance in the proximal sensors. The magenta band then shifts distally in association with an antegrade propagating contraction. This propagating contraction terminated in a synchronous pressurisation across the distal channels in the sigmoid colon. Note that the magenta (gas) temporally precedes the green (pressure). The volunteer reported flatus 8 seconds after this event. In (B), a similar pattern is observed occurring in a retrograde direction. It appears that, in this instance, gas was moved away from the rectum. No flatus or urge was reported at the time of this event.

Impedance Events

When compared to the control period, the meal induced a significant increase in the number of impedance events (antegrade: Z=-2.80, p=0.005; retrograde: Z=-2.80, p=0.005; **Figure 7.3**). However, there were no significant differences in frequency, amplitude, length of propagation, or pace of impedance events before and after the meal (**Table 7.1**).

7.5.2 Protocol 2: Characterisation of Distal Colonic Motility in Response to Intraluminal Gas Insufflation in the Sigmoid Colon

7.5.2.1 Characteristics of Pressure and Impedance Events

The characteristics of pressure and impedance events in the pre- and post-gas insufflation periods are detailed in **Table 7.2**.

Propagating Contractions

There were no significant differences in the characteristics of individual propagating contractions between the pre- and post-gas insufflation periods (**Table 7.2**). There was, however, an increase in the prevalence of the cyclic motor pattern after gas insufflation (see **7.5.3 Time Occupied by the Cyclic Motor Pattern Pre- and Post-Intervention**). Of the propagating contractions, 17 terminated in a pressurisation in the distal (n=3 antegrade) or proximal (n=14 retrograde) pressure channels. Two of these events occurred during the control period (n=1 antegrade, n=1 retrograde), with the remaining 15 occurring in the post-gas insufflation period. There was an equal distribution of events during the first, second, and third post-gas insufflation periods indicating that the abundance of these events did not increase with accumulating volumes of gas.

Synchronous Pressure Increases

As found in protocol 1, no events were identified that were consistent with the description of colonic pressurisations by Corsetti et al.(166) For the synchronous pressure increases that were observed, there were no significant differences in the amplitude, number, or frequency of these events between the pre- or post-gas insufflation periods (**Table 7.2**).

Impedance Events

Following gas insufflation, impedance events increased significantly in number (antegrade: Z=-2.67, p=0.008; retrograde: Z=-2.67, p=0.008) and frequency (antegrade: Z=-2.36, p=0.018; retrograde: Z=-2.36, p=0.018; example depicted in **Figure 7.3**). There were no significant differences in amplitude, length of propagation, or pace when comparing impedance events between the pre- and post-gas insufflation periods.

7.5.3 Time Occupied by the Cyclic Motor Pattern Pre- and Post-Intervention

The 1-minute time epochs occupied by 2-8/minute cyclic motor pattern increased significantly following both the meal and gas insufflation. This included an increase from 5.89%±4.68% pre-prandial to 29.29%±12.02% post-prandial (t(9)=-6.42, p<0.001), and an increase from 16.11%±8.79% pre-gas insufflation to 27.65%±16.86% post-gas insufflation (t(8)=-3.13, p=0.01).

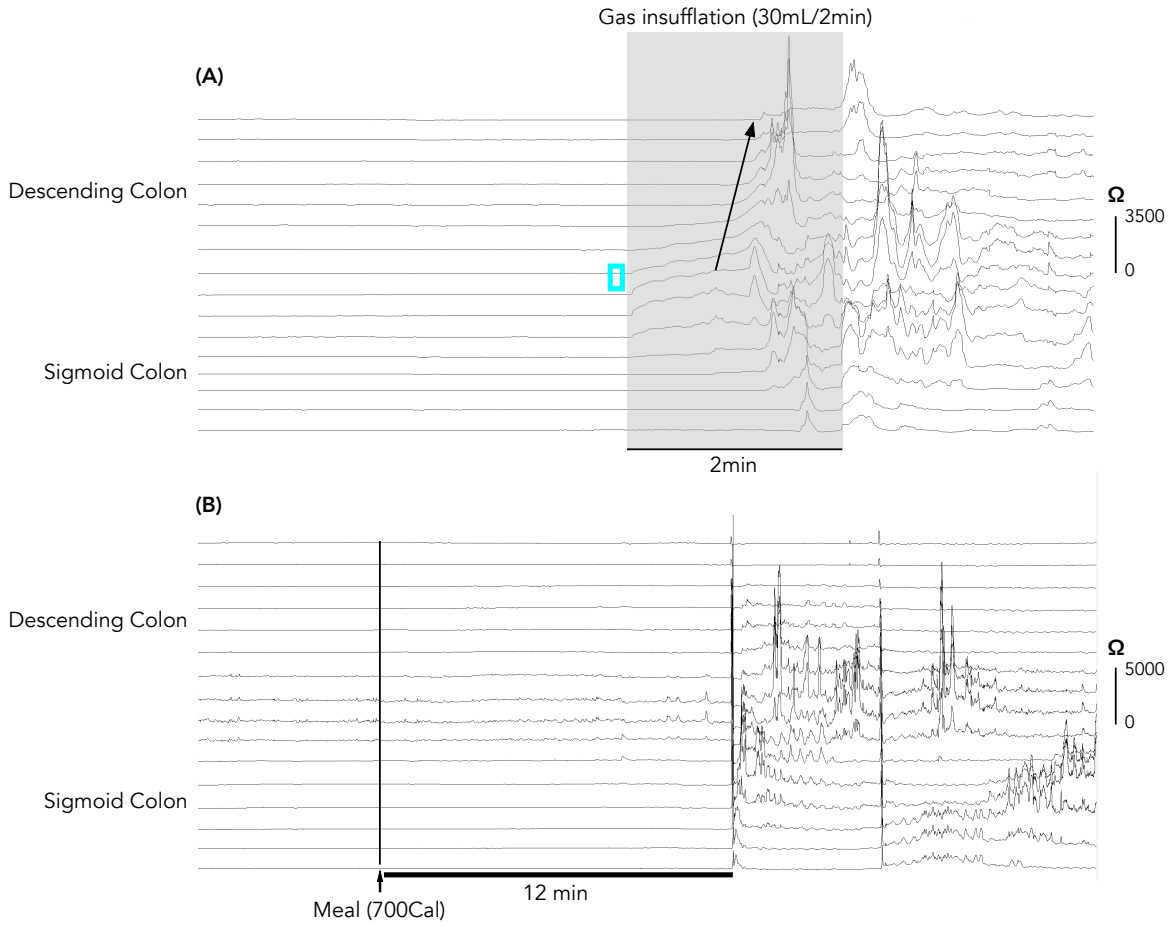

Figure 7.3 (A) An impedance trace demonstrating the characteristic increase in impedance during gas insufflation. Gas is introduced at the location of the aqua box and insufflation continues over the duration of the grey shaded area. The gas moves initially in a retrograde direction (black arrow), followed by a series of antegrade and retrograde movements. In (B), an impedance increase is seen approximately 12 minutes after the commencement of a meal. The similarity in impedance pattern to (A) would suggest that the impedance changes are related to the presence of intraluminal gas.

7.5.4 Temporal Associations Between Pressure and Impedance; Inter-event Interval Distribution Analysis

The temporal associations between pressure and impedance data were assessed using an inter-event interval distribution analysis. The findings were similar after a meal and after gas insufflation, as detailed below.

Propagating Contractions

There were temporal associations between propagating contractions and impedance events. This relationship was bidirectional after a meal and after gas insufflation, meaning that a propagating contraction was temporally associated with a subsequent impedance event (post-prandial p=0.008; post-

gas insufflation p=0.010), and an impedance event was temporally associated with a subsequent propagating contraction (post-prandial p=0.010; post-gas insufflation p=0.012). However, during the control period, this association was unidirectional only, with propagating contractions being temporally associated with subsequent impedance events (p=0.008).

Propagating Contractions Which Terminated in a Pressurisation

When analysis was restricted to propagating contractions which terminated in a pressurisation, a unidirectional association was observed. Both after a meal and after gas insufflation, impedance events were associated with subsequent propagating contractions terminating in a pressurisation (post-prandial p=0.008; post-gas insufflation p=0.005). This would suggest that gas transit temporally preceded propagating contractions which terminated in a pressurisation.

Synchronous Pressure Increases

Synchronous pressure increases and impedance events were temporally associated bidirectionally after a meal or after gas insufflation (post-prandial: synchronous pressure increase $\rightarrow$ impedance event p=0.012; impedance event $\rightarrow$ synchronous pressure increase p=0.007; post-gas insufflation: synchronous pressure increase $\rightarrow$ impedance event p=0.015; impedance event $\rightarrow$ synchronous pressure increase p=0.013). These associations were not present during the control period.

7.5.5 Timing of the Colonic Meal Response and Distal Colonic Gas Accumulation: Hierarchical Change-Point Model

The probability that the colonic meal response commenced prior to gas accumulation in the distal colon was 94%. It is therefore most likely that the meal response commences prior to the arrival of gas, suggesting that the meal response is mediated by extrinsic neural inputs.

7.5.6 Discrete Wavelet Transform Analysis

A 2-D cross-wavelet transform analysis was performed to assess the temporal delay between impedance and pressure data at each spatial location in the colon (**Figure 7.4**). The phase offset reflects the temporal delay between the two variables, with a phase of zero indicating pressure and impedance events occurring simultaneously. In all study periods, there was an overall phase offset between the two variables, weighted to the left of the midline. This demonstrates a consistent temporal delay, with impedance events preceding pressure events, most marked between frequencies of 0.5-4cpm. This may represent gas being displaced ahead of propagating contractions (examples of this are shown in **Figure 7.4A**: antegrade propagating contractions, and **Figure 7.4B**: retrograde propagating contractions). At

higher frequencies (8-16cpm), it appeared that pressure and impedance events occurred almost simultaneously, with a slight offset weighted to the right of midline, demonstrating that pressure events preceded impedance events (**Figure 7.4C**).

A 2-D cross-channel wavelet transform analysis was performed to assess the propagation direction of impedance events (**Figure 7.5**). During all study periods (pre-intervention, post-prandial, post-gas insufflation), there was a significantly greater proportion of impedance events propagating in a retrograde direction, most marked between frequencies of 0.25-2cpm. Following a meal or gas insufflation, impedance signals were of higher amplitude when compared with the control period. This suggests that higher volumes of gas transit occurred after meals and after gas insufflation.

7.5.7 Symptoms

One participant in the meal protocol reported the passage of flatus, which occurred immediately following an antegrade propagating contraction which terminated in the distal descending colon, followed by a pressurisation across the distal channels, located in the sigmoid colon. This occurred concurrently with an antegrade propagating impedance event (**Figure 7.2A**).

Most participants in the gas insufflation protocol reported mild symptoms (symptomatic n=8, asymptomatic n=1). Symptoms descriptors included bloating, pressure, aching, cramping, rumbling, bubbling, gurgling, and butterflies. These ranged in intensity from 1/10-3/10 on a visual analogue scale and were localised to the left lower quadrant (n=3), pelvis (n=3), epigastrium (n=1), or periumbilical (n=1) regions. The symptoms were usually intermittent and of short duration, lasting a median of three minutes (range 1-113 minutes). The symptom of longest duration (113 minutes) was described as a left upper quadrant/epigastric "ache", of 2/10 severity, which commenced during the control period, persisted for the remainder of the study, and did not change in location or severity by subsequent gas insufflation events. Overall, participants were asymptomatic for 78.8%±25.3% of the total study period.

The urge to pass flatus was experienced by two participants in the gas insufflation protocol. On one occasion this occurred following the third gas insufflation (60mL total insufflation volume). The other volunteer reported a brief flatal urge during the control period, which resolved prior to the first gas insufflation.

Association of Symptoms to Pressure and Impedance Events

There were no significant differences in the characteristics of propagating contractions, synchronous pressure increases, or impedance events when comparing symptomatic and asymptomatic periods.

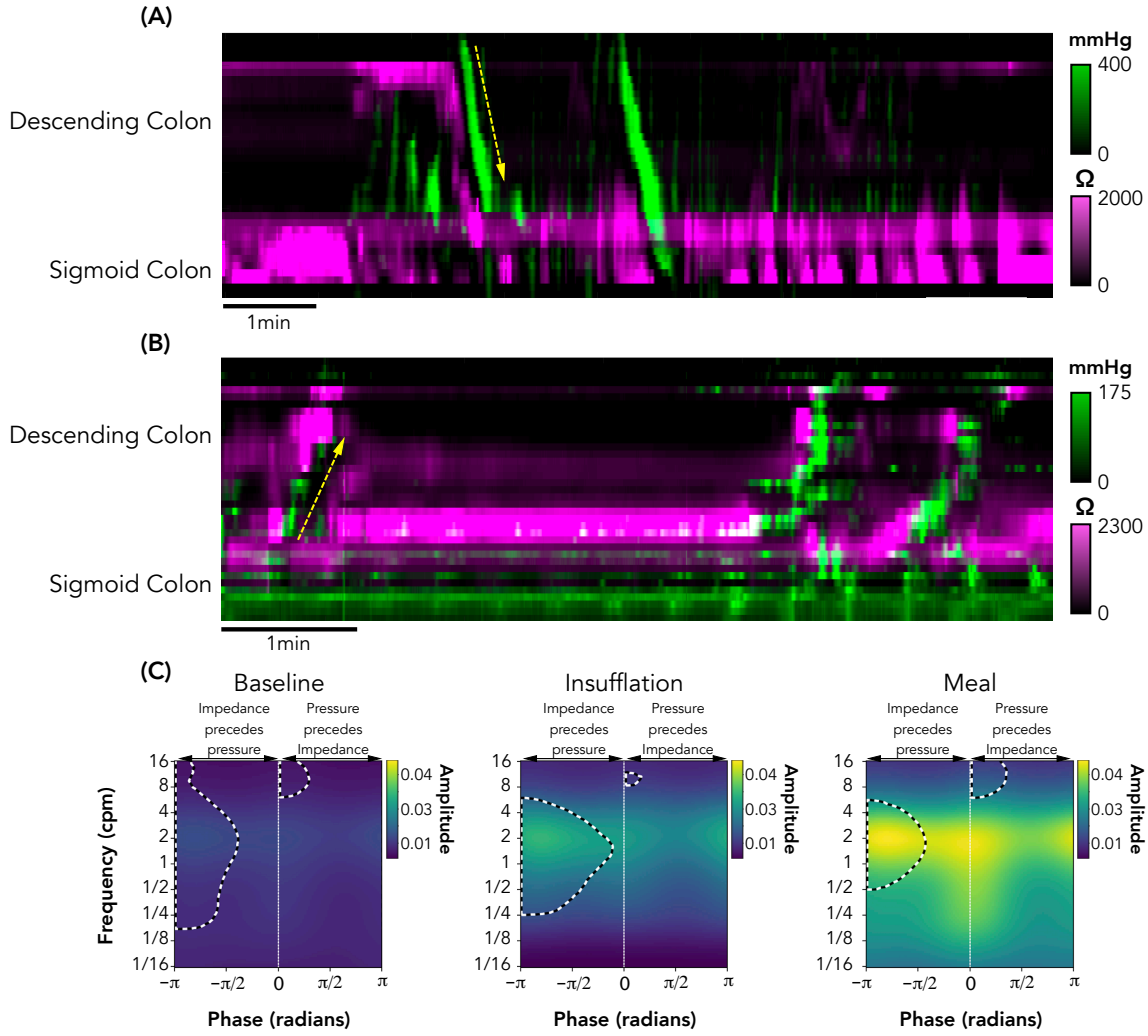

Figure 7.4 (A) and (B), displaying composite colour maps with overlaid pressure (green) and impedance (pink) data. (B) demonstrates antegrade propagating contractions. The first of these (yellow hatched arrow) is preceded by an antegrade magenta streak, indicating gas moving from proximal to distal. Note the band of magenta prior to the propagating contraction is greatly diminished after the propagating contraction, indicating that the gas did not return to its original position. (B) demonstrates retrograde propagating contractions preceded in time by retrograde gas transit. (C) 2-dimensional, cross-wavelet transform analysis demonstrating the temporal associations between impedance and manometry data. The three images in (C) reflect each study period; (left) control period (for both protocol 1 and 2); (middle) post-gas insufflation period; and (right) post-prandial period. In each image, the y-axis displays the frequency of propagating contractions and impedance events. The x-axis reflects the phase offset which reflects the temporal delay between the two variables, with a phase of zero indicating pressure and impedance events occurring simultaneously. A phase offset to the left of the central white vertical line indicates impedance events occurring prior to propagating contractions; and a phase offset to the right of the central white vertical line indicates propagating contractions occurring prior to impedance events. Amplitude is represented by a colour spectrum from yellow (high) to blue (low) and reflects the changes in the average wavelet coefficient. In each of the figures in (C), black and white hatched areas indicate significant findings. In the image of the left, a large black and white hatched area can be seen from 16cpm to 1/6cpm of the left side of the white vertical line. This indicates that, within the hatched region at those frequencies, impedance is significantly more likely to temporally precede pressure than pressure preceding impedance. This association is evident in all three images, indicating that - at most frequencies - impedance temporally precedes pressure as demonstrated in examples (A) and (B).

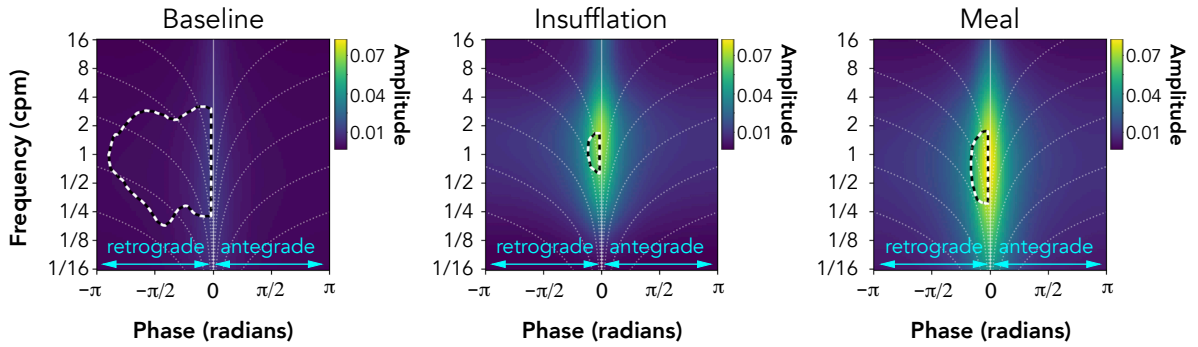

Figure 7.5 A 2-dimensional cross-channel wavelet transform analysis demonstrating the propagation direction of impedance events. The three images reflect each study period; (left) control period (for both protocol 1 and 2); (middle) post-gas insufflation period; and (right) post-prandial period. In each image, the y-axis displays the frequency of impedance events. The x-axis reflects the phase offset which reflects the temporal delay between impedance events in adjacent channels. A phase offset to the left of the central white vertical line indicates retrograde propagation, and to the right of the central white vertical line indicates antegrade propagation. Amplitude is represented by a colour spectrum from yellow (high) to blue (low) and reflects the changes in the average wavelet coefficient. During all study periods, the black and white hatched regions are to the left of the vertical central line. Within these black and white regions, retrograde propagation is significantly greater than antegrade propagation at the same frequency. This is most marked between frequencies of 0.25-2cpm. Following a meal or gas insufflation, impedance signals were of higher amplitude when compared with the control period. This suggests that higher volumes of gas transit occurred after meals and after gas insufflation.

7.6 Discussion

This study describes the temporal associations between colonic motility (pressure) and gas transit (impedance) in the human colon. Both a meal and gas insufflation resulted in a significant increase in the number of impedance events and an increase in the prevalence of the cyclic motor pattern in the distal colon. Impedance events most commonly preceded propagating contractions which is likely to represent a column of luminal gas being propelled ahead of a propagating contraction.

These findings are based upon the assumption that impedance events represented gas transit. This interpretation is based upon preliminary studies performed in rabbit colon ex vivo with concurrent impedance manometry and video imaging(152). In these experiments, manual infusions of liquid or gas were compared to establish the characteristic changes elicited by either media on the impedance recording. While it was not possible to perform concurrent prolonged imaging on human participants in this study, the impedance events that we recorded were similar to those recorded in the ex vivo rabbit

colon in the presence of gas. Consistent with this, we reliably observed an immediate increase in impedance during each gas insufflation (**Figure 7.3**), when we were certain that gas was present in the colon.

Colonic motility can be altered by meals, sleep, exercise, and stress(2, 80, 100, 111, 112, 132, 153, 156, 160-164), as well as by mechanical and chemical stimuli(112, 130, 136, 169). However, it is yet to be determined how motility patterns are related to intraluminal gas. In the human distal colon, the post-prandial period is associated with both an increase in the cyclic motor pattern(2) as well as the accumulation of gas(384). Both responses were demonstrated in this study, with an increase in the cyclic motor pattern following the meal as well as an increase in impedance events, reflecting gas accumulating in the distal colon.

Given this finding, the question arises as to whether post-prandial gas in the distal colon acts as a mechanical stimulus for the cyclic motor pattern. The colonic transit of gas, liquid, or solid content has been suggested in several previous studies to stimulate rectosigmoid motility with characteristics similar to the 2-8/minute cyclic motor pattern(116, 168, 173, 176, 316). This led to the hypothesis that this motor pattern functions as an "intrinsic colonic gatekeeper"(116) or "rectosigmoid brake"(176) which controls delivery of colonic content into the rectum (see **1.6.3 The Cyclic Motor Pattern**). Protocol 1 addressed one aspect of this potential relationship. Our findings demonstrate (with a 94% probability) that the increase in the cyclic motor pattern occurred prior to the arrival of gas in the distal colon. This would suggest that extrinsic neural inputs prime the distal colon in preparation for the arrival of content from the proximal colon. This may either reflect an excitatory input, or removal of tonic neural inhibition, as described in ex vivo human colonic specimens(388).

Our data also indicate that gas insufflation can elicit a colonic motor response. The increase in time occupied by the cyclic motor pattern after gas insufflation (28%) was similar to the post-prandial increase (29%). This finding suggests that the cyclic motor pattern can also be initiated by a localised sensorimotor response to gas, which would further support the rectosigmoid brake hypothesis. Colonic transit occurs continuously between meals(98) and, therefore, local stimulation of the cyclic motor pattern by intraluminal content may prevent rectal filling in the absence of a meal stimulus.

Further support for the presence of a rectosigmoid brake is that most of our participants experienced no urge to pass flatus following gas insufflation, despite the site of insufflation being approximately 30cm from the anal verge. It is unlikely that the gas was absorbed, because room air is predominantly composed of N_2 (78%), which is poorly absorbed. In a study by Hernando-Harder et al., gas insufflated into the rectum reached the caecum within six minutes(717). Retrograde gas transit was also demonstrated in our findings.

While the cyclic motor pattern increased in prevalence after the gas insufflation, in contrast to the meal response, there was no significant increase in the overall number, frequency, and amplitude of all individual propagating contractions. This may be due to the relatively small volume of gas introduced in protocol 2 (60mL total over 42-minutes). The colonic meal response is well established(2, 516, 700, 718) and it has been shown that the intensity of the response is related to the caloric content of the meal (**2.6.6 Colonic Motor Response to a Meal**). For example, a meal of 350Cal has only a minimal effect on colonic motility, whereas a 1000 Cal meal significantly increases colonic motility(155, 157). A similar, graded colonic response may be elicited with insufflation of increasing volumes of gas. The 60ml volume of gas insufflation used in this study is relatively small in comparison to normal colonic gas volume (100-200mL(81)). Previous studies using gas insufflation have used much larger volumes (200mL-720mL(719, 720)), and insufflation volumes are also considerably larger in computed tomography colonography imaging (>2L)(721, 722). Nevertheless, the small volumes used in the present study consistently resulted in increases in the cyclic motor pattern. Our chosen volume was also related to ongoing studies in our laboratory examining the colonic response to gas insufflation in specific patient populations who may not tolerate larger volumes, such as patients with irritable bowel syndrome or faecal incontinence.

Chen et al.(167) proposed that simultaneous pressure waves (pan-colonic pressurisations(166)) could be used as a functional biomarker for gas transit. In our study, the characteristics of simultaneous pressure waves (which we referred to as *synchronous pressure increases*) were not altered after either a meal or gas insufflation. Synchronous pressure increases can be caused by abdominal strain, diaphragmatic movement during phonation, or re-positioning in bed. Corsetti et al.(166) discriminated between synchronous pressurisations caused by colonic motor activity and abdominal wall muscle activity using abdominal wall electromyography (EMG). In their study, the synchronous pressurisations had a mean duration of >20s. The maximum duration of any synchronous pressure increase in our data was 12s, with mean of <5s. This suggests that the synchronous pressure increases that we observed may not have been generated by colonic smooth muscle, but rather by increases in intra-abdominal pressure caused by contraction of the abdominal wall musculature. This is supported by our finding that the characteristics of synchronous pressure increases were unchanged after a meal or after gas insufflation. Given the temporal association between synchronous pressure increases and impedance events in the post-intervention periods (when there was also an increased presence of gas in the distal colon), extraluminal mechanical compression of the colon (by abdominal wall muscle contractions) may cause movement of gas without contractions of the colonic smooth muscle.

In addition to synchronous pressure increases, we also recorded pressurisations at the termination of propagating contractions. These pressurisations were also associated with impedance events. In the

isolated rabbit small intestine and colon, synchronous pressurisations were regarded as "common cavity" phenomena(152, 183), in which a propagating contraction causes colonic dilation ahead of the contraction. The common cavity occurs when accumulating luminal contents are trapped by a blind end. This could occur as a result of a closed anal sphincter (for antegrade propagating contractions) or a more proximal lumen-occlusive contraction (for retrograde propagating contractions). In one healthy control, a pressurisation following an antegrade propagating contraction was associated with flatus (**Figure 7.2A**). The volunteer reported flatus 8 seconds after this pressurisation event.

While the focus of our analyses was the cyclic motor pattern, the associations between gas transit and other propagating motor patterns (such as HAPCs and single motor patterns) were not analysed. However, it needs to be emphasised that our study design was very much biased towards the recording of the cyclic motor pattern. We have shown previously that the cyclic motor pattern dominates the propagating activity in the sigmoid colon particularly in the period during and after a meal(2, 316). The cyclic motor pattern also made up the majority of the propagating activity observed after gas insufflation. In our previous study(2), nearly half of the single motor patterns occurred in the proximal colon; a region not examined in this study. In addition, due to the prevalence of the cyclic motor pattern in the sigmoid colon, isolated propagating contractions separated from other propagating contractions by >1min were rarely seen. High amplitude propagating contractions (HAPCs) are infrequent and are seldom seen in short duration studies of the prepared human colon(2). In this study, we recorded only five HAPC, all of which terminated in a synchronous pressure increase and associated with gas transit. Prolonged studies of >8 hours duration would provide greater insight into the relationships between all colonic motor patterns and gas transit.

In conclusion, these findings demonstrate a post-prandial increase in the 2-8/minute cyclic motor pattern and a subsequent increase in the number of impedance events, reflecting retrograde gas movement. Similar increases in cyclic motor activity and impedance events were induced by gas insufflation into the sigmoid colon. The absence of a conscious urge to pass flatus, and the temporal association between propagating contractions and impedance events may suggest that the cyclic motor pattern acts as a physiological "brake" which modulates rectal filling. Whether there is a reduction or absence of the cyclic motor pattern in conditions such as diarrhea-predominant IBS and faecal incontinence is the subject of on-going investigation.

7.7 Tables

Table 7.1 Protocol 1: Meal Response; The Characteristics of Manometry and Impedance Events (Mean±SD), Separated by Study Period (Pre- and Post-Prandial)

	Pre-prandial						Post-prandial					
	Impedance events		Propagating contractions		Propagating contraction with pressurisations	Synchronous pressure increases	Impedance events		Propagating contractions		Propagating contractions with pressurisations	Synchronous pressure increases
	Antegrade	Retrograde	Antegrade	Retrograde			Antegrade	Retrograde	Antegrade	Retrograde		
Total number	21	20	723	745	0	842	504	430	2081	2480	34	803
Number per participant	3.00±4.20	2.86±5.40	72.30±61.47	74.50±45.49	0	84.20±76.64	50.40±31.62	43.00±32.71	208.10±108.64	248.00±98.24	3.40±2.59	80.30±66.47
Frequency	0.03±0.04/min	0.02±0.05/min	0.59±0.49/min	0.61±0.37/min	N/A	0.68±0.60/min	0.41±0.25/min	0.35±0.26/min	1.70±0.77/min	2.03±0.64/min	0.03±0.02/min	0.69±0.56/min
Amplitude	365.31±480.04Ω	381.67±331.01Ω	8.26±5.91mmHg	8.92±5.59mmHg	N/A	8.80±2.97mmHg	1374.01±1261.36Ω	1087.24±1176.79Ω	11.62±3.58mmHg	13.64±4.13mmHg	24.40±11.51mmHg	11.47±3.42mmHg
Length of propagation	4.23±0.83cm	4.53±1.56cm	4.86±1.08cm	5.18±0.83cm	N/A	N/A	6.20±1.50cm	5.12±0.73cm	6.71±1.24cm	6.95±1.20cm	24.39±11.51cm	N/A
Pace	0.17 ± 0.22s/cm	0.16±0.27s/cm	1.08±0.47s/cm	1.30±0.81s/cm	N/A	N/A	0.46±0.13s/cm	0.49±0.13s/cm	0.61±0.31s/cm	0.77±0.35s/cm	-0.02±0.63s/cm	N/A
Duration						3.30±1.22s						3.30±1.40s

Table 7.2 Protocol 2: Gas Insufflation; The Characteristics of Manometry and Impedance Events (Mean±SD), Separated by Study Period (Pre- and Post-Gas Insufflation)

	Pre-gas insufflation						Post-gas insufflation					
	Impedance events		Propagating contractions		Propagating contraction with pressurisations	Synchronous pressure increases	Impedance events		Propagating contractions		Propagating contractions with pressurisations	Synchronous pressure increases
	Antegrade	Retrograde	Antegrade	Retrograde			Antegrade	Retrograde	Antegrade	Retrograde		
Total number	27	26	644	701	2	565	570	536	709	899	15	569
Number per participant	3.86 ± 4.02	3.71 ± 3.90	71.56 ± 38.89	77.89 ± 36.51	1 ± 0	62.78±41.05	63.33 ± 23.97	59.56 ± 18.53	78.78 ± 51.99	99.89 ± 45.86	3.75 ± 2.06	63.22 ± 39.40
Frequency	0.06 ± 0.06/min	0.06 ± 0.06/min	1.09 ± 0.56/min	1.19 ± 0.54/min	N/A	0.99±0.67/min	0.92 ± 0.40/min	0.85 ± 0.28/min	1.15 ± 0.84/min	1.44 ± 0.73/min	0.05 ± 0.03/min	0.91 ± 0.59/min
Amplitude	315.44 ± 238.47 Ω	268.31 ± 240.27 Ω	11.86 ± 5.11mmHg	15.20 ± 6.71mmHg	28.39 ± 12.91mmHg	8.92±3.58 mmHg	682.62 ± 368.07Ω	633.94 ± 421.46Ω	15.52 ± 5.51mmHg	18.27 ± 8.71mmHg	55.40 ± 29.71mmHg	10.88 ± 2.78mmHg
Length of propagation	4.5 8 ±2.49cm	4.26 ± 1.98cm	4.22 ± 0.69cm	4.95 ± 0.92cm	15.50 ± 6.36cm	N/A	5.71 ± 0.88cm	5.59 ± 1.11cm	5.74 ± 1.65cm	6.14 ± 1.74cm	21.76 ± 2.14cm	N/A
Pace	0.34 ± 0.33s/cm	0.72 ± 0.75s/cm	1.91 ± 0.99s/cm	1.95 ± 1.10s/cm	0.10 ± 2.16s/cm	N/A	0.51 ± 0.15s/cm	0.49 ± 0.11s/cm	1.29 ± 0.91s/cm	1.54 ± 0.92s/cm	-0.79 ± 0.64s/cm	N/A
Duration						2.56 ± 0.94s						2.83 ± 1.32s

Chapter 8: The Mechanisms Responsible for the Generation of Propagating Motor Patterns in the Human Colon

8.1 Statement

The content of this chapter has been published in *Neurogastroenterology & Motility.*

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The co-authors have provided permission for the inclusion of the study in this thesis. The percentage contributions of each author to this study were as follows:

- Research design: **PH 25%**, RMR 25%, MC 25%, PD 25%.
- Data collection and analysis: **PH 45%**, RMR 18.33%, LW 18.33%, PD 18.33%.
- Writing and editing: **PH 40%**, MC 10%, LW 10%, DW 10%, SB 10%, NS 10%, PD 10%.

8.2 Abstract

Background: Colonic high-resolution manometry (HRM) has been used to reveal discrete, propagating colonic motor patterns. To determine the mechanisms underlying these motor patterns, we used HRM to record contractile activity in human distal colon ex vivo. **Methods**: Surgically excised segments of descending (n=30) or sigmoid colon (n=4) were immersed in oxygenated Krebs solution at 36°C (n=34; 16 female; 67.6±12.4 years; length: 24.7±3.5cm). Colonic motor patterns were recorded by HRM catheters. After 30 minutes of baseline recording, 0.3mM lidocaine and/or 1mM hexamethonium were administered. Ascending neural pathways were activated by electrical field stimulation (EFS; 10Hz, 0.5ms, 50V, 5s duration) applied to the anal end before and after drug administration. **Results**: Spontaneous propagating contractions were recorded in all specimens (0.1-1.5 cycles/minute). Most contractions occurred synchronously across all recording sites. In five specimens, rhythmic antegrade contractions propagated across the full length of the preparation. EFS evoked local contractions at the site of stimulation (latency: 5.5±2.4 seconds) with greater amplitude than spontaneous contractions (EFS=29.3±26.9, spontaneous=12.1±14.8mmHg; p=0.02). Synchronous or retrograde propagating motor patterns followed EFS; 71% spanned the entire preparation length. Hexamethonium and lidocaine modestly and only temporarily inhibited spontaneous contractions, whereas TTX increased the frequency of contractile activity while inhibiting EFS-evoked contractions. **Conclusions:** Our study suggests that the propagated contractions recorded in the organ bath have a myogenic origin which can be regulated by neural input. Once activated at a local site, the contractions do not require the propulsion of faecal content to sustain long-distance propagation.

8.3 Introduction

Contractile activity in the human colon plays a critical role in the absorption of water and electrolytes and in the propulsion and excretion of colonic content. Recent development of high-resolution manometry catheters has enabled detailed descriptions of propagating motor patterns throughout the colon in healthy adults(2, 166, 167) and the identification of motor abnormalities in adults and children with severe constipation(166, 250, 267, 723). The relationships between motor patterns recorded in vivo and those recorded in long, isolated ex vivo segments of colon(256, 724) remains to be established.

Using high-resolution manometry, at least six motor patterns have been described in the human colon in vivo(2, 166, 267, 725), including the cyclic motor pattern(2) and pancolonic pressurisations(166, 167) (see **1.6 Colonic Motor Patterns**). The cyclic motor pattern is characterised by repetitive, propagating pressure events with a frequency of 2-6 cycles/minute. Pancolonic pressurisations are characterised by synchronous increases in pressure across all recording sensors in the colon. They occur at frequencies between one per minute to one every four minutes(166, 167). The physiological roles of these two motor patterns remain uncertain. The cyclic motor pattern, found predominantly in the distal colon, has been proposed to act as a brake that mediates rectal

filling(116, 316). It has been suggested that pancolonic pressurisations are associated with the transit of gas and liquid(166, 167).

The mechanisms underlying these motor patterns are not well understood. It is likely that the cyclic motor pattern is driven by the pacemaker networks of the interstitial cells of Cajal (ICC) which exhibit a similar slow-wave frequency of 2-6 cycles/minute(30). These slow waves have been temporally associated with small amplitude variations in intraluminal pressure(726). This myogenic cyclic activity appears to be influenced by extrinsic nerves; the cyclic motor pattern rapidly increases in prevalence and amplitude following a high-calorie meal(2).

The mechanisms underlying pancolonic pressurisations are even more poorly defined. Corsetti et al.(166) hypothesised that pancolonic pressurisations may require colonic distension. Organ bath studies using colonic specimens from guinea pigs or mice have shown that constant distension can induce synchronous colonic contractions at a similar frequency to pancolonic pressurisations in human studies(727, 728). Constant stretch, applied by force transducers attached to the serosa of human colon in an organ bath, can initiate repetitive contractions that occur synchronously along the length of the colon(724).

In this study, using a technique developed by Spencer et al.(724) with the addition of high-resolution manometry(256), we provide a detailed characterisation of spontaneous motor patterns and their responses to pharmacological agents and electrical stimuli in ex vivo specimens of human colon.

8.4 Methods

8.4.1 Specimen Retrieval and Initial Handling

The study was approved by the Flinders Clinical Research Ethics Committee No. 50/07. Segments of descending colon (n=40) or sigmoid colon (n=6) were obtained from 46 patients who underwent elective colorectal surgery (anterior resection) for non-obstructing colorectal cancer (n=43) or recurrent sigmoid diverticulitis (n=3) after obtaining prior informed consent. The length of colon resected was determined by the surgeon based upon the vascular territory of the inferior mesenteric artery. The inferior mesenteric artery is routinely ligated during an anterior resection, resulting in a length of macroscopically normal colon being resected along with the diseased segment.

The timing of ligation of the inferior mesenteric artery varied, depending on the surgeon involved in each case and the case complexity. Five consultant colorectal surgeons were involved in experimental tissue donation. One surgeon who contributed most specimens (DW, 50%) opted for a late ligation where possible to minimise ischaemia time.

To reduce time between specimen removal and recording, an investigator (RMR or PH) was notified of surgery timing and retrieved the specimen immediately upon excision. The tumour and at least 5cm margin of macroscopically healthy tissue was isolated and sent for histological review by a surgical pathologist. The remaining segment of tissue proximal to the diseased segment (length=24.7±3.5cm; range: 15-36cm) was placed into warmed, pre-oxygenated Krebs solution (in mM: 118 NaCl, 4.7 KCl, 1.0 NaH_2PO_4, 25.0 $NaHCO_3$, 1.2 $MgCl_2$, 11 D-glucose, 2.5 $CaCl_2$) and taken to the laboratory. Prior to placement in the organ bath, the specimen was cleared of residual blood and faeces by washing with Krebs solution. In 10 additional experiments (n=8 descending colon; n=2 sigmoid colon), a 2cm wide ring was cut from the proximal end of the specimen. From these segments, circular muscle strips were excised from an inter-taenial region.

All specimens were placed in the organ bath within 20 minutes of removal from the patient. Following recordings, the intact tubular colonic specimens were required to be returned to the pathology department. Experimental recordings ranged from 1-5.5 hours (median=2.13 hours). All specimens had normal colouration and did not appear to be cyanotic.

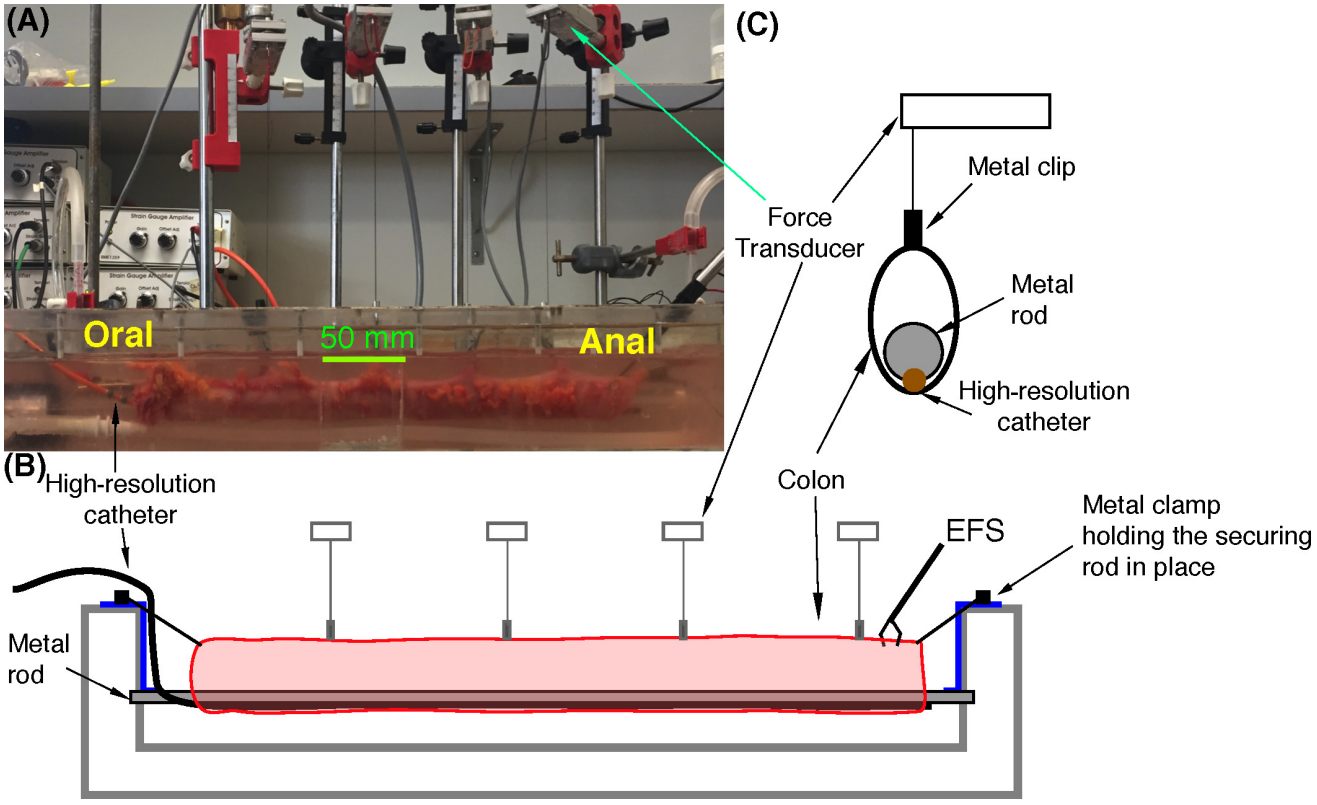

Figure 8.1 (A) Ex vivo experimental setup of excised human descending colon. The specimen was immersed in an organ bath filled with Krebs solution, bubbled with 95% O_2/5% CO_2. The manometry catheter, affixed to a Perspex rod, was passed through the colonic lumen. Force transducers applied tension to the colonic wall, ensuring close contact between the manometry catheter and the mucosa. (B) A schematic of the setup with the electrical field stimulation (EFS) applied at the anal end of the specimen via platinum electrodes insulated to within 2mm of their tips. (C) A sagittal profile of the setup.

8.4.2 Experimental Setup

8.4.2.1 Intact Colonic Specimens (n=36)

Using an experimental setup similar to that described in previous studies(256, 623, 724) (**Figure 8.1**), the specimen was placed into a water-jacketed organ bath filled with 2L of Krebs solution at 36-37°C. Krebs solution was bubbled continuously with 95% O_2/5% CO_2. A tube attached to small aquarium pump was also positioned in the base of the organ bath. A high-resolution manometry catheter was taped with wax film (Parafilm "M" Laboratory Film; Bemis, Neenah, WI, USA) to a Perspex rod which was inserted through the lumen of the specimen. The rod was fixed in the organ bath with the catheter abutting the mucosa on the lower side of the specimen. The oral and anal ends of the specimen were gripped by alligator clips, from which a thread ran to either end of the organ bath, anchoring the specimen (**Figure 8.1**). Either three or four isometric force transducers (depending on specimen length) were attached to the uppermost side of the specimen using alligator clips tied to silk threads, spaced evenly along the length of the specimen (~5-7cm intervals; details below; **Figure 8.1**). The clips were attached to the serosa between the taenia coli. A resting tension of ~5g was applied to each transducer which lifted the colon, ensuring close contact between the manometry catheter and the mucosa. The high-resolution manometry catheter and force transducers both recorded mechanical colonic activity, but manometry data was preferentially used for analysis given the higher spatial resolution (1cm sensor spacing).

Specimens were equilibrated in the organ bath for 20 minutes. A control period was then recorded during which no drugs were administered for a minimum of 30 minutes to record spontaneous contractions. To establish the dependence of spontaneous activity on nicotinic synaptic transmission in the enteric circuits, we first added 1mM hexamethonium to 16 preparations. After hexamethonium, we tested further requirements for neural activity. Previously, the neurotoxin tetrodotoxin (TTX) has been used(39); however, given the size of the 2L organ bath used for the intact tubular specimens, it was not feasible to use TTX for all studies on account of the volume required to achieve an effective concentration. In place of TTX, after the 30-minute control period, lidocaine (0.3mM), an alternative voltage-dependent sodium channel antagonist, was added to 16 preparations. TTX (0.6μM) was only added to the final two preparations. Drugs were added along the length of the preparation. Continuous mixing of the Krebs solution via the carbogen (95% O_2/5% CO_2) insufflation and the aquarium pump ensured that the drugs were rapidly mixed through the preparation.

To activate ascending neural pathways, electrical field stimulation (EFS; Grass SD9 Stimulator; Grass, Quincy, MA, USA; 2mm Pt electrodes, insulated up to the last 5mm of their length, spaced 2mm apart, 10Hz, 0.5ms pulse width, 50V, 5-second train duration) was applied at the anal end of the preparation. To avoid confusion between spontaneous and EFS-evoked contractions, stimulation was applied after a spontaneous contraction, with a delay of ≥ half the average inter-contraction interval. Due to a technical fault, EFS was not applied to the preparations with TTX.

8.4.2.2 Mechanical Recording Techniques: Force Transducers and High-Resolution Manometry (HRM)

Isometric force transducers (Grass FT-03C; Grass, Quincy, MA, USA) were connected to custom-made preamplifiers (Biomedical Engineering, Flinders University) to a PowerLab (Model 16/35, ADInstruments, Bella Vista, NSW, Australia) and a Macintosh computer running LabChart version 6 (ADInstruments, NSW, Australia). Two high-resolution manometry catheters were used to record intraluminal muscle contractions. The first, used for 12 recordings, was a fibreoptic catheter with 72 pressure sensors spaced at 1cm intervals(729). The fibreoptic catheter was attached to a spectral interrogator unit (FBG-Scan 804; FOS&S, Geel, Belgium), and pressures were recorded in real time on a custom-written LabVIEW program (National Instruments, Austin, TX, USA). The second catheter, used for 24 recordings, was a commercially available, solid state high-resolution impedance manometry catheter (Sandhill Scientific, Unisensor USA Inc) with 32 pressure sensors spaced at 1cm intervals. Pressure data was acquired at 10Hz using InSIGHT^{TM} (Sandhill Scientific, Unisensor USA Inc). Impedance data was not used in this study. Pressure data acquired by either catheter were exported as text (*.txt) files for analysis in custom-made PlotHRM software developed by the authors (LW), written in MATLAB (MathWorks, MA, USA) and Java (Sun Microsystems, CA, USA)(730).

8.4.2.3 Circular Muscle Strip Studies (n=10)

To further test the neural contributions to activity in colonic smooth muscle, studies of circular muscle strips were also conducted. In 10 specimens, the proximal disease-free end of the specimen was removed and placed in Krebs solution (see **8.4.1 Specimen Retrieval and Initial Handling**), warmed to 36-37.5°C, and bubbled with 95% O_2/5% CO_2. The specimen was cut open along the taeniae coli and pinned out as a flat sheet. Excess fat, mesocolon, and mucosa were excised via sharp dissection. Two inter-taenial, transmural tissue strips of 5mm width and 10-15mm length were cut parallel to the orientation of the circular muscle fibres. These circular muscle strips were placed into a warm-jacketed 100mL organ bath filled with Krebs solution.

Each strip was passed through a pair of ring electrodes (Biomedical Engineering, Flinders University). One end of the preparation was sutured to a fixation rod at the base of the organ bath and the other end was attached to a force transducer (Grass FT-03C; Grass, Quincy, MA, USA). Each strip was then tensioned to 10g in the direction of orientation of muscle fibres. Force transducers were connected to custom-made preamplifiers (Biomedical Engineering, Flinders University), a PowerLab (Model 8/30, ADInstruments, Bella Vista, NSW, Australia), and a Macintosh computer running LabChart version 6 (ADInstruments, NSW, Australia).

After a 20-30-minute equilibration period, a 30-minute basal period of spontaneous contractile activity was recorded. During this period, EFS (10Hz, 0.5ms, 20V, for a duration of 10 seconds) was additionally applied to the strips to induce contractile activity. TTX (0.6 μM) was then applied in the organ baths.

8.4.3 Data Analysis

8.4.3.1 Intact Tubular Preparations

Contractile activity was manually identified in PlotHRM. Individual events were characterised in terms of; (a) the direction of propagation; antegrade, retrograde, or synchronous (ie. all pressure waves starting within a period of 1 second at all points along the specimen); (b) length of propagation, expressed as a % of the length of the preparation; (c) amplitude of component pressure waves; (d) speed of propagation, and; (e) frequency of propagating events per minute ("cycles per minute"/cpm). These characteristics were obtained for the control period and for each period after administration of drugs.

For each of the electrical field stimulations (EFS), the following information was collected; (a) time from the start of stimulation to the start of the next contraction adjacent to the stimulating electrode, and; (b) whether the contraction at the stimulation site was associated with a propagating contraction. If so, we then determined; (c) the extent of propagation, expressed as a % of the length of the preparation; (d) the direction; (e) speed of propagation, and; (f) amplitude of propagation.

Finally, using an analysis technique similar to one we have published previously(39), we determined whether EFS could reset the timing of spontaneous propagating contractile activity. This was achieved by determining the time interval between; (a) spontaneous propagating contractions prior to EFS; (b) the last spontaneous propagating contraction prior to EFS and the next spontaneous propagating contraction following EFS, and; (c) the EFS-induced propagating contraction and the next spontaneous propagating contraction.

8.4.3.2 Circular Muscle Strip Preparations

The frequency of spontaneous contractions and resting tone was calculated during the basal period and after application of TTX. In addition, the amplitude of EFS-induced contractile activity was calculated prior to and after TTX. To quantify the response to EFS, the change in force was derived from mean force during stimulation minus the mean force in the 60s prior to stimulation.

8.4.4 Drugs

Lidocaine (0.3mM) and hexamethonium (1mM) were purchased from Sigma-Aldrich, Castle Hill, Australia. These drugs were dissolved in Krebs solution. TTX was purchased from Alomone Labs, Jerusalem, Israel, and dissolved in deionised water.

8.4.5 Statistical Analysis

For the intact tubular preparations, comparisons of characteristics (numbers, amplitude, velocity, extent of propagation) for propagating events before and after drug administration were made using a non-parametric Wilcoxon matched-pairs signed-rank test.

The intervals between electrical field stimulation (EFS) and the next contraction were compared to the average intervals between the five preceding spontaneous contractions at the same location using a non-parametric Wilcoxon matched-pairs signed-rank test. The characteristics of the event evoked by EFS were also compared to the five preceding propagating events. In addition, the characteristics of the evoked propagating events were compared before and during exposure to lidocaine or hexamethonium. A one-way ANOVA with Tukey's range test post hoc analysis was used to determine whether there was a significant difference between the time interval between spontaneous propagating contractions prior to EFS and the time interval between an EFS-induced propagating contraction and the next spontaneous propagating contraction.

For the muscle strip studies, statistical comparisons of the spontaneous contractile frequency, tone, and EFS-induced contraction amplitude prior to and after TTX were made using a non-parametric Wilcoxon matched-pairs signed-rank test.

Statistical analysis was performed using IBM SPSS (Version 19.0, Released 2010; IBM Corp., Armonk, New York, USA).

8.5 Results

As there was no significant difference between the recording pressures captured by the two manometry catheters, the data recorded by the fibreoptic and solid-state catheters were combined.

8.5.1 Mechanical Recordings of Colonic Contractions During the Control Period

Spontaneous contractile events were recorded in every specimen during controeriods. Most contractions propagated the full length of the specimen and occurred at a frequency of 0.1-1.5cpm (median=0.6cpm). In 24 specimens, contractions appeared to occur simultaneously across most or all of the recording sites (**Figure 8.2A**). In five of these specimens, the propagating contractions were in an unequivocal, antegrade direction (**Figure 8.2B**). The characteristics of the propagating activity during the control period are shown in **Table 8.1**.

8.5.2 Electrical Field Stimulation (EFS) During the Control Period

EFS was applied to the distal end of 19 specimens (**Figure 8.3**). In 18 specimens, a local contraction was observed with short latency after the onset of EFS (5.5±2.4 seconds). In one specimen, no local contraction occurred. An attempt was made to apply EFS mid-way between contractions, with a delay of approximately half of the interval between spontaneous contractions. Thus, we could test whether electrical stimulation altered the timing of the next contraction. This was indeed the case; the interval between the onset of EFS and the following contraction was significantly less than the average interval between the preceding five spontaneous contractions at the same location (5.5±2.4 seconds vs 173±154 seconds; range: 31-600 seconds; $p<0.0001$). This indicates that the next contraction was hastened by EFS. The interval between the spontaneous contractions prior to EFS and the delay between the EFS-induced contraction and the next spontaneous propagating contraction were similar (173±154 seconds vs 198±137 seconds; NS). This suggests that the EFS reset the rhythm of the propagating contractions throughout the preparation and that selective stimulation of the enteric nervous system can interfere with the intrinsic pacemaker frequency.

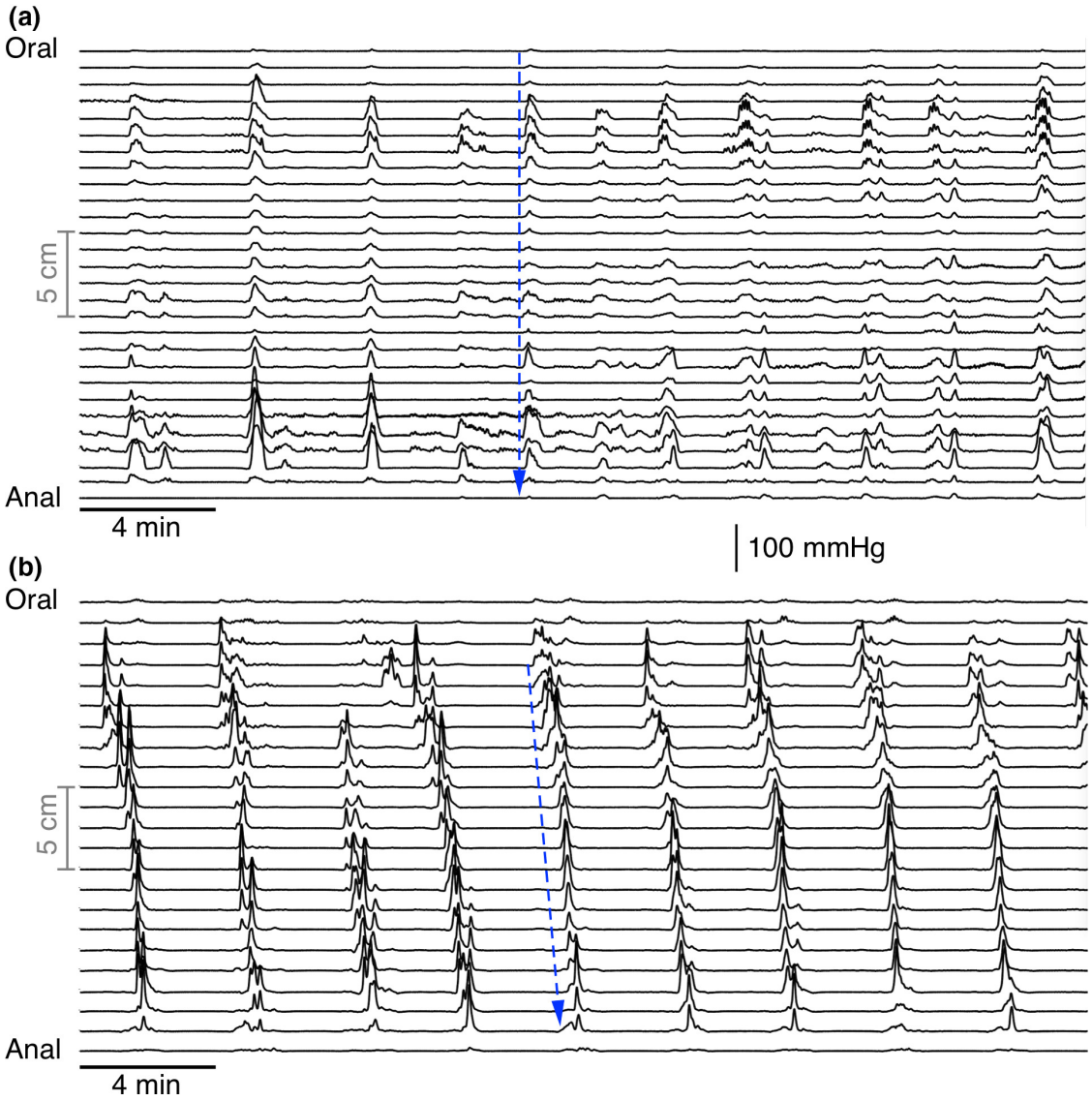

Figure 8.2 Spontaneous cyclical contractile events during the control period occurred with two distinguishable patterns. (A) Contractions were synchronous along the preparation (n=24), or, (B) contractions showed a clear antegrade propagation (blue hatched arrows denote direction; n=5).

In all cases, contractions evoked by EFS became the start of a propagating event. Most of these evoked propagating events (79%) spanned the entire length of the preparation (up to 30cm). In the remaining specimens, the propagating contractions were observed over at least 19% of the length. Overall, the length of propagation after EFS did not differ from the preceding spontaneous propagating contraction (EFS; 17.9±5.7 v spontaneous 17.4±7.3cm). Following EFS, 85.8% of the evoked propagating contractions travelled in a retrograde direction at an average velocity of 44.2±39.9mm/s. This contrasts with spontaneous contractions prior to stimulation which mostly appeared simultaneously along the length of colon.

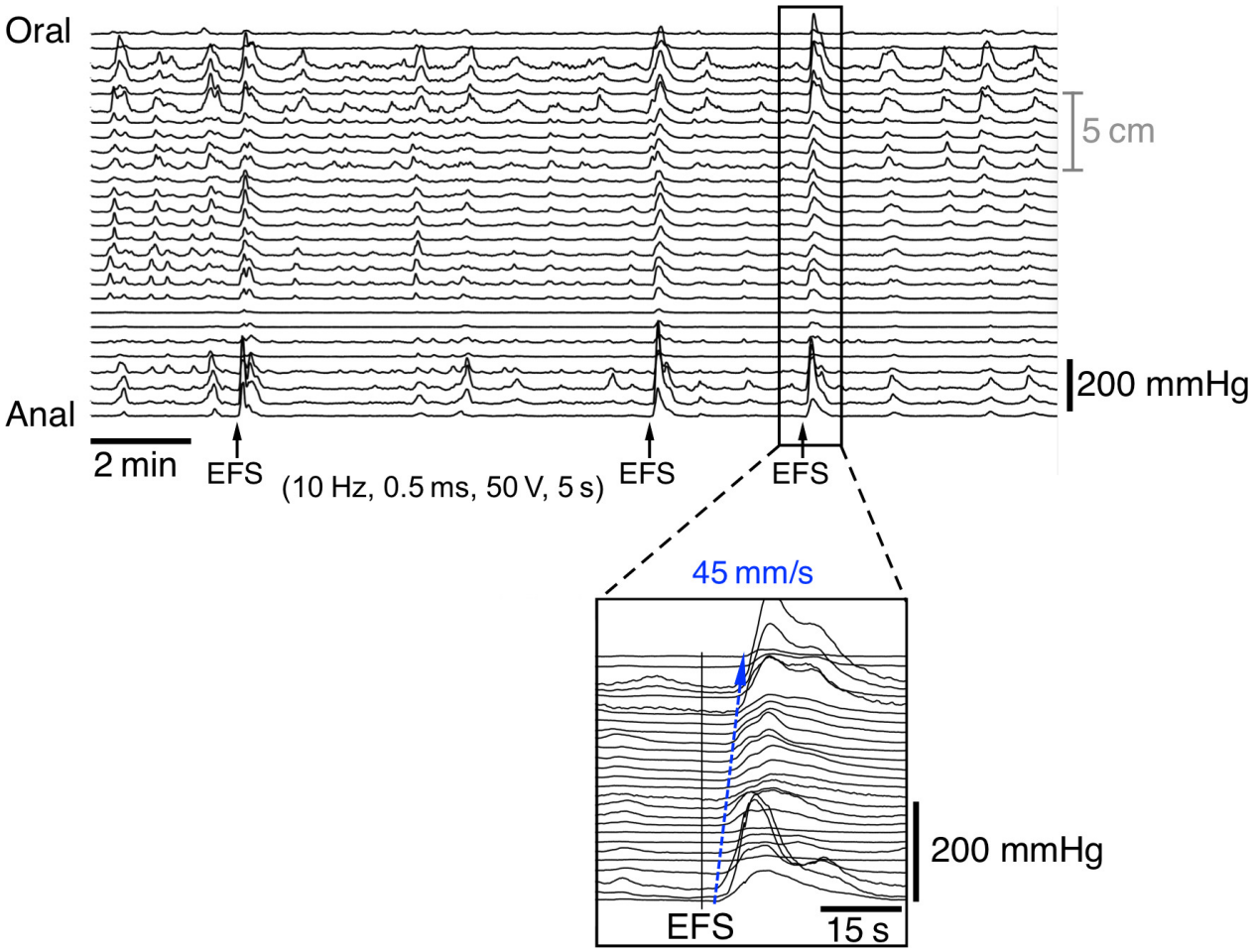

Figure 8.3 EFS-induced propagating contractions that involved the entire 25cm length of the preparation. The bottom image contains an expanded image of the region within the black rectangle. EFS resulted in a propagating contraction that travelled in a retrograde direction at 45mm/s.

The amplitude of the local contraction evoked by EFS was significantly greater than the amplitude of spontaneous contractions at the same location (EFS 29.3±26.9 vs spontaneous 12.1±14.9mmHg; p=0.02). The contractions 10mm and 20mm oral to the site of the EFS that formed part of the initiated propagating contract were also of significantly greater amplitude than spontaneous contractions at those locations (10mm, EFS: 23.6±19.9 vs spontaneous 54.7±42.6; p=0.003; 20mm, EFS: 24.6±20.1 vs spontaneous 47.3±43.4; p=0.03). However, when the average amplitude of the EFS contractions throughout the entire propagating event was compared to the average amplitude of the spontaneous propagating contractions, there was no significant

difference (EFS; 17.2±16.2 vs spontaneous; 21.1±22.7mmHg). This indicates that the EFS induced a large, localised contraction before triggering a propagating contraction that was indistinguishable from spontaneous propagating contractions.

8.5.3 Effects of Nerve Conduction Blockade on Colonic Motility Using Lidocaine (n=14)

In 14 specimens, lidocaine (0.3mM) was added after the control period. In another four specimens, it was added after hexamethonium (1mM). In two of the 14 (where lidocaine was added first), there was a brief (<5 minutes) interruption of the spontaneous propagating contractions. This was followed by a return of propagating activity in the continuing presence of lidocaine (**Figure 8.4**). Apart from this brief interruption, lidocaine had no effect upon the characteristics of the propagating activity compared with the control period (**Table 8.1**). Lidocaine applied after hexamethonium also had no additional effects on spontaneous propagating contractions.

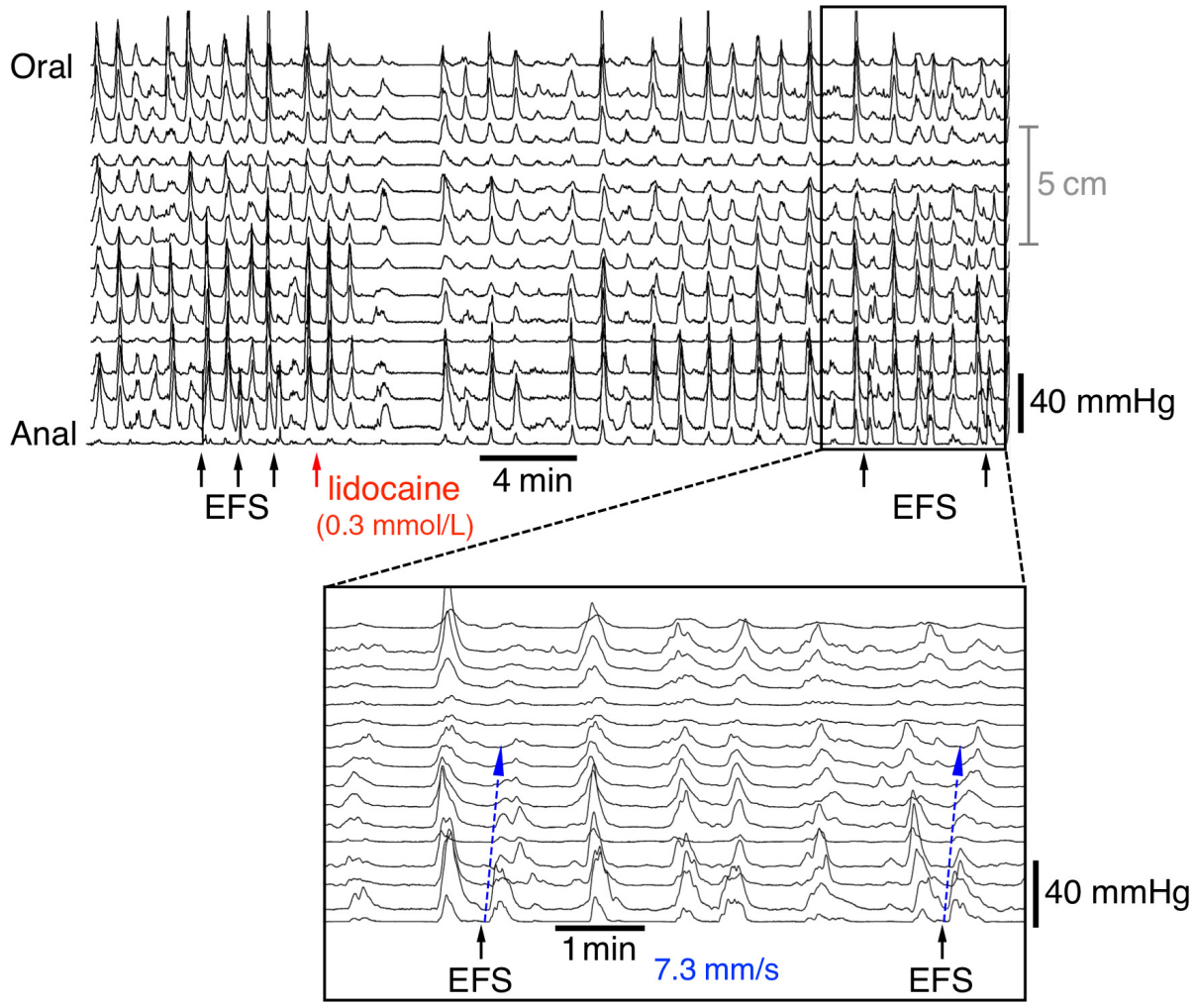

Figure 8.4 Administration of lidocaine (0.3mM) caused a brief cessation of spontaneous activity in two specimens, followed by a return of activity. In another 12 preparations, lidocaine had no apparent effect. As shown in the expanded section of the trace, EFS still elicited retrograde propagating contractions after lidocaine.

Electrical field stimulation was applied to six specimens in the presence of lidocaine (**Figure 8.4**). In five of these, EFS was followed by a contraction at the site of stimulation with an average delay of 14 seconds (7.7±2.9 seconds). This interval was significantly shorter than the interval between spontaneous propagating contractions recorded in the presence of lidocaine (74.8±57.3 seconds; p=0.03). Similar to the control period, EFS in the presence of lidocaine evoked a local contraction that was significantly larger than spontaneous contractions at the same site (EFS; 23.4±14.1 vs spontaneous; 9.2±8.3mmHg; p=0.03). In the presence of lidocaine, EFS-evoked local contractions initiated a propagating event that spanned the length of the preparation. The majority of these evoked events (78%) propagated in a retrograde direction at 44.2±30mm/s. The characteristics of the EFS-evoked propagating events during lidocaine did not differ from those stimulated during the control period (**Table 8.1**).

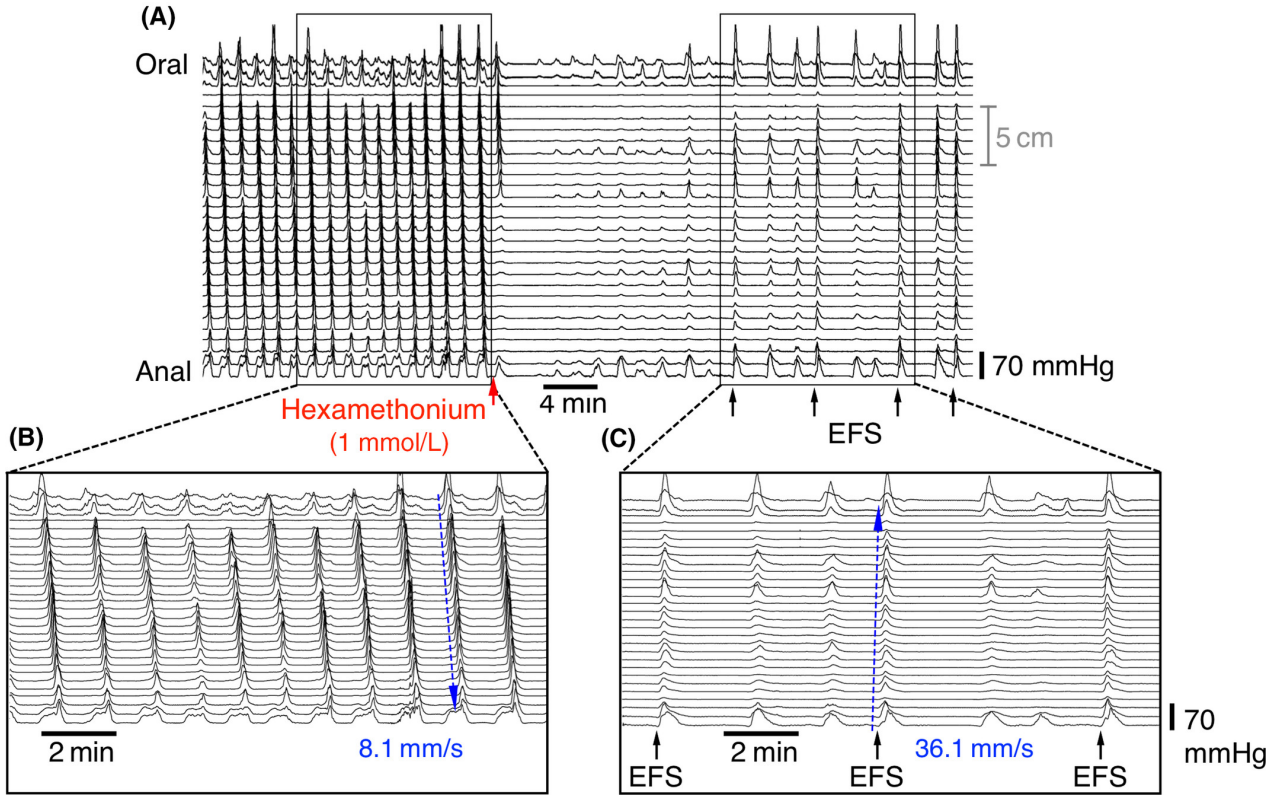

Figure 8.5 (A) In this specimen, application of hexamethonium (1mM) caused cessation of the regular spontaneous activity. After a brief period of inhibition, the propagating contractions recommenced. EFS was still able to induce propagating contractions that spanned the length of the preparation. (B) and (C) show expanded sections of the trace from the box outlined in (A). Note that prior to hexamethonium (B), the propagating contractions travelled in an antegrade direction. After hexamethonium (C), the propagating contractions travelled in a retrograde direction. In seven preparations, hexamethonium had no detectable effect at all on spontaneous contractions.

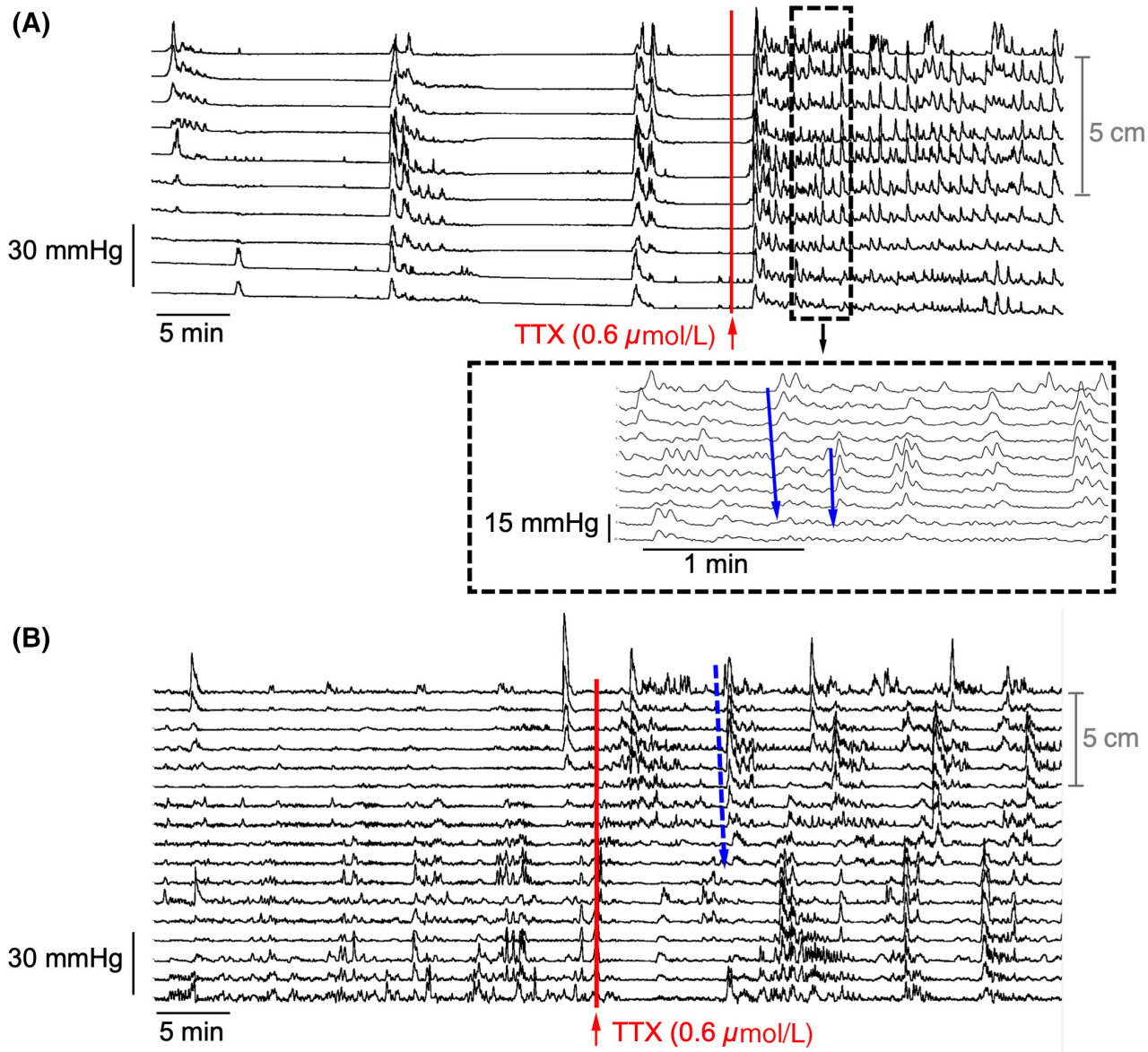

Figure 8.6 The two preparations in which TTX was added. (A) TTX resulted in a dramatic increase in basal tone and the frequency of the contractile activity occurring at 2-4cpm. The post-TTX contractions regularly propagated along the segment of colon. Some examples are shown by blue arrows in expanded selection of trace. (B) TTX resulted in an increase in propagating contractions (blue hatched line).

8.5.4 Effects of Nicotinic Receptor Blockade on Colonic Motility Using Hexamethonium (n=16)

The nicotinic receptor antagonist hexamethonium (1mM) produced complete or near-complete inhibition of spontaneous propagating contractions in 3/16 specimens tested. In six other preparations, hexamethonium was associated with a brief (<5 minutes) inhibition of spontaneous propagating activity, followed by a return of activity (**Figure 8.5**). In the remaining seven preparations, hexamethonium had no apparent effect on contractility. Where they occurred, spontaneous propagating contractions in the presence of hexamethonium did not show significant differences in their characteristics, compared with the control period (**Table 8.1**).

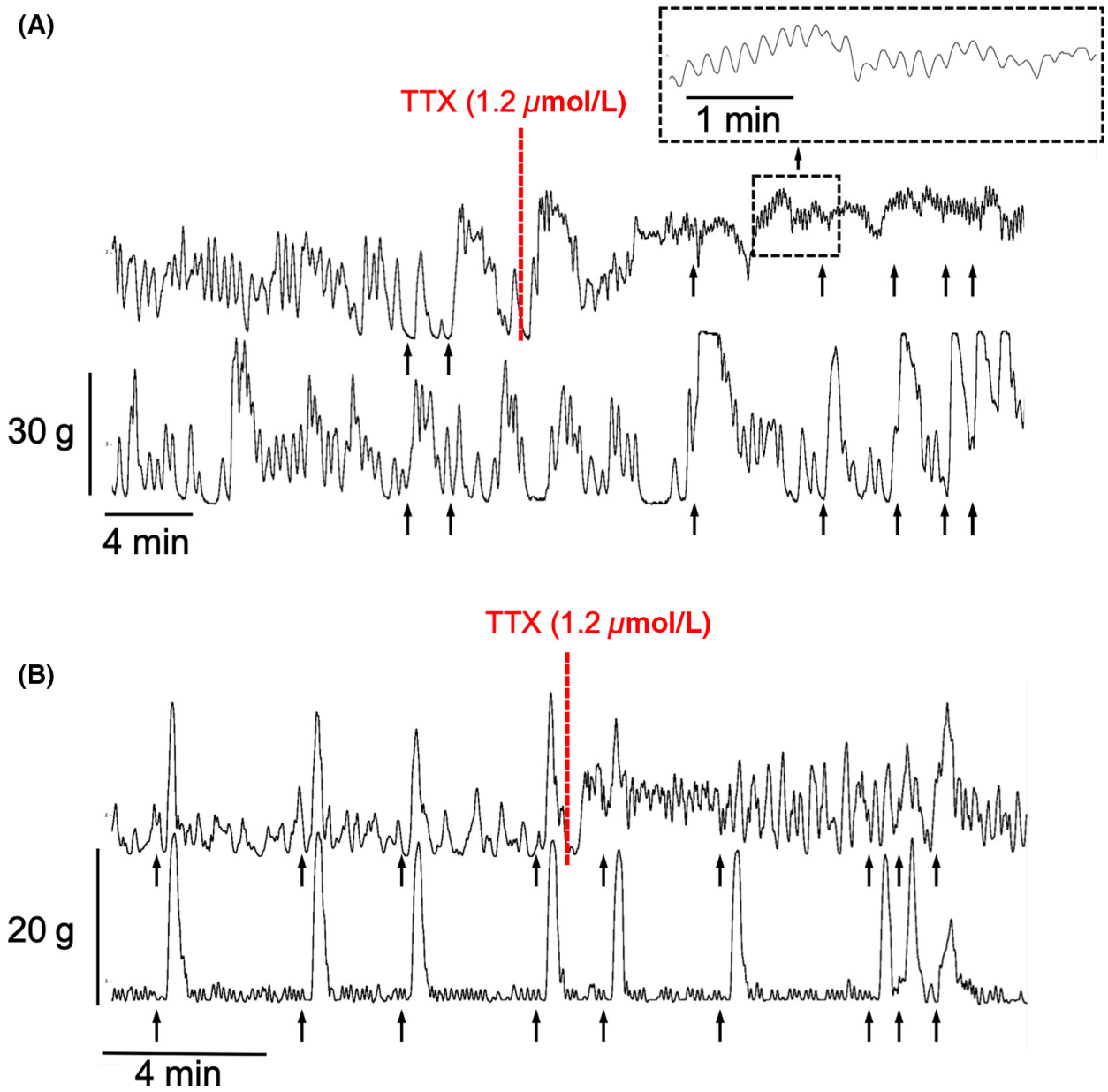

Figure 8.7 Altered motility following administration of tetrodotoxin (TTX) in circular muscle strip preparations. In both (A) and (B), TTX was only added to the top trace (red hatched line). Application of TTX abolished the response to EFS, while increasing the frequency of spontaneous contractions.

EFS was applied to all 16 specimens in the presence of hexamethonium (**Figure 8.5**). In all preparations, a contraction at the stimulation site was recorded within 14 seconds of the start of the stimulus (mean±SD; 5.6±2.4 seconds). This delay was significantly shorter than the interval between spontaneous propagating events in hexamethonium preceding EFS (145.4±57.6 seconds; $p<0.0001$). EFS after hexamethonium induced a localised contraction with a similar amplitude to spontaneous contractions (EFS; 23.2±19.5 vs spontaneous; 16.1±17.5mmHg; $p=0.3$). The EFS-evoked localised contraction developed into a propagating contraction in all but one instance; 63% of these propagating contractions spanned the full length of the colonic segment. The remainder spanned at least 18% of the specimen length. The majority (77%) of these propagating events travelled in a retrograde direction at 31.3±27mm/s. In the three preparations in which spontaneous propagating

contractions were inhibited by hexamethonium, EFS still evoked both local and propagating contractions. The characteristics of the EFS-evoked propagating events during hexamethonium did not differ from EFS-evoked propagating events during the control period (**Table 8.1**).

8.5.5 Effects of Nerve Conduction Blockade on Colonic Motility Using Tetrodotoxin (TTX; n=2)

The addition of TTX to two preparations did not inhibit spontaneous propagating contractions. On the contrary, TTX dramatically increased the frequency of these contractions (**Figure 8.6**) and, in one preparation, increased the basal tone (**Figure 8.6A**).

8.5.6 Circular Muscle Strip Studies: Effects of Nerve Conduction Blockade on Colonic Motility Using Tetrodotoxin (TTX; 20 Preparations from 10 Patients)

As with the intact tubular preparations, the application of TTX to muscle strips did not inhibit spontaneous contractions. Instead, TTX induced a significant increase in frequency (basal; 1.7±0.9 vs TTX; 3.2±1.7cpm; paired t test, $t(19)=-4.75$, $p<0.001$) (**Figure 8.7**). A small increase in basal tone after TTX did not reach significance (basal 14.2±5.6g vs TTX 15.7±8.8g; Wilcoxon signed-rank test, $Z=-0.97$, $p=0.33$).

Prior to TTX, EFS induced contractions in all specimens (Δforce pre-TTX=10.2±6.7g). TTX abolished the response to EFS in 16 of 20 preparations tested (80%). In the remaining (n=4) preparations, the response to EFS was significantly diminished by TTX, but a contraction of reduced amplitude persisted (Δforce post-TTX = 0.7±4.6g; Wilcoxon signed-rank test, $Z=-3.55$, $p<0.001$). These results indicate that TTX, a low molecular weight water-soluble drug, penetrates colonic tissue effectively in the majority of preparations.

8.6 Discussion

The primary aim of this study was to provide a detailed description of a distinctive propagating motor pattern observed in long specimens (15-36cm) of excised, intact human colon. In each preparation, we recorded spontaneous, semi-regular propagating contractions spanning the length of the colonic specimen. These propagating events either occurred simultaneously at all recording sensors or, in some specimens, propagated in an antegrade direction. These findings confirm and extend the results of two previous studies(256, 724). In one study, colonic motor patterns were recorded with widely-spaced force transducers which prevented accurate determination of the direction of propagation(724). The second study focused primarily on excised colonic specimens from four patients with slow transit constipation(256). Here, our aim was to provide a detailed account of these events and identify some of the underlying mechanisms.

8.6.1 Origin of Low-Frequency, Propagating Contractions Ex Vivo

In most experimental animals, neurally-dependent repetitive motor complexes have been observed in isolated ex vivo preparations of distal colon(13, 731-734). In rabbit and rat, repetitive myogenic contractions extended over large proportions of the colon, becoming most prominent after pharmacological blockade of neural activity(732-734). In the majority of our human specimens, pressure waves occurred simultaneously across all recording channels. The frequency of these events varied but, by the end of the 30-minute equilibration period, they occurred approximately 1cpm. Spontaneous contractions at similar frequencies have been recorded in ex vivo human circular(38, 39, 735-738) and longitudinal(101, 103, 737-740) muscle strips, and in short (4cm) tubular segments(738). This slow phasic activity is tetrodotoxin-resistant(38, 39, 101, 736, 740) and present in aganglionic segments(739), suggesting that it is myogenic in origin. However, these myogenic contractions appear to be modulated by neural pathways, as activation of ascending myenteric neural pathways triggered premature contractions with similar time course and amplitude(39) (also see **8.6.2 Drug Penetration** below).

Similar motor patterns have also been recorded during in vivo colonic manometry studies. Corsetti et al.(166) described pancolonic pressurisations which occurred in healthy adults at close to 1-minute intervals, a finding supported Chen et al.(725) Corsetti et al.(166) proposed that the generation of pancolonic pressurisations requires colonic distension. Previous studies in animals have shown that constant distension of excised animal colon can result in contractile events of a similar frequency and appearance of the human pancolonic pressurisations(728, 741). In our previous ex vivo studies of excised human colon(256, 724) and in this current study, clips were attached to the serosa to apply constant tension to the gut wall, a stimulus that mimics distension. In all of these studies, regular synchronous pressurisations were observed.

Pancolonic pressurisations have not been reported in all in vivo colonic manometry recordings in humans(2, 250, 256, 693). This inconsistency may be explained by differences in study protocols. In studies where pressurisations were present, either tap water enemas had been given(166) or recordings were made with water-perfused catheters(725). Tap water enemas often fail to remove stool from the entire colon, so a degree of distension caused by luminal contents persists. In the study using water-perfused manometry(725), up to four litres of water was introduced into the colon over a seven-hour period which may have also resulted in luminal distension. Studies in isolated mouse and guinea pig colon have shown that colonic motor complexes occurring at ~1cpm are generated by distension(727, 731). In contrast, in human in vivo studies where pancolonic pressurisations were not regularly observed, participants underwent a full bowel preparation and a solid-state catheter was used, so these recordings were performed in an empty colon(2, 256, 693).

The propagating contractions recorded in the present study at approximately 1cpm do not resemble the high-amplitude propagating contractions recorded in human colon in vivo, which have a much lower frequency, a higher amplitude, and propagate much more slowly (0.4-0.1cm/s) than the events recorded in the present study (4.4±4.0 cm/s)(2, 100, 111, 112). Another colonic motor pattern, the cyclic motor pattern, is also commonly

recorded in the human distal colon in vivo(2). The cyclic motor pattern comprises pressure waves at 2-6cpm which often propagate in a retrograde direction. This pattern was not observed prior to the addition of drugs or after the application of hexamethonium or lidocaine, nor was it present in other studies of isolated large tubular preparations(256, 724). However, in the present study, contractile events with a frequency of 2-6cpm did emerge after application of TTX in both tubular and circular muscle strip preparations. The frequency of the cyclic motor pattern (2-6/min) is similar to the dominant type of slow waves generated by interstitial cells of Cajal in the colon(30, 283). The cyclic motor pattern is present in vivo before a meal and is greatly increased (within a minute) after consumption of a meal(2). Previously, we had hypothesised that the amplitude of contractions at slow wave frequency are modulated by extrinsic neural input after eating via activation of enteric excitatory neurons. Extrinsic neural pathways to the colon are obviously not functional in resected specimens however(256). This hypothesis could explain the conspicuous absence of 2-6cpm cyclic propagating motor patterns throughout most of the present study. However, the appearance of the cyclic motor pattern after TTX suggests that the cyclic motor pattern is normally suppressed by inhibitory motor activity. Application of TTX to segments of the proximal colon of mice and dogs also causes a significant increase in contractile activity(742, 743), indicating the presence of tonic inhibition. Human colonic manometry studies have also shown that proximal colonic propagating contractions increase after partial removal of tonic nitrergic inhibition(744). It should also be noted that repetitive contractions and underlying electrical activity in the 2-6cpm range have previously been recorded from small muscle strips of human colon ex vivo(38, 737).

8.6.2 Drug Penetration

In this study, our specimens were largely unaffected by high concentrations of lidocaine and hexamethonium. There are two possible explanations to account for this; (1) either large rapidly propagating contractions do not require neural activity for their expression, or; (2) the drugs failed to penetrate the tissue. Electrophysiological recordings from human colonic circular muscle have shown, in some preparations, intermittent long depolarisations (at 1-3-minute intervals) with superimposed action potentials(38, 736) which are not blocked by TTX. Similarly, Carbone et al.(39) recorded slow, intermittent, phasic spontaneous contractions that were resistant to TTX. In our current study, application of TTX to both muscle strips and tubular preparations caused an increase in contractile frequency and, in the latter, failed to abolish propagating contractions. As lidocaine also works by antagonism of neural voltage-sensitive sodium currents, this suggests that the generation of large, phasic contractions do not require neural innervation.

Electrical stimulation potently evoked premature contractions that propagated rapidly over long distances in our large, tubular preparations. This is consistent with fast neurotransmission in ascending neural pathways. Therefore, while neural input may not be required, neural inputs can still alter muscle activity and activate a premature contraction. Surprisingly, the effects of the electrical stimulation persisted after the addition of lidocaine or hexamethonium. This led us to question whether small molecular weight drugs were adequately

penetrating into the tissue during our experiments. However, in the circular muscle strip studies, TTX abolished the EFS response. This suggests that water-soluble, low molecular weight drugs do penetrate the tissue specimens. Lidocaine has been shown to inhibit motor patterns in several studies of colonic motility in ex vivo preparations from animals(733, 734, 745). However, in the current study, it seems likely that lidocaine did not fully access the myenteric plexus and cause complete neuronal blockade. This may be because lidocaine is lipid-soluble(746), so it may also have been absorbed into the large fatty epiploic appendages along the colonic specimen which we could not excise, as the specimen had to be returned intact at the end of the recording period to the surgical pathologist.

Arguments about fat solubility do not apply to hexamethonium, which also failed to abolish the synchronous contractions. In some preparations, hexamethonium had no effect at all. In others, hexamethonium caused a temporary inhibition of activity, but contractions reappeared after a few minutes. This suggests that hexamethonium did access nicotinic receptors within the enteric plexuses. However, it is possible that it was unable to access some ganglia, perhaps those located under the thick muscle of the taenia coli. Such hexamethonium-inaccessible neural pathways (which would also be lidocaine-inaccessible) may trigger spontaneous myogenic contractions along the entire preparation, which appear to be tightly coordinated. Alternatively, it is possible that hexamethonium has a limited effect upon motor patterns in the distal colon as reported in the rabbit(747). The effects of TTX, on the other hand, may have been mediated primarily by blockade of neural activity outside the ganglionated plexuses, for example, in the axons of motor neurons.

Carbone et al.(39) also described hexamethonium-resistant, long-duration, large contractions that occurred spontaneously and which could also be triggered prematurely by electrical stimulation in human colonic muscle strips. The mechanism underlying the generation of this activity is not likely to be due to direct stimulation of excitatory motor neurons, which have orally directed projections up to 10-12 mm(45). Ascending interneurons can project up to 30mm proximally and descending interneurons have even longer projections and can be activated antidromically by electrical stimulation(748). If either pathway impinged on circular muscle motor neurons, it could then trigger the contractions(39). It was speculated that neural stimulation worked via non-nicotinic pathways. This, together with poor drug penetration by lidocaine, may explain the present results.

8.7 Conclusion

Large segments of ex vivo human distal colon demonstrated spontaneous, coordinated, propagating contractions that were detectable using high-resolution manometry. These contractions resemble previously described low-frequency, myogenic phasic contractions. Electrical nerve stimuli potently evoked premature propagating contractions across the entire colonic specimen, suggesting smooth muscle excitation via neural release of excitatory neurotransmitters from ascending neural pathways. The preparations used in the present

study appeared resistant to hexamethonium and lidocaine, showing only temporary inhibition of spontaneous contractions while EFS-evoked propagating activity persisted. In the presence of TTX, contractile activity increased in both tubular and muscle strip studies and effects of EFS were diminished or abolished. These data suggest that spontaneous, slow, phasic contractions are fundamentally myogenic but can be initiated by EFS-induced local neurotransmitter release.

8.8 Tables

Table 8.1 Characteristics of Propagating Contractions

	Spontaneous Propagating Contractions					Electrical Field Stimulation				
	Propagation Length (cm)	Local Contraction Amplitude (mmHg)	Propagating Contraction Amplitude (mmHg)	Velocity (Antegrade only) (mm/s)	Time Between Propagating Contractions	Propagation Length (cm)	Local Contraction Amplitude (mmHg)	Propagating Contraction Amplitude (mmHg)	Velocity (Retrograde only) (mm/s)	Time Between EFS and First Contractions (s)
Control	17.4±7.3	12.1±14.9	21.1±22.7	12.8±8.2	173.1±154.0	17.9±5.6	29.3±26.9	17.2±16.2	44.2±32.9	5.6±2.4
Hexamethonium	18.3±6.2	16.1±17.5	12.6±7.0	N/A	145.4±57.7	18.9±7.5	23.2±19.5	17.8±11.1	37.6±27.3	4.7±1.6
Lidocaine	13.5±3.5	9.2±8.3	9.1±7.6	N/A	74.8±57.4	17.1±4.6	24.1±14.1	10.6±4.3	31.3±27.1	7.5±2.9

Chapter 9: The Effects of Loperamide on Excitatory and Inhibitory Neuromuscular Transmission in the Human Colon

9.1 Statement

The content of this chapter is under review for publication in *Neurogastroenterology & Motility* as of September, 2021.

Heitmann PT, Keightley LJ, Wiklendt L, Wattchow DA, Brookes SJH, Costa M, Dinning PG (2021). The effects of loperamide on excitatory and inhibitory neuromuscular function in the human colon. *Neurogastro Motil.* Under review as of August, 2021.

The co-authors have provided permission for the inclusion of the study in this thesis. The percentage contributions of each author to this study were as follows:

- Research design: **PH 50%**, MC 50%.
- Data collection and analysis: **PH 90%**, LK 10%.
- Writing and editing: **PH 90%**, LW 2%, DW 2%, SB 2%, MC 2%, PD 2%.

9.2 Abstract

Background: The actions of opioids on enteric neurons have mostly been described in animal studies, with a consensus that opioids act in most species by inhibiting excitatory enteric motor neurons to reduce intestinal motility. In humans, the mechanisms by which opioids alter motility are less certain. The aim of this study was to describe the effects of loperamide on excitatory and inhibitory neuromuscular actions in the human colon. **Methods:** Tissue specimens of human colon were received from patients (n=30 preparations from n=10 specimens) undergoing elective colorectal surgery (anterior resection). Three inter-taenial, circular muscle strips (5mm width) were dissected from each specimen. Three separate organ baths were used to investigate neuromuscular transmission; (1) both excitatory and inhibitory transmission (no drug additions); (2) excitatory transmission only (selective blockade of inhibitory transmission using L-NOARG/MRS2179), and; (3) inhibitory transmission only (selective blockade of excitatory transmission using hyoscine hydrobromide). Frequency-response curves were performed as well as analyses paired by specimen, stimulation parameters, and study period. Specimens of guinea pig ileum and distal colon were additionally studied to validate the efficacy of the loperamide and naloxone preparations. **Results:** In human preparations with L-NOARG/MRS2179, loperamide had no significant effects on the isometric contractions during or following electrical field stimulation (EFS; 20V, 10Hz, 0.5ms for 10s). In preparations with hyoscine hydrobromide, loperamide reduced the isometric relaxation during EFS (median difference +0.40g post-loperamide, Z=-2.35, p=0.019). The same loperamide preparation inhibited neuromuscular activity in guinea pig ileum and colon (Z=-4.08, p<0.001). **Conclusions & Inferences**: In contrast to guinea pig ileum and colon, loperamide appeared to have no effect on excitatory neuromuscular transmission in the circular muscle of the human colon. Loperamide, however, did reduce inhibitory neuromuscular transmission in the human colon. These findings may suggest that, in humans, loperamide alters intestinal motility by acting on premotor enteric neural circuits rather than on the final excitatory enteric motor neurons.

9.3 Introduction

Synthetic opioids, such as loperamide, are used therapeutically to reduce the frequency of bowel motions in diarrhoeal illnesses, faecal incontinence, and to reduce ileostomy output(286, 287, 749-751). Opioid use for analgesia is also complicated by the common, undesirable side effect of constipation(288, 289). Up to 45% of patients taking regular opioids report a stool frequency of less than three times weekly(289). To reflect this, an additional category was included in the Rome IV criteria for functional bowel disorders; opioid-induced constipation(231) (see **1.8.3 Opioid-Induced Constipation**).

The site and mechanism of action of opioids in the gastrointestinal tract have mostly been described in animal studies, with a consensus that opioids act in most species by inhibiting excitatory enteric motor neurons to reduce intestinal motility(297-302, 752-757). Loperamide is a potent μ-opioid receptor agonist(758-760). In the guinea pig ileum, both loperamide and morphine act via μ-opioid receptors to inhibit enteric excitatory

neurons(761, 762). This effect is competitively antagonised by the opioid receptor antagonist naloxone(761, 762).

In humans, opioid agonists have been demonstrated to significantly alter colonic and anorectal function, including a reduction in the frequency and coordination of colonic propagating activity, increased colonic transit time, and increased anal sphincter tone(141, 231, 287, 290, 291, 763). In a study using the magnetic capsule tracking system, opioid agonists resulted in an increase in non-propulsive colonic activity and reduced the number of long fast antegrade movements(141), which are likely to relate to the high-amplitude propagating contractions recorded in high-resolution colonic manometry studies(2) (**1.6.2 High-Amplitude Propagating Contractions**). As seen in animal studies, it is widely assumed that the mechanism of action of opioid agonists in the human gastrointestinal tract is also due to the inhibition of excitatory enteric motor neurons. However, the site and mechanism of action of opioids on human colonic motility is less certain. Only a few pharmacology studies using isolated preparations of human small and large intestine have been published(302-305, 764). Benko et al.(303) demonstrated that morphine did not reduce the cholinergic-mediated excitatory contractile response to electrical field stimulation (EFS) in preparations of human small intestine. This was despite being able to demonstrate a clear inhibition of neuromuscular transmission with morphine using guinea pig preparations in the same study. Hoyle et al.(292) demonstrated the inhibition of non-adrenergic, non-cholinergic (NANC) inhibitory neuromuscular transmission in human colonic circular muscle by both endogenous enkephalins and δ-opioid receptor agonists.

The aim of this study was to assess the effect of loperamide on excitatory and inhibitory neuromuscular transmission in the human colon. Given the expected simultaneous activation of excitatory and inhibitory neurons by EFS, we used selective antagonists of excitatory and inhibitory transmission to separate the two components(765-767). Primarily, our intent was to gain insight into the physiological role of opioid receptors in colonic neuromuscular function, and the pharmacological changes that occur following the administration of exogenous opioids.

9.4 Methods

Ethics approval was received from the Southern Adelaide Clinical Human Research Ethics Committee (Project 50.07) and Animal Welfare Committee of Flinders University (Projects 844/12, 908/12, 916/12).

9.4.1 Human Studies

Human colonic tissue specimens were obtained from patients undergoing an elective anterior resection, whom all provided consent for tissue donation pre-operatively. The inferior mesenteric artery is routinely ligated

during an anterior resection, resulting in a length of macroscopically normal colon being resected along with the diseased segment.

Twelve tissue specimens were collected. One specimen was excluded which was pathologically dilated due to recurrent sigmoid volvulus. One experiment was aborted after the specimen exhibited no spontaneous activity after 30 minutes in the organ bath. Of the remaining ten specimens, n=30 circular muscle preparations were performed (three separate organ bath preparations from each specimen). The tissue donors included four women and six men with a median age of 68 years (range 43-85 years). The indications for surgery included rectal cancer (n=8), colonic cancer (n=1), or sigmoid diverticulitis (n=1). Surgery was performed either via an open (n=8) or laparoscopic-assisted approach (n=2). All tissue used for experimentation was macroscopically normal in appearance with no evidence of tumour involvement, colitis, obstruction, or diverticulae.

Colonic tissue was provided from the surgical team to the research team immediately upon excision from the patient. A 2cm-width ring of tissue was excised from the proximal end of the specimen and placed in Krebs solution containing (mmol L^{-1}) NaCl 118.0; KCl 4.7, $NaH_2PO_42H_2O$ 1.0; $NaHCO_3$ 25.0; $MgCl_26H_2O$ 1.2; D-Glucose 11.0; $CaCl_2$ $2H_2O$ 2.5. The solution was warmed to 36-37.5°C and bubbled with 95% O_2/5% CO_2.

The specimen was divided through the taeniae libera to lay open flat sheets of inter-taenial tissue. Fat, mesocolon, and mucosa were excised using sharp dissection. Inter-taenial strips of circular muscle of approximately 5mm width and 15mm length were dissected.

The circular muscle strips were placed into three separate, warm-jacketed 100ml organ baths (see **Figure 9.1**). One end of the strip was sutured to a fixation rod and the strip was passed through a ring electrode (Biomedical Engineering, Flinders University). The other end of the strip was attached to a force transducer (Grass FT-03C; Grass, Quincy, MA, USA). Each strip was subjected to 10-15g of basal tension. Force transducer data was recorded using custom-made preamplifiers (Biomedical Engineering, Flinders University), a PowerLab (model 8/30, AD Instruments, Bela Vista, NSW, Australia), and an Apple computer with Labchart software (version 6, AD Instruments, NSW, Australia).

An equilibration period of 20-30 minutes was allowed until the specimen demonstrated consistent, spontaneous, phasic activity. EFS was delivered by an electrical stimulator (SD9 stimulator, Grass Instruments) via a pair of ring electrodes, delivering stimulation parameters of 20V, 0.5ms pulse duration, for 10s, at three-minute intervals, with frequencies of 1Hz, 5Hz, 10Hz, & 20Hz. Tetrodotoxin (TTX; 0.6μM) was added at the end of the study to confirm the neural origin of responses. It was expected that no neural response would be observed post-TTX to 0.5ms pulse duration, but that 5ms pulse duration would elicit muscle contraction by causing muscle tetany.

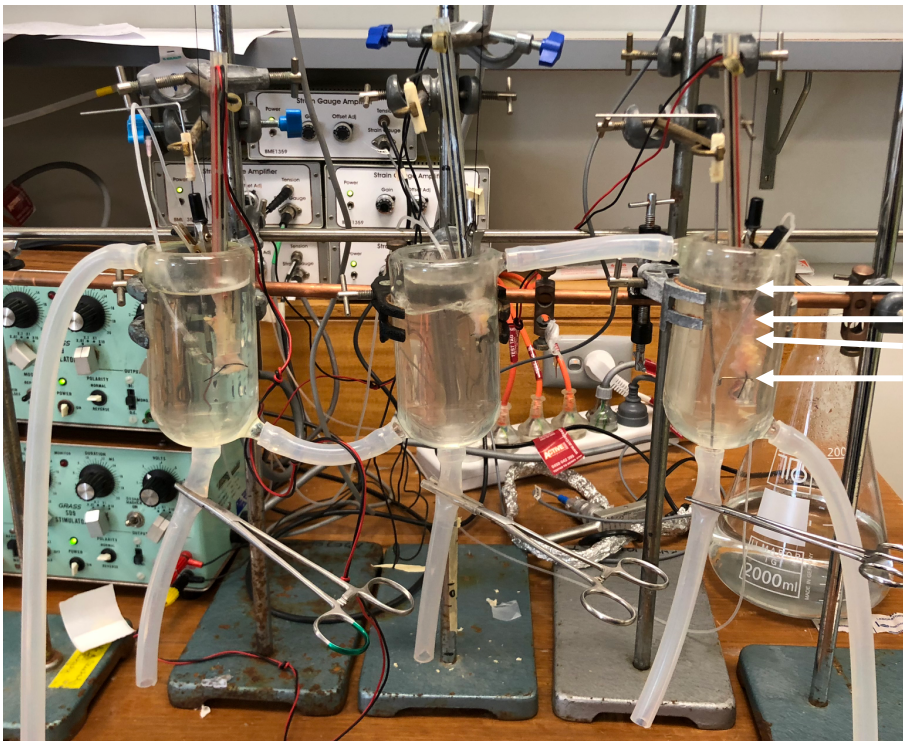

Figure 9.1 An example experimental setup using the three warm-jacketed 100mL organ baths containing circular muscle tissue specimens immersed in Kreb's solution. One end of the tissue strip was sutured to a fixation rod and the strip was passed through a ring electrode. The other end of the strip was attached to a force transducer using an alligator clip and subjected to 10-15g of basal tension.

Three separate organ baths were used to investigate neuromuscular transmission in preparations with; (1) both excitatory and inhibitory transmission present (no drug additions); (2) excitatory transmission only (selective blockade of inhibitory transmission; L-NOARG and MRS2179), and; (3) inhibitory transmission only (selective blockade of excitatory transmission with hyoscine hydrobromide).

Of the 30 preparations, n=18 specimens were used for testing stimulation parameters and TTX responses, and n=21 were used with loperamide/naloxone (nine preparations were used for both protocols, with the addition of TTX at the end of the study).

Frequency-response curves were performed for three study periods; (1) before loperamide; (2) after the addition of loperamide, and; (3) after loperamide and naloxone (**Figure 9.2**). "Drugs were administered in sequence with no wash out performed between dose administrations".

9.4.2 Guinea Pig Studies

Specimens of guinea pig ileum and distal colon were used to validate the efficacy of the loperamide and naloxone preparations. Duncan-Hartley guinea pigs (n=6 preparations of ileum and n=2 preparations of distal colon from n=3 guinea pigs) were euthanised by exposure to isoflurane followed by exsanguination, as per the

protocol approved by the institutional animal welfare committee. The small intestine and colon were excised and placed in Krebs solution, warmed to 36-37.5°C and bubbled with 95% O_2/5% CO_2. The specimen was gently flushed with Krebs solution to expel any residual content.

We used study protocols described in a previous study(768). Briefly, 3-4cm preparations of ileum and colon were placed into an organ bath (volumes 150-400mL). L-shaped connectors were attached at both ends of the preparations with a ligature. Krebs solution was infused at the proximal end and a Microtip catheter with a single pressure sensor was placed at the distal end (Millar Pressure Catheter; 3.5Fr; 0.73mm diameter; Ny, Model: SPR-524). Distension by slow infusion of Krebs solution induced intraluminal pressure peaks, indicative of peristaltic contractions. Single EFS pulses at 0.1Hz elicited classic cholinergic twitches(297) and short longitudinal muscle contractions which were recorded by a force transducer (Grass FT-03C; Grass, Quincy, MA, USA) attached to the proximal end of the specimen via a hook. The catheter and force transducer were connected to custom-made preamplifiers (Biomedical

Engineering, Flinders University), PowerLab (model 16/35, AD Instruments, Bela Vista, NSW, Australia) and an Apple computer with Labchart software (version 7, AD Instruments, NSW, Australia).

9.4.3 Drug Additions

The following drugs were dissolved in de-ionised water and used for all experiments:

- Loperamide hydrochloride (Aspen Pharmacare Australia Pty Ltd, St Leonards, Australia), 1uM.
- Naloxone hydrochloride (Hameln Pharmaceuticals GmbH, Hameln, Germany), 1uM.
- Tetrodotoxin (Alomone, Jerusalem, Israel), 0.6uM, used to abolish neurally-mediated smooth muscle contraction.
- Hyoscine hydrobromide (Sigma-Aldrich Pty Ltd, North Ryde, Australia), 1µM, used to inhibit cholinergic excitatory transmission to the intestinal smooth muscle.
- N_ω-Nitro-L-arginine methyl ester hydrochloride (L-NOARG, Sigma-Aldrich Pty Ltd, North Ryde, Australia), 1mM, used to inhibit nitregic inhibitory transmission to the intestinal smooth muscle.
- MRS2179 tetra-ammonium salt (In Vitro Technologies Pty Ltd, Noble Park, Australia), 10µM, used to inhibit purinergic inhibitory transmission to the intestinal smooth muscle.

9.4.4 Analysis

9.4.4.1 Frequency-Response Curves

The change in mean force (Δforce) was calculated during EFS and in the 10s-period immediately following EFS. This was calculated using; (1) the mean force during the one-minute period prior to EFS; (2) the mean force during EFS, and; (3) the mean force during the 10-second period immediately following EFS. The Δforce

during and post-EFS and 95% confidence intervals are displayed in frequency-response curves, separated by bath (Bath 1: L-NOARG/MRS2179, Bath 2: hyoscine hydrobromide, Bath 3: control) (**Figure 9.2**).

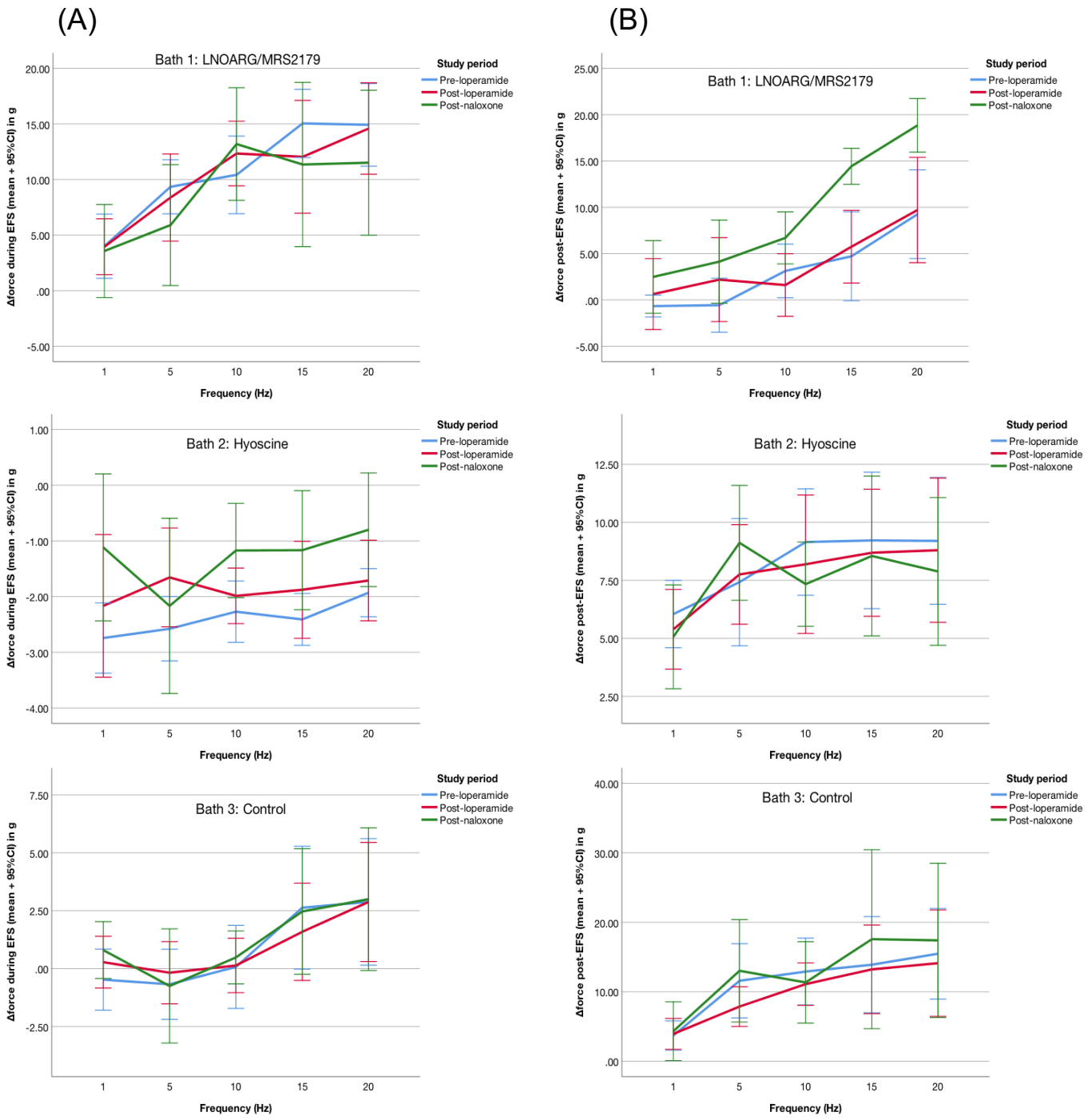

Figure 9.2 Frequency-response curves displaying the mean Δforce during EFS (y-axis) across a frequency range of 1-20Hz (x-axis). The study periods are differentiated by colour; pre-loperamide (blue), post-loperamide (red), and post-naloxone (green). Stimulation parameters included a pulse duration of 0.5ms for 10s at 20V. (A) Δforce during EFS: no significant differences were observed when comparing the three study periods, with error bars (95%CI) overlapping in all three baths across the frequency range. (B) Δforce post-EFS: the contractile response generated a significantly higher force during the post-naloxone period at stimulation >15Hz in Bath 1 (L-NOARG/MRS2179). No significant differences were observed when comparing the three study periods at <15Hz in Bath 1, or across the frequency range in Baths 2 and 3.

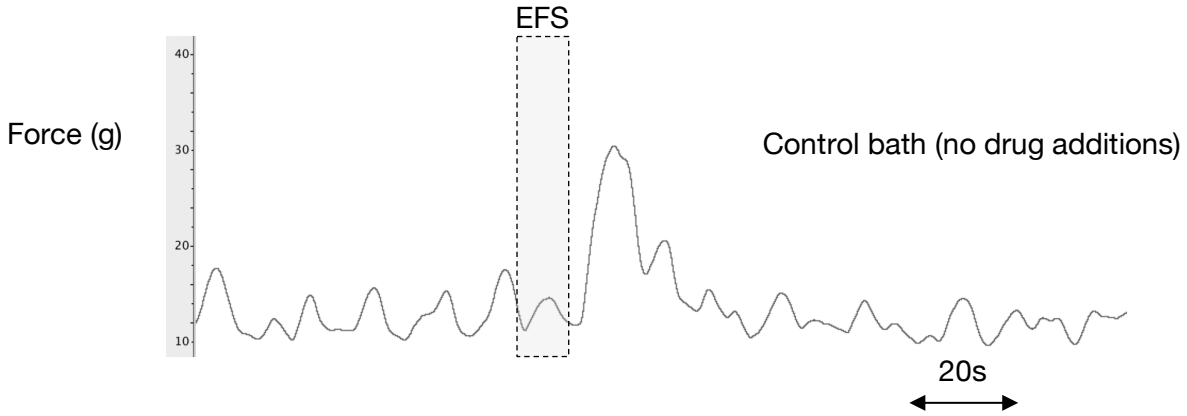

Figure 9.3 An example recording from a human colonic circular muscle preparation, obtained in the absence of any drugs (control bath). Force (g) is displayed on the y-axis and time (seconds) on the x-axis. There was negligible change in force during EFS (20V, 10Hz, 0.5ms for 10s; grey hatched box), followed by a contraction following the cessation of EFS.

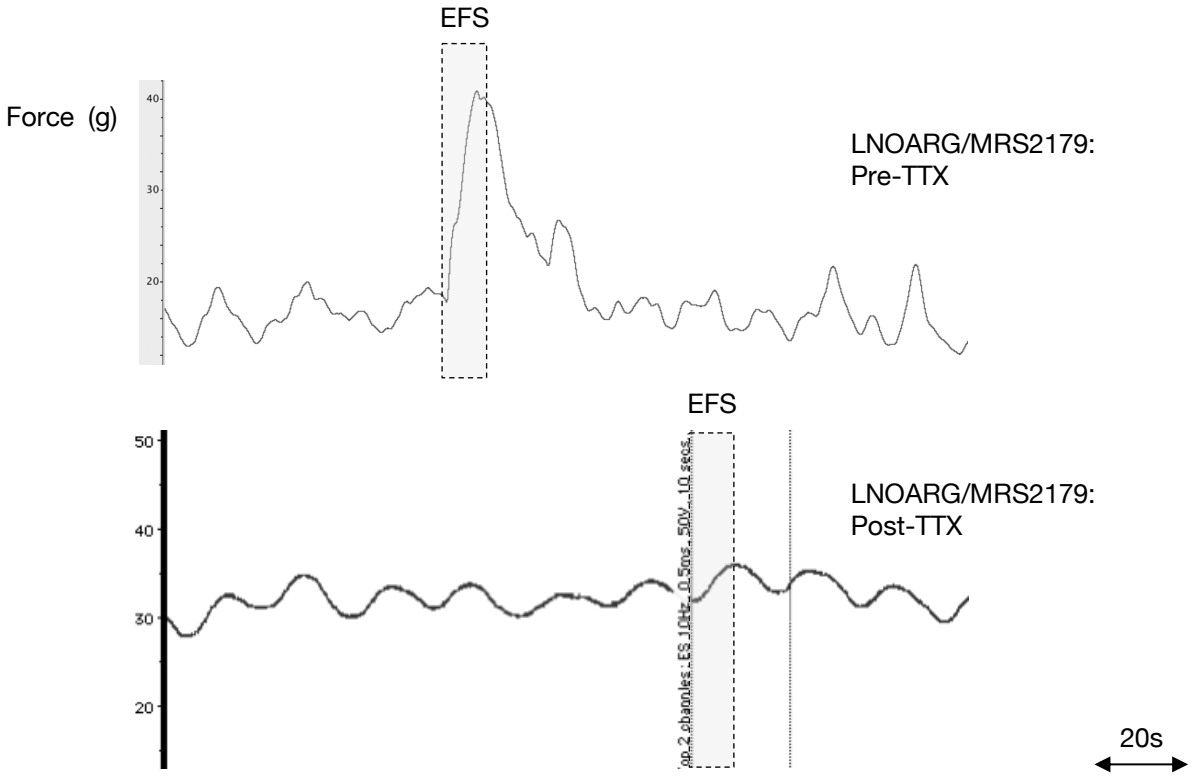

Figure 9.4 Examples of isometric force recordings from human colonic circular muscle preparations, obtained in the presence of L-NOARG/MRS2179. Force (g) is displayed on the y-axis and time (seconds) on the x-axis pre- and post-TTX. EFS (20V, 10Hz, 0.5ms for 10s) elicited an isometric contraction, with a less marked post-stimulus contraction. TTX abolished the contractions both during and post-stimulus, suggesting that both responses are neurally-mediated.

9.4.4.2 Paired Analyses

Analyses paired by specimen, stimulation parameters, and study period were additionally performed to assess changes in responses to EFS in each specimen following drug additions. The Δforce during and following EFS

were not normally distributed (Shapiro-Wilk test $p<0.01$), so non-parametric paired analyses were used for these comparisons (Wilcoxon signed-rank test). The analyses included; (1) comparisons between the pre- and post-TTX study periods in the preliminary studies to confirm that responses were abolished by TTX, and; (2) comparisons between the pre-loperamide, post-loperamide, and post-naloxone study periods. Statistical analysis was performed using IBM SPSS (Version 19.0, Released 2010; IBM Corp., Armonk, New York, USA). The Holm-Bonferroni procedure was applied to multiple comparisons(716).

9.5 Results

9.5.1 Human Studies

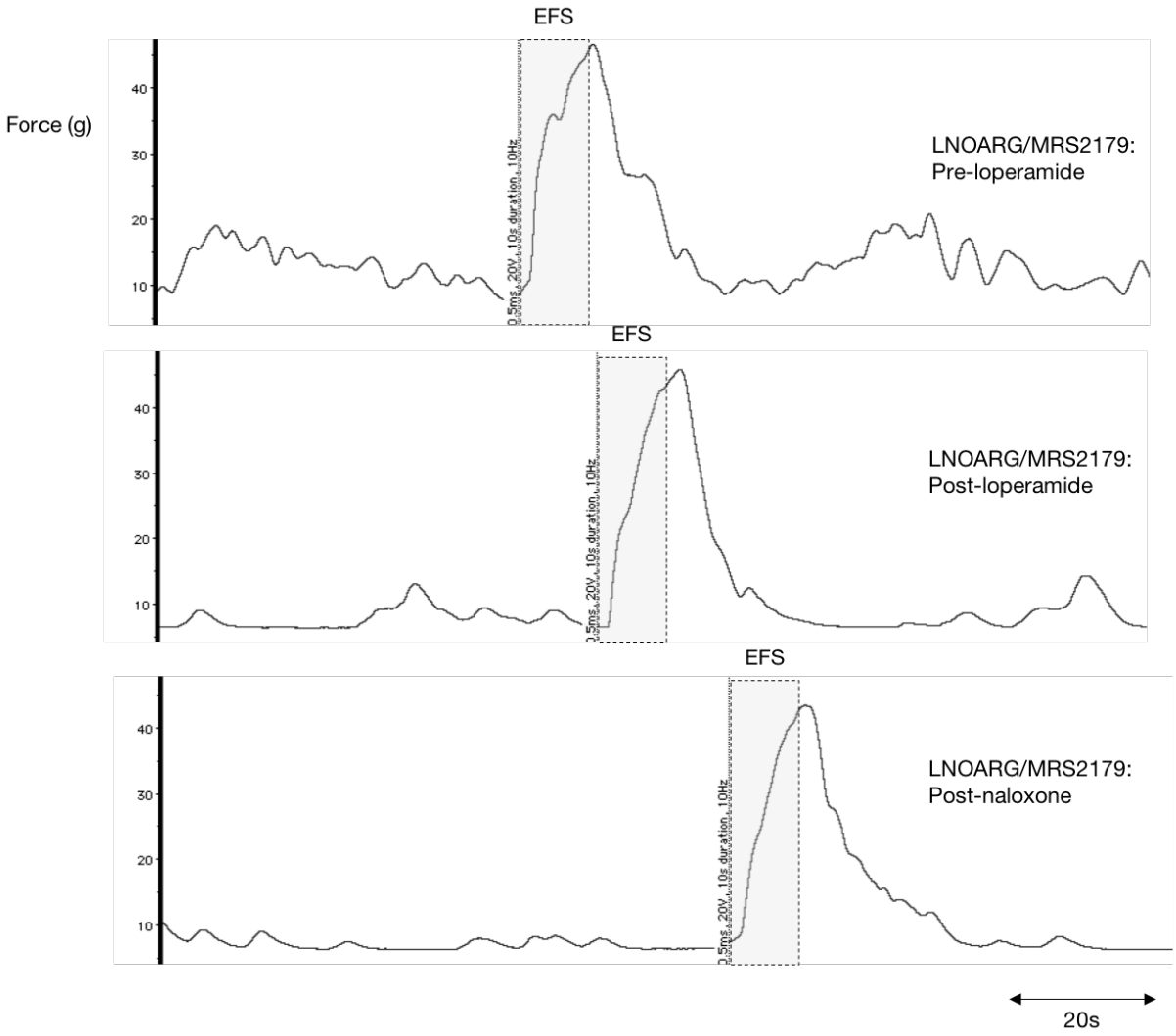

Figure 9.5 Example isometric force recordings from human colonic circular muscle preparations obtained in the presence of L-NOARG/MRS2179. Force (g) is displayed on the y-axis and time (seconds) on the x-axis during the control period and following the sequential additions of loperamide and naloxone. Loperamide had no significant effects on the isometric contractions during or following EFS. Naloxone significantly reduced the isometric contraction during stimulation, and increased the post-stimulation contraction.

In control preparations with no drugs, there was negligible change in muscle force during EFS (20V, 10Hz, 0.5ms for 10s; Δ0.07±3.11g), but there was a clear post-stimulus contraction (Δ12.91±8.33g) (**Figure 9.3**).

In preparations with L-NOARG and MRS2179, used to reveal excitatory neuromuscular transmission, EFS (20V, 10Hz, 0.5ms for 10s) elicited isometric contractions (Δ10.42±6.05g), with a less marked post-stimulus contractions (Δ3.13±5.00g). TTX abolished the contractions both during and post-stimulus (during EFS: Z=-3.89, p<0.001, median difference=-4.00g post-TTX; post-EFS: Z=-2.42, p=0.015, median difference=-1.50g post-TTX; **Figure 9.4**), suggesting that both responses are neurally-mediated.

In preparations with L-NOARG and MRS2179, loperamide had no significant effects on the isometric contractions during or following EFS (**Figure 9.5**). After loperamide, naloxone significantly reduced the isometric contraction during stimulation (Z=-3.51, p<0.001, median difference=-2.70g post-naloxone) and increased the post-stimulation contraction (Z=-3.59, p<0.001, median difference=+4.10g post-naloxone).

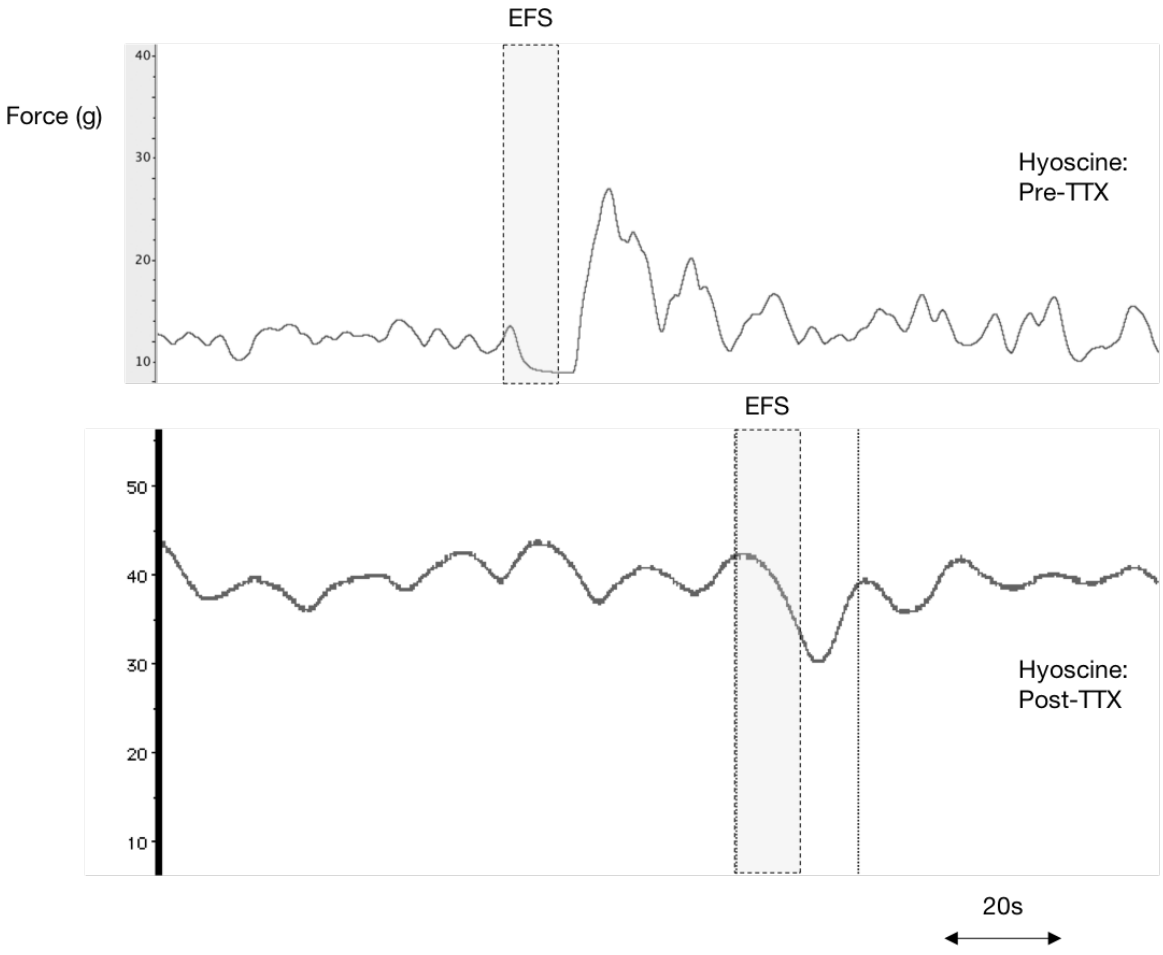

Figure 9.6 Example isometric force recordings from human colonic circular muscle preparations, obtained in the presence of hyoscine hydrobromide. Force (g) is displayed on the y-axis and time (seconds) on the x-axis pre- and post-TTX. EFS (20V, 10Hz, 0.5ms for 10s) elicited isometric relaxations, followed by a contraction following the cessation of EFS. TTX reduced or abolished both the relaxation during EFS and the post-stimulus contraction.

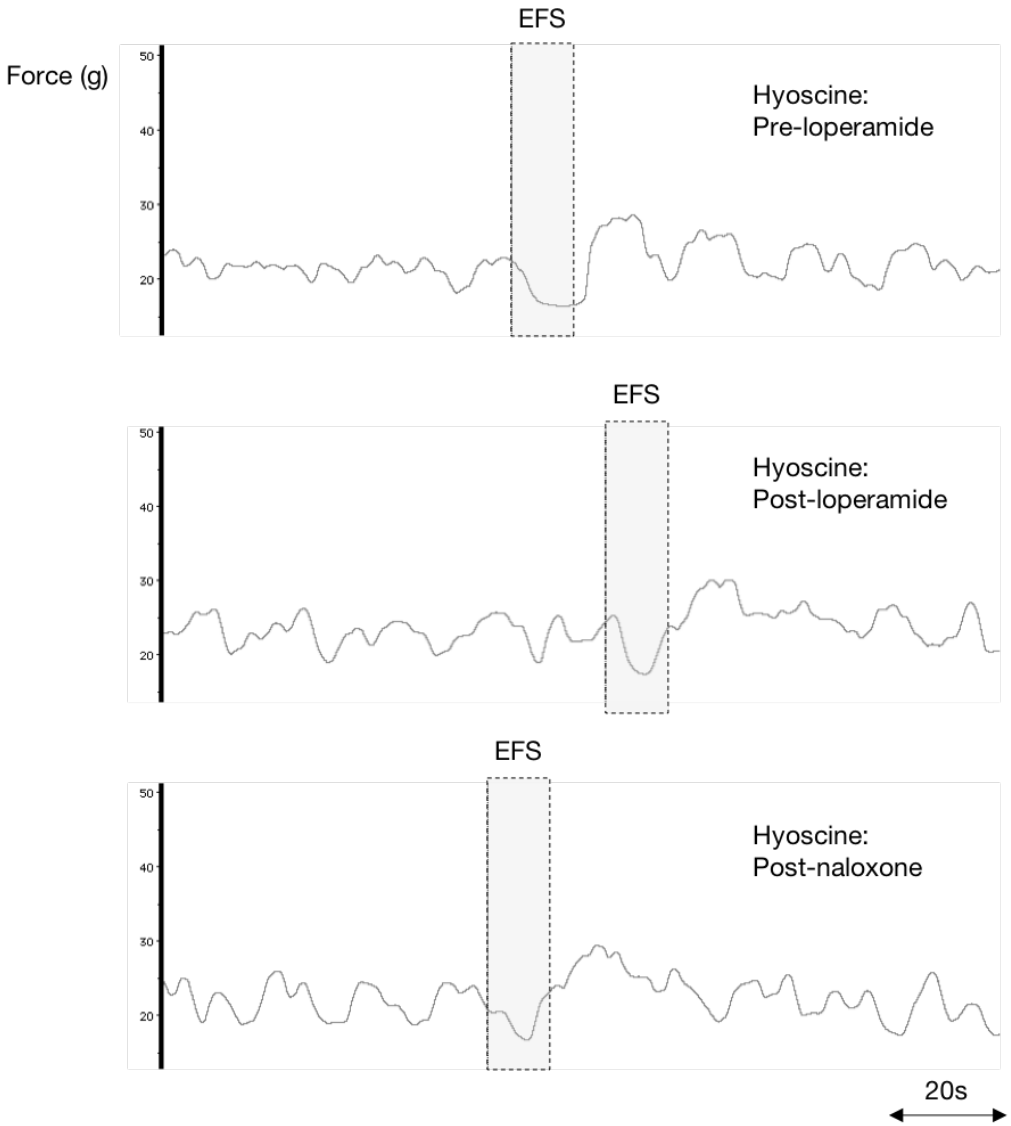

Figure 9.7 Example isometric force recordings from human colonic circular muscle preparations, obtained in the presence of hyoscine hydrobromide. Force (g) is displayed on the y-axis and time (seconds) on the x-axis during the control period and following the sequential additions of loperamide and naloxone. Loperamide reduced the isometric relaxation during EFS. Naloxone, added after loperamide, further reduced the isometric relaxation. No significant differences in the post-stimulus contractions were observed after loperamide or naloxone.

In preparations with hyoscine hydrobromide, used to reveal inhibitory neuromuscular transmission, EFS (20V, 10Hz, 0.5ms for 10s) elicited isometric relaxations (Δ-2.27±0.95g). Post-stimulus contractions were also recorded in these preparations (Δ9.15±3.97g). TTX reduced or abolished both the relaxation during EFS (Z=-2.85, p=0.004, median difference=+1.20g post-TTX) and the post-stimulus contractions (Z=-2.49, p=0.013, median difference=-2.40g post-TTX; **Figure 9.6**).

In preparations with hyoscine hydrobromide, loperamide reduced the isometric relaxation during EFS (median difference +0.40g post-loperamide, Z=-2.35, p=0.019). Naloxone, added after loperamide, further reduced the

isometric relaxation (median difference +1.20g post-naloxone, Z=-3.36, p=0.001; **Figure 9.7**). No significant differences in the post-stimulus contractions were observed after loperamide or naloxone.

9.5.2 Guinea Pig Studies

Loperamide (3μM) significantly reduced the amplitude of the post-EFS contractile response (50V, 10Hz, 0.5ms, 3s; Z=-4.08, p<0.001). The addition of naloxone did not alter the EFS response (Z=-1.11, p=0.27) but did increase the basal tone of the specimen (Z=-3.62, p<0.001). Loperamide also significantly reduced the amplitude of the peristaltic contractions in the small intestine elicited by 10Hz EFS (Z=-4.08, p<0.001) and abolished the cholinergic twitches of the longitudinal muscle to 0.1Hz EFS in the small intestine.

9.6 Discussion

Loperamide had no effect on excitatory neuromuscular action in specimens of human colonic circular muscle, but did reduce inhibitory neuromuscular action. Despite this, we demonstrated that loperamide inhibited neurally-mediated motor events in the longitudinal muscle of guinea pig ileum and colon, as has been reported by others(303, 761, 762). A similar absence of a loperamide effect on excitatory neuromuscular transmission was reported by Benko et al.(303) in longitudinal muscle strips of human small intestine. The findings of this study and those of Benko et al.(303) may suggest that, in humans, loperamide inhibits intestinal motility by acting on premotor interneurons rather than on the final excitatory enteric motor neurons. Similarly, in the guinea pig small intestine, opioids can act presynaptically in myenteric ganglia(769).

These findings may also suggest that agonism of the μ-opioid receptor of human enteric excitatory motor neurons alone is not sufficient to alter motility. Human enteric motor neurons are responsive to other opioid agonists. For example, Angel et al.(305) reported a reduction in contractile response to EFS in preparations of human ileum using the μ-opioid receptor agonist D-alaglymepheglyol (DAMGO) but not the post-EFS contractile response, both of which are considered to be predominantly mediated by cholinergic neurons(389, 765-767, 770). However, this was only observed at high concentrations, at which co-activation of both μ and κ receptors can occur(771). Secondly, Chamouard et al.(304) demonstrated a reduction of excitatory neural transmission in longitudinal and circular muscle strips of human sigmoid colon using a δ-opioid receptor agonist. Thirdly, Yuan et al.(764) found that morphine inhibited the excitatory transmission in strips of human small intestine. And, finally, Bauer et al.(772) demonstrated that opioid peptides inhibit neuromuscular transmission from enteric inhibitory motor neurons by acting on δ-receptors.

Loperamide reduced inhibitory neuromuscular transmission, demonstrated by a reduction in isometric relaxation during EFS. These results are similar to the inhibition of neuromuscular transmission in enteric inhibitory motor neurons observed using enkephalins and δ-opioid receptor agonists in preparations of human

sigmoid colon described by Hoyle et al.(292). However, in our work, this effect was only demonstrated on the grouped paired analyses and not replicated on the frequency-response curves. This may indicate a relatively minor effect of loperamide on inhibitory transmission or a type I error.

It is perplexing that naloxone caused a further reduction in inhibitory neuromuscular activity, an effect counterintuitive to what would be expected following the sequential additions of an agonist and antagonist of the same receptor. It has been previously reported that the antimotility effects of loperamide are inhibited by naloxone(761, 773). Since naloxone is non-selective and also acts at δ and κ receptors, a likely explanation is that, during EFS, there is release of endogenous opioids from enteric neurons that act on opioid receptors other than μ-receptors. In experiments to localise the opioid receptors, Sternini et al.(774) found that the distribution and density of μ-opioid receptor immunoreactivity was comparable in the human jejunum and colon, localised to neuronal cell bodies in both submucosal and myenteric ganglia.

During EFS, the addition of naloxone reduced the force of contraction during excitatory neural transmission and increased the amplitude of the post-stimulus contraction. An increase in the post-EFS contractile response was replicated on the frequency-response curves at frequencies >15Hz. The post-EFS contractile response is considered to be mediated by acetylcholine and tachykinins(305, 765-767, 770). Antagonism of opioid receptors would be likely to remove the inhibition by endogenous opioids of acetylcholine release(775). This could explain the increased amplitude of the "off contraction" post-EFS that we observed. This is reflected in findings from in vivo human studies, in which administration of naloxone in the absence of opioids results in a reduction in colonic transit time(776). Similarly, the use of alvimopan, a peripherally-acting μ-opioid receptor antagonist, reduces colonic transit time in the absence of opioid use(777). Collectively, these findings may suggest that that, in the human colon, endogenous opioid peptides have a tonic inhibitory effect on enteric motor neurons.

There are several limitations in comparing findings from our human and guinea pig studies. These include; (1) longitudinal muscle contractility was recorded in guinea pig preparations versus circular muscle in human preparations; (2) a higher concentration of loperamide used in the guinea pig studies (3μM vs 1μM); (3) differing EFS parameters (human: 20V, 10Hz, 0.5ms for 10s; guinea pig: 50V, 10Hz, 0.5ms for 3s), and; (4) different tissue preparations (human: circular muscle strips; guinea pig: 3-4cm tubular segments of small/large intestine). Whilst we would still expect to see alterations in neuromuscular response to EFS in both preparations, it must be conceded that these differences in study protocol may confound our findings.

Chapter 10: Discussion

10.1 Overview

The findings of this thesis address several aspects of physiology and pathophysiology related to continence and defaecation. A combination of experimental techniques were undertaken to address the thesis aims, including clinical, in vivo, and ex vivo human studies. Specifically, the original contributions to scientific knowledge in this field include:

- **Chapters 1 & 2:** A contemporary literature review on the functional physiology of colonic motility and defaecation.
- **Chapters 4 & 5:** The discordance between conventional and contemporary anorectal investigation results and symptom severity in faecal incontinence. No single diagnostic investigation or combination of investigation results was strongly associated with faecal incontinence severity. These findings also highlight the limitations of current diagnostic investigation and analysis techniques as well as the limitations of quantitative symptom scoring. These findings suggest that the severity of symptoms in faecal incontinence is not solely attributable to anorectal dysfunction.
- **Chapter 6:** A characterisation of the colonic motor response during stimulated defaecation with bisacodyl in children with severe, treatment-refractory constipation. The majority of these children generated an "all-or-nothing" response to bisacodyl, with high-amplitude propagating contractions occurring within 12 minutes of bisacodyl infusion. Prior to this study, these children were already being treated with a regular laxative regime, which often included bisacodyl. Despite their ability to generate HAPCs in response to bisacodyl, these children still experience refractory symptoms. This indicates that defaecation requires more than just the ability to generate HAPCs. As such, our current approach to investigation and analysis cannot identify the causation of symptoms in the majority of these children. These findings highlight the limitations of our current diagnostic investigation and analysis techniques.
- **Chapter 7:** The first application of high-resolution impedance manometry in the human colon in vivo, providing a description of the associations between distal colonic motility and gas transit. Most participants reported no conscious urge to pass flatus despite gas insufflation into the distal colon. Our impedance recordings demonstrated an increase in gas in the distal colon after a meal. The prevalence of the rectosigmoid cyclic motor pattern increased in response to a meal or intraluminal gas insufflation. This suggests that colonic motility, and specifically the cyclic motor pattern, is related to the regulation of gas storage, continence, and evacuation. These findings also suggest that the cyclic motor pattern can be initiated by a localised sensorimotor response to intraluminal gas as well as by extrinsic neural inputs.
- **Chapter 8:** A description of the generation and regulation of spontaneous colonic motor patterns using high-resolution manometry in ex vivo human colon. Propagating contractions in specimens of excised human colon are likely to be myogenic in origin given that they persist following the administration of hexamethonium or lignocaine. Additionally, spontaneous contractions increase in frequency following the addition of tetrodotoxin. This is likely to represent the removal of tonic neural inhibition which allows myogenic-generated motility patterns to occur. This provides a physiological basis to appreciate how colonic motility patterns are generated and regulated.

- **Chapter 9:** Loperamide, a μ-opioid receptor agonist, causes a reduction in cholinergic-mediated contraction during electrical field stimulation in guinea pig ileum and colon, but appears to have no effect on excitatory neuromuscular transmission in human colonic circular muscle. This may suggest that loperamide inhibits intestinal motility by acting on premotor enteric neural circuits rather than on the final excitatory enteric motor neurons in humans.

The collective interpretations of these findings and the implications for further research are discussed below.

10.2 Colonic and Anorectal Dysfunction in Disorders of Defaecation

10.2.1 Faecal incontinence

Symptoms are the reason that patients with faecal incontinence seek medical attention. The goals of treatment in faecal incontinence are – at best – to achieve a full curative resolution of symptoms or, failing that, to aim for a significant reduction in symptom severity. This could include a reduced frequency of incontinence episodes, reduced use of pads, and/or increased confidence to leave home and socialise. Given that anorectal investigations are often performed both pre- and post-intervention, it is important to appreciate how these results relate to symptom severity in order to assess the outcomes of treatments.

No single anorectal investigation result, or combination of investigation results, is strongly associated with faecal incontinence severity (**Chapters 4 & 5**). Furthermore, the strength of associations did not substantially improve with an upgrade from conventional diagnostic equipment (low-resolution anorectal manometry and two-dimensional endoanal ultrasound) to contemporary diagnostic equipment (high-resolution anorectal manometry and three-dimensional ultrasound). Despite this, the majority of patients did return multiple abnormal results on different tests, supporting the multifactorial pathogenesis of faecal incontinence(190-192). These findings highlight the difficulties in identifying causation of symptoms in faecal incontinence and, therefore, directing targeted treatments. This is important for both; (1) clinicians, who use these results to guide management decisions and assess treatment outcomes, and; (2) scientists working in this field, as a means to better understand the complex, multifactorial pathophysiology of faecal incontinence.

Symptoms aside, there were clear associations between the findings of anorectal investigation results. For example, approximately 40% of the cohort had anal sphincter defects on endoanal sonography, which were correlated with a reduction in anal canal resting and squeeze pressures on anorectal manometry (**Chapter 4**). Associations between investigation results were consistent with recognised patterns of anorectal dysfunction in faecal incontinence (**Chapter 5**), including:

- Anal sphincter injury resulting in functional sphincter weakness.
- Global anal sphincter weakness.
- Global anal canal hyposensitivity.

- Global rectal hyposensitivity.
- Pudendal motor neuropathy and anal sphincter weakness.

Importantly, the findings of **Chapters 4 & 5** suggest that a focus solely on the anorectum is inadequate to encapsulate symptom causation in faecal incontinence for many patients. There are a multitude of processes occurring extrinsic to the anorectum which are integral to the normal physiology of defaecation and continence (**Chapter 2**). These include, but are not limited to, voluntary and involuntary processes occurring within the central nervous system (cerebral cortex, brainstem, spinal cord), extrinsic sympathetic/parasympathetic innervation to the colon and anorectum, stool consistency, and colonic motility, among other factors(28, 128, 778). As such, if there is a disturbance to the normal physiology of defaecation and continence and a cause is not identified following diagnostic assessment of the anorectum, consideration must be given to other contributing pathophysiological mechanisms.

When considering colonic dysfunction in faecal incontinence, Bharucha et al.(199, 201) reported that bowel disturbances, including constipation, diarrhoea, and/or abdominal pain, were a major risk factor for developing faecal incontinence. In my findings, a high proportion of patients with a presenting complaint of faecal incontinence also had concurrent constipation, with >40% of the cohort reporting a Cleveland Clinic constipation score of ≥9/30(257). This is consistent with other recent studies which also reported that >40% of patients referred with either isolated faecal incontinence or constipation actually have concurrent symptoms of both disorders(108, 306-308, 586).

10.2.2 Constipation

In some instances, constipation can primarily be an anorectal disorder, such as in the setting of a rectal evacuation disorder (eg. faecal impaction, rectocoele). However, like faecal incontinence, the symptoms of constipation can also be the result of pathophysiological mechanisms which occur extrinsic to the anorectum. The subset of children in my study included those with severe, treatment-refractory symptoms being managed by paediatric gastroenterologists in quaternary paediatric hospitals. The vast majority of these children demonstrated high-amplitude propagating contractions (>90%) and defaecated in response to bisacodyl, which is considered to be a “normal” response(264-269). Some of these children were previously being treated with bisacodyl as part of their outpatient management prior to the colonic manometry study and reported ongoing, severe symptoms, despite demonstrating a “normal” colonic response to bisacodyl during the manometry study. The ability of most children in our sample to generate a bisacodyl response (HAPCs), yet still report persistent, treatment-refractory constipation, demonstrates that our current approach to investigation of colonic dysfunction in these children is inadequate.

An assessment of colonic neuromuscular function based on the bisacodyl response alone may too superficial and overlook more subtle patterns of dysfunction. While the absence of high-amplitude propagating

contractions (HAPCs) following bisacodyl is an important clinical finding, this only accounted for <10% of the children studied in my study. In the remaining children, where HAPCs were present, there was no ability for my analysis to discriminate between pathological findings and normality (**10.2.4 Development and Standardisation for Analysis of Colonic Investigations**). A more detailed assessment and understanding of colonic motility is required to elucidate symptom causation in the majority of these children.

Wessel et al.(250) described "a look beyond high-amplitude propagating sequences", with a more detailed, descriptive analysis of colonic manometry studies in 18 children with constipation. In their study, the authors described alterations in colonic motility which may be of pathological significance which are not routinely assessed in clinical practice. One example of this was an abnormal colonic meal response, with a reduction in the prevalence of the retrograde cyclic motor pattern. This finding has also been reported in adults with slow transit constipation(723). However, the functional implications of these findings and how they relate to the pathogenesis of symptoms remain unclear.

Collectively, the clinical findings from patients with faecal incontinence and constipation (**Chapters 4-7**) highlight the limitations in; (a) our understanding of the relevant physiology and pathophysiology, and; (b) our current approaches to the clinical investigation of defaecation disorders. The development and standardisation of analysis techniques for functional colonic and anorectal assessments, as well as the development of new investigative technologies, will be crucial to address these issues in future.

10.2.3 Future Direction: Development and Standardisation for Analysis of Anorectal Investigations

During the last decade, there have been significant advances in available technologies for the assessment of colonic and anorectal function. These include high-resolution colonic and anorectal manometry(2, 166, 316-318, 637, 645, 725), ingestible wireless capsule devices(98, 138, 139), magnetic resonance imaging (MRI) defaecography(320), and cine-MRI(319, 321). Despite the substantial progression in available diagnostic technologies, the utility of the information gleaned from these techniques is ultimately limited by our data analyses and interpretation. In anorectal assessment, conventional low-resolution anorectal manometry and two-dimensional endoanal ultrasound have been superseded by high-resolution anorectal manometry (HRAM) and three-dimensional endoanal ultrasound(671-673). However, in many anorectal clinics (including our own), reporting of results has not substantially changed with the update from conventional to contemporary techniques (**Chapters 4 & 5**). For example, each patient's results include the absolute values of anal canal resting pressure and maximal squeeze pressure, which are compared to the published normal ranges obtained from healthy controls.

Rather than reporting absolute values, some authors have reported improved sensitivity for detecting anorectal dysfunction using the calculation of dynamic, functional measures from HRAM results(121, 318, 643). These

include the squeeze increment, contractile integral, resting average, rest integral, and functional anal canal length(120, 346). For example, the contractile integral is derived using the mean of all pressure values from the anal canal during a five-second squeeze, multiplied by the functional anal canal length, minus the mean pressure during a five-second resting period(120). This combination of measures may provide a more accurate reflection of the functional capabilities of the anal sphincter, but this is yet to be determined.

Other authors have used area-pressure and area-tension loops to describe the dynamic biomechanics of the anorectum(779, 780). This is similar to the volume-pressure loops used to describe cardiac physiology. The Frank-Starling mechanism describes the length-tension and force-velocity relationships in cardiac muscle. Specifically, this details the ability of the myocardium to alter the force of contraction and stroke volume in response to alterations in venous return. Using length-tension loops to describe anorectal function can assess how these parameters are altered by increasing rectal volumes. In one study, this allowed the authors to distinguish normal from dysfunctional anorectal motor activity in healthy volunteers and patients with faecal incontinence(780).

While these analysis techniques have been reported in several recent studies(120, 121, 318, 346, 643, 780), their uptake is not yet widespread. Study protocols, analysis, and reporting techniques vary widely between specialist anorectal centres(638). This complicates any comparisons between patient and control data, comparisons of findings between centres, and collation of data for large, multicentre studies. Recently, the International Anorectal Physiology Working Group reported a standardised testing protocol(781) which was intended to improve the consistency in study protocols between centres. However, this was largely focused on the performance of anorectal manometry, rectal sensory testing, and balloon expulsion testing, rather than data analysis and interpretation.

10.2.4 Future Direction: Development and Standardisation for Analysis of Colonic Investigations

Colonic manometry is performed less commonly than anorectal investigations and there is likely to be even greater variability in protocols and equipment between centres. The only current clinical application of colonic manometry is the assessment of colonic neuromuscular function in treatment-refractory constipation(79, 122-124), as described in **Chapter 6**. Outside of this, colonic manometry is predominantly a research tool, with differing study protocols, equipment, and analysis techniques between research centres.

Standardised terminology for commonly identified colonic motor patterns were described in a recent expert consensus statement(11). However, standardised techniques for the analysis of colonic manometry are yet to be developed. Most analysis techniques which are used for identifying and characterising colonic motor patterns are limited to either a motility index, area under the curve analyses, or descriptive, observational methods(107). While area under the curve analyses or motility indices do quantify phasic activity, these

analyses provide no descriptive characterisation of motor patterns. This restricts their utility for describing heterogenous, multi-faceted, or subtle patterns of dysfunction. I utilised both visual identification of high-amplitude propagating contractions and area under the curve analysis techniques in **Chapter 6**. Using these techniques, my manual analysis did not have the sensitivity to identify any patterns of colonic dysfunction in >90% of children with severe constipation. Presuming that these children do have an element of colonic motor dysfunction, the development of more sensitive analysis techniques for colonic manometry studies is required to identify the pathogenesis of their symptoms. Ultimately, our understanding of colonic motility disorders is unlikely to improve until we substantially improve our measurement technologies, protocols, and analysis techniques.

To address the limitations of manual analysis techniques, there have been several previous approaches to automate the analysis of colonic manometry data(782-786). No single automated analysis technique is yet to have widespread uptake and each have their own limitations. The automated analysis techniques described to date have included:

- Methods of automated waveform detection, but with no descriptive analysis of motility patterns(783).
- Pattern-recognition algorithms with manually-defined parameters of health and disease states(786) or characteristics of motility patterns(782). However, if identified motor patterns are based upon pre-conceived descriptions of motility patterns, these approaches will be subject to the same bias of manual analysis approaches.
- Independent component analyses to discriminate between motility patterns in healthy volunteers and patients with slow transit constipation(784). Each study was classified into one of three subtypes (regular rhythm, slow rhythm, disorder) based upon a pre-defined frequency and duration of colonic activity which, again, is subjective and introduces bias.
- Cross-correlation analyses based on the temporal delay between pressure waves in adjacent manometry channels(785). This approach was able to discriminate between healthy controls and patients with slow transit constipation using pancolonic data, but not when examining data obtained from the distal colon only.

Most recently, automated wavelet transform analyses have been used to analyse colonic manometry data(714, 715). Phasic, oscillatory signals, such as those recorded by colonic manometry, can be converted from temporal data to time-frequency data using the wavelet transform. This allows for; (a) the identification of varying frequencies of colonic pressure waves; (b) comparisons to ascertain how stimuli such as a meal alters the pressure wave frequencies, and; (c) comparisons between the characteristics of motility patterns in healthy volunteers and patients. This approach was implemented in **Chapter 7** to describe the temporal associations between pressure and impedance events, as well as the direction of propagation of impedance events. Wavelet transform analyses may be beneficial for future research in this field, without the limitations of the previous automated analysis techniques described above. Wavelet transform analyses have broad applications in other

medical and scientific fields(787), including neurology(788), meteorology(789), and geoscience(790), among others.

10.2.5 Future Direction: Development and Application of New Investigative Technologies

Recording colonic smooth muscle activity and colonic transit is complicated by the requirement for prolonged recording given the involuntary and infrequent nature of motor events, among other factors (**1.5 Colonic Motility: Recording Techniques**). As such, the ongoing refinement of existing technologies and development of new assessment techniques will be crucial to further scientific knowledge in this field.

Gregersen et al.(446) recently developed an anorectal diagnostics device, named Fecobionics. This is a synthetic stool which contains data sensors which provide information on anorectal geometry and manometry during simulated defaecation. This integrates the information received from several existing anorectal investigations and, in doing so, addresses some of their limitations. This includes; (1) anorectal manometry, which is not performed during defaecation; (2) defaecography, which does not provide intraluminal pressure data, and; (3) the balloon expulsion test, which does not provide data on anorectal geometry. Whether Fecobionics can replace these tests in clinical practice is yet to be determined but, at this stage, it remains a promising research tool for the dynamic assessment of defaecation in health and disease.

During the course of this candidature, our laboratory began collaborating with a research group at the University of Auckland who have designed a device for non-invasive body surface electrode recording of colonic activity, or electrocolonography(791). The device includes a 64-channel electrode array sticker applied to the skin surface of the abdominal wall and connected to a portable data logger. Similar to colonic manometry, electrocolonography is intended to record the occurrence of propagating contractions and their characteristics. Using one of their devices, I concurrently recorded colonic motor activity using both impedance manometry and electrocolonography in one healthy volunteer (electrocolonography data not included in results), with the aim of contributing further studies to their database to validate this recording technique.

If electrocolonography is demonstrated to have the same sensitivity to detect colonic motor patterns as high-resolution colonic manometry, this may allow us to preferentially use electrocolonography instead of colonic manometry for future studies. This would avoid the requirement for bowel preparation, fasting, intravenous sedation, and colonoscopy for catheter placement. This could allow us to perform recordings in normal physiological conditions (unprepared colon) and significantly increase the ease of collecting human data, both of which are highly desirable for our participants and ongoing research endeavours. The validation of electrocolonography is ongoing at the time of writing.

10.3 The Functional Role of Colonic Motility in Transit and Continence

10.3.1 High-Resolution Colonic Impedance Manometry in Healthy Adult Volunteers

In **Chapter 7**, I performed in vivo colonic impedance manometry studies in healthy adult volunteers to investigate the functional role of distal colonic motility in gas transit. This was a new application of an existing technology, which had previously been used to describe the relationships between motility and transit in the oesophagus, small intestine, and ex vivo rabbit colon(142-145, 152). My findings demonstrated that impedance (gas) and pressure (motility) events were associated, with impedance events temporally preceding pressure events. In all study periods (control, post-prandial, post-gas insufflation), impedance events propagated in both antegrade and retrograde directions, with a predominance of retrograde propagation. These findings also confirm that impedance manometry is a viable investigative tool to record aspects of colonic physiology. Furthermore, a study duration of <4 hours can be useful if provocation with gas insufflation is used as part of the assessment process, comparable to physiological stimuli used elsewhere in human pathophysiological testing (eg. oral glucose tolerance test, exercise stress test etc.).

The observations of retrograde gas transit provide insights into the normal regulation of gas storage, continence, and evacuation. Retrograde propagating contractions associated with retrograde transit have been demonstrated in animal models including rabbits(792), dogs(26, 793), sheep(129), and horses(794, 795). Only in the last decade, since the advent of high-resolution colonic manometry(729), retrograde cyclic motor activity was demonstrated to be the most prevalent motor pattern in the human colon(2). In my study, the site of gas insufflation was approximately 30cm proximal to the anal verge. Despite this, only two participants reported a conscious flatal urge, with one participant reporting a single episode of flatus. The room air used for insufflation has a higher concentration of N_2 than that of colonic gas. Given the poor mucosal absorption of N_2, I anticipated a higher number of flatus events. These findings demonstrate the compensatory mechanisms of the colon to manage and store gas in the event of increasing gas volumes in the distal colon, as well as the role of the cyclic motor pattern in the regulation of gas storage, continence, and evacuation.

10.3.2 Future Direction: High-Resolution Colonic Impedance Manometry in Patients with Defaecation Disorders

To follow on from the impedance manometry studies performed in healthy volunteers (**Chapter 7**), the same study protocol will be performed in patients with defaecation disorders. This will allow for comparisons between healthy volunteers and patients, to investigate the contribution of colonic dysmotility to the pathogenesis of defaecation disorders. Specifically, this would include investigating the hypothesis that a reduction or absence of the cyclic motor pattern in the distal colon leads to uncontrolled rectal filling, urgency, and faecal incontinence(176). If this is the case, patients who demonstrate a reduction in cyclic motor activity could be used for more selective applications of sacral nerve stimulation, which increases the cyclic motor pattern(173, 181) (**1.6.3 The Cyclic Motor Pattern**). High-resolution impedance manometry may also be

useful in characterising pressure-transit relationships in other functional bowel disorders where dysmotility is implicated, such as diarrhoea-predominant irritable bowel syndrome.

Two patients with faecal incontinence were studied using high-resolution impedance manometry during this candidature (data not included in results). Completion of data collection for patients with faecal incontinence (n=10) was planned to be conducted during this candidature. Unfortunately, the timing of these experiments coincided with the COVID-19 global pandemic, during which a temporary cessation of elective, non-urgent colonoscopy bookings was enacted by the Australian Government. The remaining data collection is planned to be completed in 2021 for publication in a subsequent study.

10.4 The Physiological Mechanisms Responsible for the Generation and Regulation of Colonic Motility

10.4.1 The Generation of the Cyclic Motor Pattern and Pathophysiological Implications

Whilst **Chapters 4-7** involved in vivo human studies, ex vivo experiments were additionally performed (**Chapters 8 & 9**) to investigate the physiological mechanisms underlying the generation of colonic motor patterns.

In **Chapter 8**, the cyclic motor pattern was only apparent following the administration of tetrodotoxin (TTX) to ex vivo human colon. This suggests that the cyclic motor pattern is myogenic in origin and is suppressed by tonic neural inhibition. Electrical stimulation (with parameters directed at neural stimulation) initiated propagating contractions, but these could be diminished or abolished following the administration of TTX, demonstrating that enteric neural innervation can additionally initiate and modulate colonic motor activity. This finding has important clinical significance, because the postprandial increase in the cyclic motor pattern is reduced in adults and children with constipation(250) (**10.2.2 Constipation**), and is also hypothesised to be reduced in faecal incontinence(176). Based upon our findings, these disorders may therefore reflect; (a) colonic smooth muscle dysfunction (inability to generate the cyclic motor pattern), or; (b) dysfunction of the enteric nervous system (causing excessive inhibition of the cyclic motor pattern).

10.4.2 Synchronous Pressurisations

With the introduction of high-resolution manometry, pancolonic pressurisations or synchronous pressure increases were described in two recent publications(166, 167) (**1.6.5 Pancolonic Pressurisations**). These motor patterns were defined as an increase in pressure occurring across all sensors simultaneously. In both studies, these events were hypothesised to be associated with gas transit and flatus, however they did not have the ability in their studies to record gas transit. Synchronous pressure increases in colonic manometry studies can also be caused by abdominal strain, diaphragmatic movement during phonation, or re-positioning in bed.

Corsetti et al.(166) discriminated between abdominal strain and colonic smooth muscle activity by using abdominal wall electromyography (EMG). Any synchronous pressure increase recorded on colonic manometry which was associated with an increase in the abdominal wall EMG tracing was excluded. This allowed them to demonstrate "true" colonic pressurisations, which had a mean duration of ~24s. In the study by Chen et al.(167), no such discrimination was performed and all synchronous pressure events were included. The mean duration of synchronous pressure increases in their study was ~10s.

In my study (**Chapter 7**), I detected no synchronous pressure events that were consistent with the definition provided by Corsetti et al.(166) Rather, the maximum duration of any synchronous pressure increase in my data was 12s, with a mean duration of <5s. These values were comparable to the findings of Chen et al.(167) The synchronous pressure events identified in my study were not altered following a meal or gas insufflation. This may suggest that the synchronous pressure events that I observed were not generated by colonic smooth muscle, but rather by increases in intra-abdominal pressure caused by contraction of the abdominal wall musculature. However, my study did demonstrate an association between these synchronous pressure increases and changes in impedance. If these pressure events are not caused by colonic smooth muscle contraction, they may instead be caused by abdominal wall movement resulting in extraluminal mechanical compression of the colon. This action could also displace intraluminal gas, which would account for the changes in impedance.

Corsetti et al.(166) and Chen et al.(167) proposed that synchronous pressurisations are associated with flatus. However, flatus in humans occurs between 10-20/day(796) while, in the study by Corsetti et al.(166), pressurisations occurred at a frequency of 12 events/hour. In my study, the mean frequency of synchronous pressure increases was 40-59 events/hour. Given this frequency, it is unlikely that all of these events are associated with flatus. Despite this, it is likely that some of these synchronous pressure events are associated with gas transit and flatus. Examples of this were evident in my study, in which a synchronous pressurisation occurred at the termination of a propagating contraction which was associated with impedance events. In one instance, a synchronous pressurisation following an antegrade propagating contraction was associated with flatus, which would support the hypotheses of Corsetti et al.(166) and Chen et al.(167) In the rabbit small intestine, synchronous pressurisations were hypothesised to be a common cavity phenomenon(152, 183), in which a propagating contraction causes dilation to accommodate transit of intraluminal content, with "back pressure" from a closed anal sphincter (antegrade) or contraction proximally (retrograde). Conversely, common occluded contractions also demonstrate a similar appearance on the manometry trace, in which an extended length of colon concentrically contracts(183).

If the synchronous pressure increases described by Corsetti et al.(166) were not caused by abdominal strain (no increase in the abdominal EMG tracing), and are not all associated with gas transit and flatus, what other factors could account for their occurrence? There is some evidence that synchronous pressurisations are stimulated by colonic distension. Previous animal studies using ex vivo guinea pig colon(728, 741) have demonstrated that sustained colonic distension can result in repetitive, synchronous pressure events that appear

to be of a similar nature to the those recorded in the human colon in vivo. In **Chapter 8**, I also recorded rhythmic synchronous pressure increases. I had applied sustained tension to the colonic wall using force transducer clips to ensure close contact between the manometry catheter and the mucosa, which may have stimulated the same mechanoreceptors which respond to luminal distension. Synchronous pressure increases can also be induced by acetylcholinesterase inhibitor neostigmine(166, 385). Neostigmine has been shown to increase colonic tone(386). Given that Corsetti et al.(166) only used a water enema for a bowel preparation prior to catheter placement, there would still be intraluminal content within the proximal colon. Therefore, the increased colonic tone induced by neostigmine could result in increased contact between the colonic wall and luminal content, thereby stimulating mechanoreceptors.

Further studies are needed to determine the cause and physiological role of synchronous pressure increases and, based upon my studies, colonic impedance manometry may help to further characterise the functional role of these events.

10.4.3 Effects of Opioids on Excitatory and Inhibitory Enteric Musculomotor Neurons

The effects of opioids on gastrointestinal function are clearly apparent in clinical practice, with a huge burden of morbidity occurring as a result of opioid-induced constipation(231, 288, 289). In animal models, opioid receptor agonists cause a reduction in acetylcholine release, resulting in a reduction in cholinergic-mediated smooth muscle contraction(297-301). I demonstrated a reduction in cholinergic-mediated contraction during electrical field stimulation in guinea pig ileum and colon in response to loperamide, a μ-opioid receptor agonist (**Chapter 9**). However, loperamide appeared to have no effect on excitatory neuromuscular transmission in human colonic circular muscle. This is similar to previous findings in response to μ-receptor agonists in human colonic circular muscle strips(305) and longitudinal muscle strips of small intestine(303) and colon(305). As such, the precise mechanisms by which loperamide causes the alterations in gastrointestinal function observed in clinical practice cannot be described by my findings. This may suggest that loperamide inhibits intestinal motility by acting on premotor enteric neural circuits rather than on the final excitatory enteric motor neurons in humans. A similar mechanism of action has also been described in the guinea pig small intestine, in which opioids can act presynaptically in myenteric ganglia(769).

10.4.4 Future Direction: Human Colonic Tissue Specimens with Opioid Receptor Agonists & Antagonists

The next stage of the ex vivo circular muscle strip experiments will be to implement a similar experimental protocol to assess the effects of κ- and δ-opioid agonists on excitatory and inhibitory neural activity. While I performed frequency-response curves to assess neuromuscular responses, it would of additional benefit to also perform dose-response curves to assess whether higher concentrations of opioid agonists elicit differing responses.

It would additionally be of benefit to assess synaptic transmission within the myenteric plexus, as described in guinea pig tissue by Cherubini et al.(769) While my study assessed the mechanical neuromuscular response to stimulation, Cherubini et al.(769) described identification of myenteric ganglia under magnification, with intracellular recording used to record synaptic potentials in response to stimulation. A similar study protocol performed in human tissue pre- and post-loperamide could assess whether loperamide alters synaptic transmission in the myenteric ganglia.

The circular muscle strip experiments described in **Chapter 9** were predominantly focused upon changes in neuromuscular activity in response to opioid receptor agonists. An additional finding from this work was the altered responses to naloxone, an opioid receptor antagonist. In a previous in vivo study, the administration of naloxone, an opioid receptor antagonist, resulted in an increase in colonic transit in the absence of opioids(776). Similarly, the use of alvimopan, a peripherally-acting μ-opioid receptor antagonist, increases colonic transit in the absence of opioids(777). These findings may suggest that endogenous opioid peptides have a tonic inhibitory effect on colonic smooth muscle, which can be altered by opioid receptor antagonists. It would be worthwhile further pursuing the effects of opioid antagonists on endogenous opioid peptides and colonic neuromuscular function. This could be achieved using a similar experimental protocol, with naloxone responses assessed in the absence of opioid receptor agonists. These experiments were planned to be conducted during this candidature. Unfortunately, the timing of these experiments coincided with the COVID-19 global pandemic, during which a temporary cessation of human tissue experimentation was enacted by Flinders University.

10.4.5 Human Colonic Tissue Specimens: Longitudinal Muscle

While the ex vivo experiments described in **Chapters 8 & 9** focussed upon colonic tubular specimens and specimens of circular muscle, little is understood regarding the actions and role of the longitudinal muscle layer in colonic function. It was recently demonstrated that the innervation of human colonic circular and longitudinal muscle differs, with motor neurons in longitudinal muscle having smaller cell bodies and shorter circumferential projections(797). Motor innervation also differs within the longitudinal muscle layer, with the taenia coli having a lower ratio of inhibitory to excitatory neurons and greater electrical coupling when compared to the intertaenial longitudinal muscle(797).

To further investigate motor function in human colonic longitudinal smooth muscle, an ongoing series of experiments were devised using flat sheets of colonic tissue with force transducer recordings and video imaging. These experiments were intended to characterise spontaneous longitudinal muscle activity, as well as responses to mechanical and pharmacological stimulation. Specifically, these experiments intended to assess whether the taenia coli exhibit different patterns of contractile activity to the intertaenial longitudinal muscle, and the relationships between circular and longitudinal muscle activity. In the small intestine, the longitudinal

muscle contracts synchronously with circular muscle during propagating contractions(52, 798). It is unclear whether this association is present in the colon. However, it was beyond the scope of my candidature to perform and include these experiments in this thesis and is an ongoing study.

10.5 Conclusion

Multidisciplinary collaboration with scientists, clinicians, patients, and biomedical engineers is crucial when studying the complex, dynamic physiology of the colon. Ideally, this work should be conducted in institutions where researchers have the ability to examine cellular mechanisms at one end of the spectrum, to clinical dysfunction in patients at the other extreme. Flinders University is an institution which currently has this unique opportunity. This, in part, is due to the original vision of a co-located teaching hospital and integrated medical school by Professor Gus Fraenkel (the founding Dean of the medical school) and, presently, to the expertise of the personnel leading the laboratories.

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