Integration of novel diagnostic techniques and in-depth characterisation of anorectal (dys)function in studies of healthy volunteers and patients with faecal incontinence

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A thesis submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy

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Statement of originality

I, Annika Maria Pauliina Rasijeff, confirm that the research included within this thesis is my own work or that where it has been carried out in collaboration with, or supported by others, that this is duly acknowledged below and my contribution indicated. Previously published material is also acknowledged below.

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Details of collaborations

Chapter 2

Karla Garcia-Zermeno (National Bowel Research Centre and GI Physiology Unit, Blizard Institute, Centre for Neuroscience, Surgery & Trauma, Queen Mary University of London, London, United Kingdom) **performed the initial data search and collaborated with data extraction.**

Gian Luca (The George Institute for Global Health, University of New South Wales, Sydney, Australia) **performed the meta-analysis and provided related figures.**

Chapter 3a

Ugo Grossi, Paul Vollebregt, and Pam Chaichanavichkij (National Bowel Research Centre and GI Physiology Unit, Blizard Institute, Centre for Neuroscience, Surgery & Trauma, Queen Mary University of London, London, United Kingdom [for all]) **compiled the database.**

Pam Chaichanavichkij (as above) **assisted with establishing the number of controls required in each group.**

Mark Vismer (MPV Technology Limited, Bournemouth, UK) **wrote the Python script for random selection of controls.**

Chapter 4

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Chapter 6

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Chapter 7a

Anisa Choudhary (The Wingate Institute of Neurogastroenterology, Blizard Institute, Queen Mary University of London, London, United Kingdom) **acted as the 2nd reviewer for inter-observer analysis.**

Chapter 8b

Klaus Krogh and his team (Neurogastroenterology Unit, Department of Hepatology and Gastroenterology, Aarhus University Hospital, Aarhus, Denmark) **provided practical training in performing EndoFLIP.**

Manuscripts associated with this thesis

Systematic evaluation of cough-anorectal pressure responses in health and in fecal incontinence: A high-resolution anorectal manometry study.

Rasijeff AMP, Garcia-Zermeno K, Carrington EV, Knowles C, Scott SM. Neurogastroenterol Motil. 2020:e13999.

Systematic review and meta-analysis of anal motor and rectal sensory dysfunction in patients undergoing anorectal manometry for symptoms of fecal incontinence.

Rasijeff AMP, Garcia-Zermeno K, Di Tanna GL, Remes-Troche JM, Knowles CH, Scott SM. [Submitted for publication in: Colorectal Disease].

Conference abstracts associated with this thesis

Anal slow wave activity in health and faecal incontinence.

Rasijeff AMP, Wiklendt L, Dinning P, Knowles CH, Scott SM.

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Abstract

Introduction

Large overlap in the range of values seen in health and disease limit the clinical utility of investigations which describe pathophysiological findings in faecal incontinence (FI).

Aims

The aims of this thesis were to:

1. investigate the prevalence of major disorders of anal motor and rectal sensory function in FI;
2. better describe stress FI;
3. expand knowledge of normal ranges and develop novel metrics to evaluate anorectal function using both contemporary and emerging diagnostic tests (Rapid Barostat Bag [RBB] pump and the functional lumen imaging probe [EndoFLIP®]);
4. develop understanding of (the role of) parity on anorectal function in health and FI;
5. investigate the interaction of continence mechanisms in healthy individuals.

Methods

Research methods used in this thesis include systematic review and meta-analysis, retrospective case-control and cohort studies, and a prospective study of anorectal function in health using contemporary and new technologies.

Results

Anal hypocontractility is the most common pathophysiological finding in FI, but rectal sensory dysfunction remains important, especially in men. Further, assessment of the cough response and amplitude of anal slow waves revealed subtle anal motor dysfunction not appreciated by traditional metrics.

Stress FI is poorly researched but common, and appears to represent a more severe FI phenotype.

For the first time, normal ranges for rectal compliance, capacity and sensation were generated using the RBB, and distensibility of the anal canal using EndoFLIP®.

Prospective studies in health demonstrated limited impact of parity on individual metrics.

Conclusion

While routine clinical tests of anorectal function are useful for evaluating FI, identification of characteristics or metrics associated with progressive decline in function may prove useful for detecting individuals at *risk* of FI. Research in healthy populations remains relevant to maintain pace with advancing technology; the concept of normality is still an important part of clinical care.

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Ethics

The following approvals by the Queen Mary University Research Ethics Committee apply to research included in this thesis:

QMREC 2010/74	Chapter 4, Chapter 5
QMREC 2013/12	Chapter 4, Chapter 5, Chapter 6
QMERC 2017/33	Chapter 5, Chapter 6, Chapter 7a, Chapter 7b, Chapter 8b

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— Mummalle—

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List of Abbreviations

3D-HRAM	High definition (3D) anorectal manometry
AI	Anal incontinence
ANOVA	Analysis of variance
ARM	Anorectal manometry
AUC	Area under the curve
BET	Balloon expulsion test; Balloon expulsion time
BMI	Body mass index
CC	Chronic constipation
CCCS	Cleveland Clinic Constipation Score
CF	Cystic fibrosis
CI	Confidence interval
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
CSA	Cross-sectional area
DD	Defaecatory desire
DDV	Defaecatory desire volume
DI	Distensibility index
EAS	External anal sphincter
EAUS	Endoanal ultrasound
EMG	Electromyography
Ep	Elastic strain pressure modulus

EUS	External urethral sphincter
FACL	Functional anal canal length
FI	Faecal incontinence
FLIP	Functional Lumen Imaging Probe
GDPR	General Data Protection Regulation
GERD	Gastro-oesophageal reflux disease
GI	Gastrointestinal
HADS	Hospital anxiety and depression scale
HAPC	High-amplitude propagating contractions
HR-ARM	High-resolution anorectal manometry
HV	Healthy volunteers
IAS	Internal anal sphincter
IAWPG	International Anorectal Working Party Group
IBS	Irritable bowel syndrome
ICC-IM	Intra-muscular Interstitial Cells of Cajal
ICS	International Continence Society
IQR	Inter-quartile range
LARS	Low anterior resection syndrome
LLN	Lower limit of normal
LoA	Limits of agreement
LOS	Lower oesophageal sphincter
MDP	Minimum distension pressure
MeSH	Medical subject headings

MMS	Medical Measurement Systems
MR-FLIP	Magnetic resonance - functional lumen imaging probe
MRI	Magnetic resonance imaging
MTV	Maximum tolerable volume
OASIS	Obstetric anal sphincter injuries
OR	Odds-ratio
P	Pressure
PACSYM	Patient assessment of constipation- symptoms (questionaire)
PFE	Pelvic floor exercise
PIS	Participant information sheet
PNMTL	Pudendal nerve terminal motor latencies
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PTNS	Percutaneous tibial nerve stimulation
QMREC/QMERC	Queen Mary University Research Ethics Committee
QoL	Quality of life
RAER	Rectoanal excitatory reflex
RAIR	Rectoanal inhibitory reflex
RBB	Rapid Barostat Bag
ROC	Receiver operating characteristic (curve)
SCI	Spinal cord injury
SD	Standard deviation
SE	Standard error

SMIS	St Mark's Incontinence Score
SNS	Sacral nerve stimulation
SS	Solid-state
STARR	Stapled Transanal Resection of the Rectum
SUI	Stress urinary incontinence
SW	Slow waves
TASR	Transient anal sphincter relaxation
UI	Urinary incontinence
USA	United States of America
USB	Universal serial bus
V	Volume
WP	Water-perfused

Chapter 1a Introduction

Overview

"Structured, systematic thinking about faecal incontinence (FI) is required to stimulate research which will, in time, improve the quality of life for a significant number of people" (Norton, NICE guideline 2007/2018). Central to this advancement is a modern approach to diagnostic assessment of the anorectum and a contemporary understanding of structural, neurological, and functional norms within healthy people. Treatment of FI in patients who fail conservative measures is largely focused on restoring function where the capacity for anal sphincter closure or effective rectal emptying has become overwhelmed. Diagnostic studies can be used to guide clinical decision making, especially when it comes to selecting a suitable surgical approach [1]. However, large overlap in the range of test results seen in health and disease limit the clinical utility of anorectal functional testing when it comes to recognising pathophysiology [2], especially in patients with physically "less severe", but emotionally debilitating faecal and flatus incontinence [3].

Stratification of normal ranges based on gender, parity, and age have been called for to reduce variability and improve diagnostic accuracy [2,4]. This requires large numbers of healthy volunteers to undergo functional assessment and analysis using a defined and systematic approach. In the future, such an endevour is likely to require collaborative, international effort, which will benefit from a blue-print for how such extensive testing may be performed. Traditionally, tests of anorectal function including anal manometry (ARM), have been approached from the perspective that males and females differ and that parity has an effect on anal sphincter function [5]. However, there is a paucity of information on the impact of demographics on anorectal function using contemporary measures. In particular, while childbirth is considered a major risk factor for women presenting for investigation of FI [6], little is known about the impact of parity on anorectal function in asymptomatic subjects. In patients with more "minor" symptoms, identification of subclinical pathophysiology could aid in halting disease progression.

Techniques like anorectal manometry (ARM) and endo-anal ultrasound remain important clinical tools especially in the assessment of anal sphincter barrier function.

However, it is rarely the case where simply fixing the barrier makes FI symptoms go away [1]. This is because FI is rarely due to a single problem [7] and diagnosis is rarely as simple as performing one test [4]. Continuing to advance our understanding of how the components of the anorectum behave as single entities as well as how they interact with each other is therefore necessary. The Rapid Barostat Bag (RBB) pump (Mui Scientific, Canada) and EndoFLIP (Medtronic) represent novel additions to the diagnostic armamentorium with the potential to provide new insights into rectal and anal dysfunction as well as serving to objectively assess treatment outcomes [8]. However, introducing any new technology into any clinical practice should first be subject to developing an understanding of findings in healthy volunteers (HV).

This thesis hopes to add to the body of knowledge related to FI using established and novel assessment tools to evaluate anorectal function. The following introductory chapter, provides an overview of the anatomy and physiology of the anorectum and the tests that are most commonly performed in the assessment of FI. The literature addressing the causes of FI are considered and these concepts are expanded on with a systematic review of diagnostic outcomes in FI patients in Chapter 2. The significance of lesser known stress FI in terms of symptomology and functional outcomes are also explored. Throughout the thesis, the role of parity and childbirth as the major cause of pathophysiology leading to FI in women is critically evaluated. The first part examines the diagnostic potential of novel biomarkers of internal anal sphincter (IAS) and involuntary external anal sphincter (EAS) function and their potentional to differentiate between nulliparous and parous subjects using retrospective data. The second part considers these biomarkers prospectively alongside traditional measures as part of a comprehensive assessment of anal and rectal function in HV. In the process, data on anal and rectal function is gathered using novel investigation tools in preparation for their implementation into clinical practice and future clinical trials.

PART 1

Anatomy and physiology of continence and defaecation

The anorectum (**Figure 1.1**) forms an integrated functional unit, the purpose of which is to preserve continence and to regulate defaecation [9-11]. In humans, such bowel control is a learned behaviour, which is inherently related to social norms and what is considered to be a convenient time and acceptable location for defaecation [12]. The ability to maintain continence relies on intact rectal and anal sensorimotor neural pathways, a capacious, passively distensible, and evacuable rectal resevoir, and an effective barrier to outflow [13].

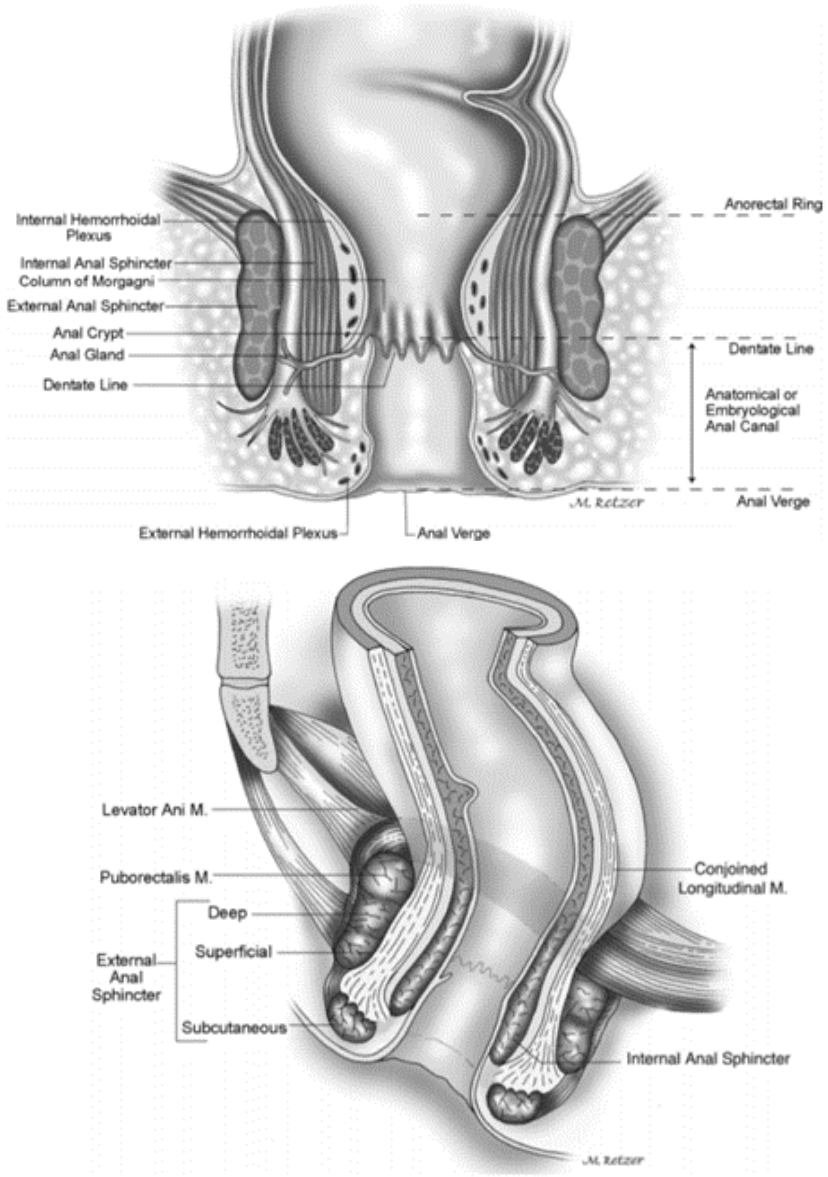


Figure 1.1 Coronal and sagittal views of the muscles of the anal canal and rectum. Reproduced with permission from Jorge *et al* [14].

The rectum

The rectum is a 13-15 cm long, sack-like, intrapelvic viscera which is continuous with the sigmoid colon [11]. It is an expandable organ for the temporary storage of faeces which starts at the level of the lower border of the third sacral vertebra conforming to its ventral concavity to create a natural dorsoventral curvature [15]. Intraluminally, three muscular thickenings known as the Valves of Houston indent the cylindrical structure. Below the middle fold, the rectum becomes slightly dilated at a region known as the rectal ampulla [16]. In contrast to the upper rectum, the rectal ampulla remains mostly empty of stool.

Like the rest of the colon, the wall of the rectum is made up of layers of smooth muscle joined together by connective tissue, and neural and vascular elements [17]. The inner rectal mucosa is lined with columnar epithelium and contains afferent nerves involved in sensing pain, particularly in the presence of inflammation [18]. The submucosa, which also contains the network of nerve fibres, sensory nerves, and parasympathetic motor neurons known as the submucosal plexus (Meissner's plexus), forms a part of the load-bearing structure of the intestinal wall [19] thanks to a concentrated presence of thick collagen fibres. The muscularis propria, the main contractile layer, is made up of an inner circular muscle layer and outer longitudinal muscle layer. Between these muscle layers is the myenteric plexus (Auerbach's plexus), which contains enteric ganglia, sensory neurons, interneurons and sympathetic post-ganglionic fibres [20].

The nerve supply to the rectum is predominantly autonomic (**Figure 1.2**). The upper rectum receives sympathetic innervation from L1, L2, and L3 via pre-ganglionic fibres that synapse in the preaortic plexus and are distributed by branches of the superior hypogastric nerve and fibres from the mesenteric plexus. Parasympathetic (motor) supply is from S2-S4 to the inferior hypogastric plexus via the pelvic splanchnic nerves (nervi erigentes) [9,21-23].

Rectal sensation is conveyed by parasympathetic, rectospinal (visceral) afferents [9,23,24]. Pressure and stretch receptors (intraganglionic laminal endings) in the myenteric plexus signal via pelvic parasympathetic nerves [25,26]. Somatic nerve endings contribute to distal rectal and upper anal sensory function via the pudendal nerve[27] where they 'sample' rectal contents [28]. Such nerves all join the dense plexus of nerves over the sacrum,

making them subject to compression and stretching of the pelvic floor and side walls such as occurs during pregnancy and childbirth (but also probably in prolonged straining and morbid obesity) [12,29].

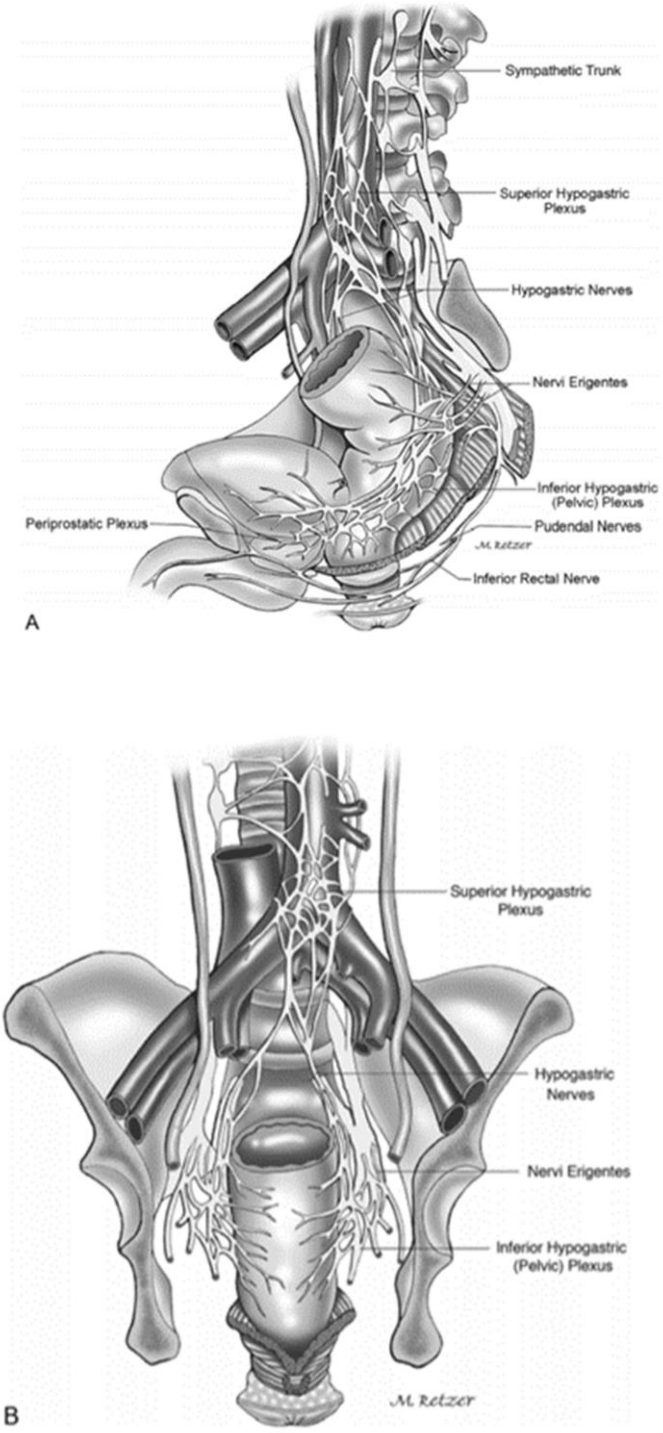


Figure 1.2 Sagittal (A) and coronal (B) views showing the innervation of the anal canal and rectum. Reproduced with permission from Jorge *et al* [14].

The anal canal

The anal canal is a short, 2-5 cm long anteroposterior slit through which stool is released to the outside. There is a slight difference in the reported length of the anal canal, depending on whether an surgical, anatomical, or functional definition is used [30]. The surgical anal canal (~5 cm) extends from the level were the rectum passes through the pelvic visceral aperture, known as the anorectal ring, to the anal margin [31] and approximates to the functional anal canal [30].The dentate line is an internal landmark of circumferentiallly arranged wavy mucosal folds known as the anal valves and which are located about half way up the surgical anal canal [32]. Above each fold are small mucosal pockets known as anal crypts [14]. Internally, the upper part of the anal canal, proximal to the dentate line, exhibits vertical folds, known as the columns of Morgagni. These are superimposed on rich submucosal vascular arrangements known as the anal cushions, which help to seal the anal canal [33]. The upper part of the anal canal is lined with columnar cells continous with the rectal mucosa and is heavily innervated by organised and free nerve endings formed by the inferior branches of the pudendal nerves [34]. These nerves are responsible for distinguishing distension (stretch or pressure) and rectal contents (solid, liquid, or air) to selectively detect and pass flatus [12]. Other afferent signals (temperature, chemical) are carried by parasympathetic nerves [24]. Below the dentate line, the anal canal is lined by non-keratinised, stratified, squamous epithelium continous with perianal skin [32], which receives nervous supply from the perineal branch of S4.

The internal anal sphincter

The IAS is a specialised, thickened (5-6 mm) downward continuation of the inner circular smooth muscle of the rectal wall, which ends with a rounded edge 6 to 8 mm above the anal orifice [14,35]. Morphologically, the muscle is divided into discrete bundles of smooth muscle separated by connective tissue septa, which are stacked next to one another in the transverse and longitudinal directions [36,37]. Consequently, there is a significant amount of connective tissue in the IAS [36]. The IAS is thought to contribute 60-75% of resting tone thus maintaining sphincter closure almost exclusively while at rest. Sympathetic (autonomic) nerve supply to the IAS comes from the hypogastric plexus via the mesenteric plexus. Parasympathetic innervation originates from S2-S4 via splanchic

nerves and through the inferior pelvic plexus [11,21]. Recent studies suggest that intra-muscular Interstitial Cells of Cajal (ICC-IM) within the circular smooth muscle may play an important role in the regulation of IAS tone [36]. The longitudinal muscle of the rectal wall extends downward alongside the IAS before splitting and diverging fanwise at its lower border; its muscle fibres attach to the skin of the anus and perianal region [35].

The external anal sphincter

The EAS is an elliptical cylinder of striated muscle that envelopes the IAS. Although it is historically subdivided into subcutaneous, superficial and deep parts, the EAS is in fact just one continuous muscle mass which extends slightly longer than the IAS, forming the most distal muscular component of the anal canal [11,14]. The EAS is between 6-13 mm thick in adults [38,39] and contributes approximately 15% of resting tone. However, its contribution to anal tone is augmented by voluntary (e.g. in response to urge) or involuntary (in response to abdominal pressure increase) contraction. Accordingly, the EAS comprises of both slow-twitch (type I) and fast-twitch (Type II) muscle fibres, with a predominance of type I, fatigue resistant fibres which allow for long sustained contraction [40,41]. Predominance of slow-twitch fibres is seen especially with ageing due to a preferential loss of type II fibres in most skeletal muscle [42].

The EAS muscle forms part of the superficial layer of the striated pelvic floor, which supports the pelvic organs as well as participating in maintaining continence. As such, there is some intermingling of EAS fibres with the transverse perineii and bulbospongiosus muscles at the perineal body anteriorly [24]. Some authors have suggested that the transverse perineii are integral to (and part of) a purse-string configuration of the EAS [43]. Hence, procedures which damage the transverse perineii (for example from episiotomy, surgery, and obstetric injury) may also result in anal sphincter dysfunction [4,44] even if the 'circular' EAS is spared [43]. Similarly, the central tendon of the perineum or perineal body, which provides stabilisation for pelvic and perineal structures, is prone to injury during childbirth resulting in a weakened pelvic floor susceptible to some types of genitourinary prolapse [24].

The EAS is innervated by the pudendal nerve which arises from the 2nd, 3rd and 4th sacral nerves and the perineal branch of the fourth sacral nerve [11,21,45]. The pudendal nerve is a mixed nerve providing afferent and efferent pathways to the EAS, urethral sphincter,

perineal musculature, mucosa or the anal canal, and perineal skin including the genitalia [27]. It thus plays a crucial role in neurological control of continence. It subserves both sensory and motor functions [46,47]. The pudendal nerve courses around the pelvic brim next to the ischial spines before passing through Alcock's canal on either side of the anal canal. From this point the nerve trunks divide into multiple branches and these are vulnerable to compression, stretch injury and subsequently, denervation of the EAS that could result in muscle weakness and incontinence [48,49]. Before the advent of ultrasound, most cases of FI that were not obviously caused by overt sphincter injury (cloaca, fistula) were believed to be a result of pudendal nerve injury [50].

The puborectalis

Posteriorly, the deepest part of the EAS is intimately related to the puborectalis. The strong, sling-like striated muscle loops around the upper anal canal and attaches to the pubic bone anteriorly demarcating the upper/inner edge of the surgical anal canal (the anorectal ring) [14]. The puborectalis preserves the acute angle between the anal canal and rectum (the anorectal angle) thus providing continuous, sphincteric occlusion-like activity, relaxing only to allow the passage of stool [14,51,52]. Division of the puborectalis during surgery has been shown to result in loss of continence [14]. The puborectalis, together with the illiococcygeous and pubococcygeus muscles (collectively known as the levator ani or pelvic diaphragm), forms the deep layer of the supportive pelvic floor; the anal canal, urethra, and the vagina (in women), transcend through its central levator hiatus, to exit the body. Like the EAS, the puborectalis is innervated by the inferior rectal branch of the pudendal nerve and the perineal branch of the fourth sacral nerve [11,21,45]. Direct muscular branches of the S3 and S4 supply the pubococcygeus and illiococcygeous components of the levator ani [24].

Continence and defaecation

The arrival of faecal material into the rectum results in active relaxation of the rectal wall facilitated by its viscoelastic properties [53]. A normal rectum can accommodate significant increases in volume (distension) while maintaining low intraluminal pressures [54]. Meawhile, cyclical, tonic contraction of the internal and external anal sphincters maintain a positive anal-to-rectal pressure gradient and the mucosal seal provided by the anal cushions and an acute anorectal angle created by tonic

puborectalis contraction, aid in sphincter occlusion so that continence is not threatened [9,55]. As the rectum reaches its elastic limit, the rectal wall becomes more resistant to stretch eliciting more regular contractions of the rectal wall [56]. Rising intrarectal pressure prompts the individual of the need to defaecate. Rectal volume tolerance varies more than 10-fold between healthy subjects [57] suggesting wide inter-individual variation in viscoelastic properties, sensory feedback mechanisms, and organ size.

Rectal distension (by stool or gas) evokes reflex IAS relaxation, known as the rectoanal inhibitory reflex (RAIR), which is an intramural reflex mediated by the myenteric plexus and modulated by the spinal cord [9,11]. As a result, rectal and upper anal canal pressures are equalised to bring rectal contents into contact with free nerve endings in the upper anal canal mucosa allowing 'sampling' of rectal contents [55]. A more persistent distal contraction, the rectoanal excitatory reflex (RAER), further compensates for relaxation of the IAS[58,59].

Transient anal sphincter relaxations (TASRs) may occur with or without conscious awareness. To compensate, the EAS contracts transiently to maintain the pressure gradient so that sphincter pressure still exceeds rectal pressure and continence is unthreatened. The involuntary contractile response of the EAS to sudden increases in intra-abdominal pressure is a polysynaptic sacral reflex [60] and compensatory guarding mechanism. It allows the anorectal pressure gradient to be maintained at all times, including instances like coughing, sneezing or exercise [61]. In patients without overt neurologic signs or evidence of spinal damage, an abnormal "cough response" may be a sign of neuropathy [62].

If the passage of stool is undesired at the time of urge (e.g. for social reasons), EAS contraction can be voluntarily enhanced to delay defaecation by closing the anal canal and forcing the sampled contents back into the rectum [24]. Alternatively, defaecation proceeds through contracting the abdominal muscles to increase rectal pressure and conscious relaxation of the EAS to allow stool passage. Cortical inhibition of puborectalis contraction straightens the anorectal angle and contraction of the lower colon and rectum aid in the complete evacuation of rectal contents [24]. The EAS contractile reflex is most likely inhibited during defaecation (bearing down/Valsalva) by descending inhibitory pathways [62]. Though some straining is usually necessary to initiate

defaecation, the need for propulsive effort probably varies according to stool form (soft vs hard) and location (upper vs lower rectum) [47]. Following defaecation, IAS, EAS and puborectalis resting tone is re-established to close the anal canal once again. **Figure 1.3** summarises the main continence mechanisms described above.

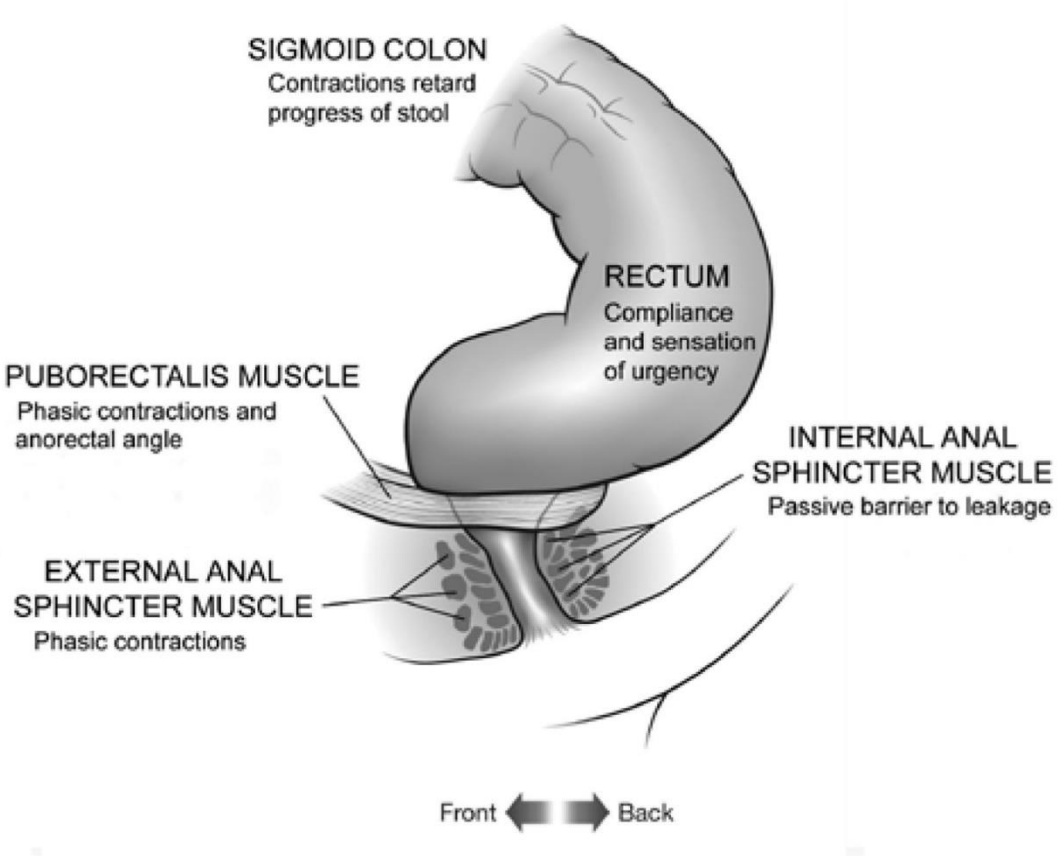


Figure 1.3 Continence mechanisms; reproduced from Whitehead and Schuster[63].

PART 2

Faecal incontinence

Definition

The unintentional loss of solid or liquid stool (FI) is an emotionally and physically debilitating condition with prevalence rates ranging between 7 to 15% in community-dwelling women [3] and comparable prevalences in men [64,65]. Wide variation in prevalence rates between studies reflect differences in data collection methods and the definition of FI used [66]. Symptoms are more frequently reported by older people [65] and can vary in severity ranging from small amounts of liquid leakage several times a month to daily gross incontinence[3]. While several validated scoring systems are available to assess symptom severity (e.g. Wexner, St Mark's Incontinence Score, FISI, Modified Manchester Health Questionnaire etc), no established threshold for defining significant FI exists [67].

Bowel disturbances, such as diarrhoea or constipation [68,69], and/or anorectal disturbances (i.e. anal sphincter weakness, reduced rectal compliance, increased or reduced rectal sensation- see later) have the potential to cause FI [46,70]. The type of FI experienced has previously been considered to be associated with certain pathophysiology. Passive incontinence i.e. leakage which occurs in the absence of a conscious need to defaecate, may be associated with IAS abnormalities [71-75] while urge incontinence, the inability to defer defaecation long enough to reach the toilet [76], has been associated with an abnormal EAS or reduced rectal capacity [72,77,78]. However, studies examining these relationships have shown conflicting results and typically exclude patients with mixed symptoms [79]. Other forms of incontinence regularly encountered in clinical practice, but poorly described in literature, include post-defaecatory leakage or soiling [4,77] and stress FI [77].

Stress FI refers to leakage upon coughing, bending, or walking and is associated with voluntary EAS muscle weakness in severe urge incontinence [77]. However, because FI symptoms tend to develop progressively over time, with the exception of post-partum FI associated with obstetric anal sphincter injuries (OASIS) [80], minor symptoms, such as flatus incontinence usually in response to stress, may have been present for a long time before patients reach the point of physiological investigation. Indeed, the prevalence of

FI in community studies increases when flatus incontinence is considered [81,82]. Because the resistance required to prevent gaseous leakage is less than that required to prevent leakage of liquid or solid stool, the pathophysiology associated with stress FI may be separate to that of urge incontinence or appear sooner. It may also be that the passage of gas occurs through shear rather then laminar flow in faeces as a results of its properties as a non-Newtonian fluid [83,84]. With appropriate measures, identifying subclinical pathophysiology in patients with flatus incontinence could enable preventive strategies (such as avoiding further vaginal deliveries or straining, optimising stool consistency, and targetted screening for FI) to be taken in certain risk groups helping to preserve continence in the long term.

Pathoaetiology of incontinence

The causes of FI are summarised in Table 1.1 and can broadly be divided into conditions causing anal sphincter weakness, anatomical disturbances of the pelvic floor, inflammation, central nervous system (CNS) disorders and bowel disturbances [3,61]. A complete review of all causes and their related pathophysiology can be found elsewhere [85].

Anal sphincter weakness	
Injury	Obstetric trauma, iatrogenic trauma
Non-traumatic	Scleroderma, IAS degeneration
Neuropathy	Stretch injury, obstetric trauma, diabetes mellitus
Anatomical disturbances of the pelvic floor	
	Fistula
	Rectal prolapse
	Descending perineum syndrome
Inflammation	
	Crohn's disease
	Ulcerative collitis
	Radiation proctitis
	Anorectal infection
Central nervous system disorders	
	Dementia
	Stroke
	Brain tumours
	Spinal cord lesions
	Multiple system atrophy
	Multiple sclerosis
Bowel disturbances	
Diarrhoea	Irritable bowel syndrome, post-cholecystectomy diarrhoea
Constipation	Constipation with or without faecal impaction/overflow diarrhoea

Table 1.1 Causes of FI. Reproduced with permission from Bharucha *et al*[3].

Pathophysiology

Since continence to faeces is dependent on sphincteric and rectal anatomy, recto-anal sensation, and rectal accomodation [47], disruption to any one of these mechanims may lead FI. However, pathophysiology is most often multifactorial [7]. Alterations in stool consistency and delivery (as a result of colonic dysfunction) [86-88] and impaired central processing of sensory information along with poor mobility may also lead to or exacerbate FI symptoms [3,61].

Rectal capacity, impaired accomodation, and rectal sensory dysfunction

Inflammatory bowel disease, tumour infiltration, rectal surgery and spinal cord injury are some of the causes associated with loss of rectal compliance or rectal wall accomodation of stool [46,89]. As a result, small volumes of stool in the rectum can cause big increases in pressure that can overwhelm anal resistance [86]. Even a small capacity rectal resevoir without evidence of pathology can predispose to FI especially if associated with anal weakness [90].

Increased low and high amplitude pressure waves in the colon and rectosigmoid have been observed in FI [87,88]. Similarly, altered rectal motility in the form of increased rectal motor complexes in response to distension may occur in FI patients [7,23]. Amitryptiline, used in patients with irritable bowel syndrome (IBS) has been shown to decrease rectal contractions [91] with the potential to reduce the likelyhood of FI episodes associated with urgency.

Hypersensitivity, or heightened sensory perception of rectal distension, is common in FI patients with urgency [92]. It may result from reduced rectal compliance, a primary afferent dysfunction (most likely a peripheral neuropathy) or both [92,93]. In another study, hypersensitivity was associated with reduced rectal compliance, repetitive rectal contractions during rectal distension, EAS weakness (see below), and exagerated anal sphincter relaxation (RAIR) during rectal distension [7]. Indeed, rectal hypersensitivity is common amongst women with a history of obstetric injury and sphincter defects, in whom anal sphincter pressures are likely to be low as a consequence. Consequently, Chan et al [92] proposed that hypersensitivity in FI patients with anal sphincter weakness may represent a learned response to an incompetent sphincter led by a fear of incontinent episodes, rather than afferent nerve or rectal wall dysfunction. Similarly,

high rates of depression and somatisation in patients with functional gastrointestinal (GI) problems may modulate cortical processing of sensation leading to hypersensitivity in the absence of pathophysiology [94].

Conversely, 10% of FI patients had reduced or blunted rectal sensitivity in one study [95] with prevalence rates generally higher in men than women [96]. Hyposensitivity in FI may be related to delayed sensory perception of urge in relation to RAIR [97], which allows stool to enter the anal canal and leak before the EAS contracts [7,98], an overly capacious rectum (megarectum), or increased rectal compliance (lax rectum) with, or without, a co-existent afferent abnormality [99,100].

In patients with co-existent constipation and/or faecal impaction, FI due to overflow may be considered [101]. Overflow incontinence is more common in elderly persons [102], physically and mentally challenged individuals, and children with FI [100]. However, classic overflow is probably only a small factor in the prejudical effects of constipation as a risk factor for FI. Rather, in the majority of patients, it is simply the presence of faeces in the rectal ampulla (which is normally empty) that poses a problem when continence mechanisms are impaired. The coexistence (of FI and constipation) is becoming increasingly recognised in adult populations [68,69] in whom findings such as functional and structural evacuation disorders and rectal hyposensitivity are common [68,99]. In addition, certain analgesics (particularly opiates) and antidepressants may also impair rectal sensation leading to FI [46]. Vollebregt et al [103] recently showed that opioid users had a significantly higher St Marks incontinence score compared to non-opioid users ($p<0.001$). Opioid usage for moderate-severe pain was also significantly more likely to have rectal hyposensitivity (odds ratio 1.74 [95% CI 1.23-2.46]; $P = 0.002$).

Anal sensory dysfunction

Sensory feedback is not only required to provide warning of imminent need to defaecate, but also to discriminate between formed stool, liquid stools, or flatus; an impaired sampling reflex may predispose to FI [104,105]. Alterations in anal sensory function can occur in relation to pressure, temperature, distension or nociception [34].

Anal sphincter weakness

Anal sphincter weakness, characterised by anal motor dysfunction affecting the IAS, EAS or both, may result from structural or neurological damage to the anal sphincters (**Figure 1.4**). Typically, sphincter weakness in FI patients is related to obstetric or iatrogenic trauma [6]. For example, reduced resting and/or squeeze pressures, which represent IAS and EAS dysfunction respectively, were found most, but not all, FI women following anal sphincter injury [106]. However, not all patients with structural defects have reduced anal tone. Conversely, many patients with reduced tone do not have evidence of sphincter defects.

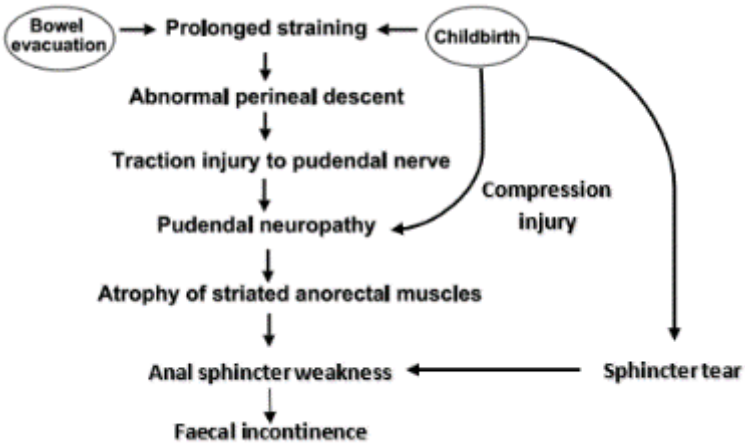


Figure 1.4 Mechanisms of injury leading to FI (modified from Sultan and Nugent[107])

Neurogenic injury, which can occur at any level of the axis extending from the CNS to peripheral nerve endings in the anal sphincter itself [108,109], has the potential to cause sphincter weakness [110]. Historically, pudendal neuropathy and subsequent denervation injury was thought to be the major cause of anal sphincter weakness in FI patients [111]. Pudendal nerve injury during childbirth can occur as a result of stretching of the nerves during elongation of the birth canal or direct trauma during passage of the foetal head [46]. The effects of compression injury within Alcock's canal were shown to be cumulative with subsequent deliveries [112,113] and often asymmetrical [114]. As previously described, pudendal neuropathy also has the potential to develop following repeated stretch injury due to chronic straining at stool[111,114,115] or pelvic surgery (including hysterectomy) [85].

Risk factors for FI

Previously identified risk factors for FI, based on community studies, include increasing age, diabetes mellitus, co-existent urinary incontinence, frequent and loose stools, poor health status, multiple comorbidities, and obesity [47,65,116].

Management of FI

In the majority of patients, symptoms of FI are resolved by conservative measures without the need for specialist evaluation [117]. Such measures may include dietary and lifestyle advice (such as optimising intake of dietary fibre, avoiding foods with laxative effects and intolerances, or pelvic floor exercise/bowel retraining), medications to increase stool bulk and anti-diarrhoeals e.g. loperamide or codeine [118,119]. In patients with co-existent issues of rectal emptying, laxatives, suppositories or enemas, or biofeedback may be employed [120-122].

In patients failing conservative measures, specialist referral may be warranted. Data are lacking on how many patients reach a specialist, but it is probably about 1 in 10 of those seeking any form of medical intervention. In this group, a thorough clinical history and physical examination will document symptoms and examination findings and depending on these, whether the patient requires specialist diagnostic tests (see below). Focus should be placed on seeking symptoms and signs referable to the urinary tract and to pelvic organ prolapse [6,46,89,112].

While the utility of physiological investigations has been questioned [123], others have demonstrated a clear benefit of investigation in aiding clinical decision making or predicting treatment response in patients with functional GI disorders [124-127]. Treatment options beyond conservative measures include physician- or nurse-led physical and behavioural therapies e.g. biofeedback, pelvic floor muscle therapy, and these may be supplemented with adjuncts such as forms of direct neuromuscular (galvanic) stimulation or non-invasive forms of neuromodulation e.g. percutaneous tibial nerve stimulation (PTNS) [128,129]. Surgery tends to be reserved for when other measures have failed although there is a place for first-line operations such as cloacal repair when there is an obvious physical breach to the barrier. Current surgical options (in very brief) include sphincter repair (sphincteroplasty) [130], sacral nerve stimulation [131-133], injection of bulking agents [134] and colostomy [89,135,136]. Previous, now abondoned procedures,

include various forms of sphincter augmentation using inflatable prothesis, rings of magnets [137] and native muscle e.g. graciloplasty [138].

Decision making for one of more of these procedures is uncertain and complicated. Diagnostic investigations are considered helpful in this context by defining the surgical target (only the starting point in decision making). Beyond this, the literature is typified by claims based on post-hoc analyses of low-quality cohort studies that were neither designed nor powered to determine covariates of treatment response. Examples include: normal EAS electromyography (EMG) has been shown to be a significant predictive factor for successful outcome of sacral nerve stimulation (SNS)[127] while alteration of rectal volume and compliance may be used to evaluate treatment success[124,139]. In patients with sphincter defects, those without pudendal neuropathy had a greater success rates following sphincter repair[140], although delayed latencies using the St Mark's electrode are not generally considered useful due to limitations in measuring pudendal nerve terminal motor latencies (PNMTL)[2]. Quite aside from the decline in use of tests like pudendal EMG, reliance on these studies to guide treatment is unproven.

PART 3

Diagnostic assessment

The aim of diagnostic tests is not to confirm a diagnosis of FI, but rather to define pathophysiology and (hopefully) direct treatment based on an in-depth assessment of anorectal sensorimotor function. Such investigations are complemented by imaging tests, which assess the structural integrity and sensorimotor function of the anorectal unit and pelvic floor [2]. Often, more than one pathophysiological finding is present[7]. The latest advances in the evaluation of anorectal function have been outlined in a recently published consensus statement by the International Anorectal Working Party Group (IAWPG) [2]. The main outcomes of the consensus are summarised in **Table 1.2**, which lists the most commonly performed diagnostic investigations judged to have good or some recognised clinical utility and the resulting diagnostic outcomes deemed to be of either major or minor importance for describing anorectal pathophysiology. In addition to those listed, the report recognises two novel tests of anorectal function ('rapid' barostat and the functional lumen imaging probe), which will be evaluated in detail as part of this thesis. To date, there has been limited clinical application of either method partly due to a lack of normal ranges [2,141].

While tests of evacuation (balloon expulsion, and barium and magnetic resonance defaecography) are of fundamental importance for the evaluation of patients with symptoms of obstructed defaecation, they are often of limited use in patients with FI *in isolation*. For this reason, defaecography is not described in detail within this thesis. A detailed description of defaecographic techniques and common abnormalities can be found elsewhere [142]. In addition, defaecographic findings from our laboratory in patients with chronic constipation (some of whom also had FI) have been published recently by Grossi et al [143].

Function	Investigation	Findings of major or minor clinical significance
Anus		
Motor	Anorectal manometry (conventional, high-resolution, 3D)	Anal hypotonia Anal hypertonia Anal hypocontractility
	Electromyography	Reduced of abnormal myogenic activity
Structure	Endoanal ultrasonography	IAS defect
	Transperineal ultrasonography	IAS degeneration or atrophy IAS hypertrophy
	Endoanal or pelvic MRI	EAS defect EAS atrophy
Rectum		
Sensory	Balloon distension	Rectal hypersensitivity Rectal hyposensitivity
Motor, sensory, and structure	Rectal barostat	Rectal hypersensitivity Rectal hyposensitivity Rectal hypercompliance Rectal hypocompliance Increased rectal capacity Decreased rectal capacity
Anorectal unit		
Motor	Anorectal manometry (conventional, high-resolution, 3D)	Pelvic akinesia (type IV dyssynergia) Poor porpulsion with dyssynergia (type II dyssynergia) Normal propulsion with dyssynergia (type I or III dyssynergia) Anorectal areflexia
	Balloon expulsion	Prolonged expulsion time
Motor, sensory, and structure	Barium defaecography	Obstructing intussusception Retaining rectocoele Rectal prolapse Enterocoele or sigmoidocoele Cystocoele Vaginal vault prolapse Excessive perineal descent Impaired rectal emptying Impaired anorectal angle opening
	Magnetic resonance defaecography	

Table 1.2 Commonly performed tests of anorectal physiological function with 'good' or 'recognised' clinical utility. Adapted with permission from Carrington 2018

Anorectal manometry

Anorectal manometry (ARM) was first used for assessing patients in the 1960's [144]. Since then, manometry has become the best established and most commonly performed investigation of anorectal function in FI patients. An evaluation of anal resting pressure (indicative of IAS function[71]), squeeze pressure (indicative of voluntary EAS function) and establishment of rectal sensory thresholds are key measurements performed during ARM which are used to establish pathophysiology in FI patients[145,146]. Low resting and/or squeeze pressures indicate anal sphincter weakness while increased or decreased sensory thresholds to rectal balloon distension help to identify patients in whom impaired sensory processing or rectal accomodation may be the cause of faecal leakage. Other dynamic manouvres such as an evaluation of the cough reflex and attempted defaecation (bearing down) are recommended as part of the manometry protocol (**Figure 1.5**), although their clinical utility is currently either unknown or heavily debated[146].

ARM can be performed using air-charged, water-perfused (WP) or solid-state (SS) catheters[147]. Traditionally, ARM has been performed using a limited number of pressure sensors to measure anal canal pressure, depicted as line tracings of pressure over time, at various levels within the anal canal. So-called 'conventional' manometry may be performed using stationary, rapid pull-through, or station pull-through techniques[9]. Since 2002, the recommended number of pressure sensors incorporated onto a manometry catheter has been >6, spanning the length of the anal canal[145]. To ensure that the highest anal canal pressure within in the anal canal is measured, irrespective of the pressure sensors' location, some catheters may incorporate a sleeve sensor. Although conventional ARM is still performed routinely in some centres[148], most modern gastrointestinal (GI) physiology laboratories have progressed to using either WP or SS high-resolution (HR-ARM) or high-definition (3D) manometry catheters.

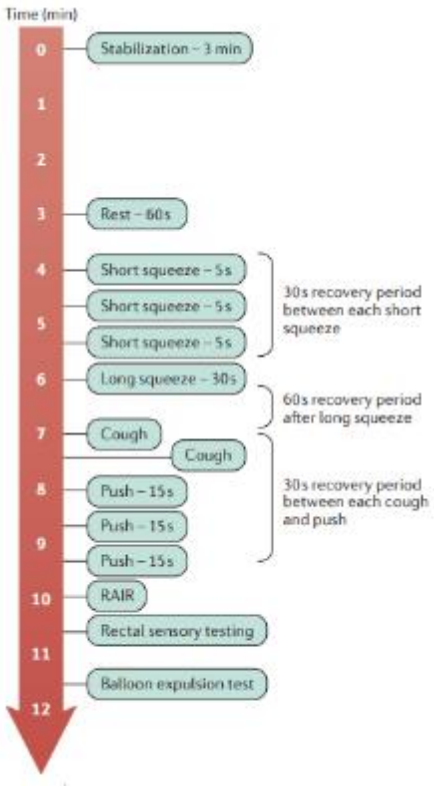


Figure 1.5 IAWPG protocol for anorectal manometry; reprinted from Carrington et al [2]

High-resolution/high-definition anorectal manometry catheters incorporate an increased number of closely spaced pressure sensors (>6, but typically between 8-12 pressure sensors for HR-ARM and up to 257 independent pressure sensors for 3D-ARM). Improved data visualisation and analysis is performed by specialist software in which pressures at each sensor are represented using colour topography with interpolation between sensors used to provide a continous depiction of pressure along the length of the catheter (**Figure 1.6**). Thus, objective, dynamic assessment of anal sphincter pressures, anorectal reflex activity (RAIR), and rectoanal coordination is facilitated, while a balloon attached to the tip of the probe may be used to evaluate rectal sensory thresholds [146]. However, despite advantages such as improved artefact recognition and the possibility to observe certain qualitative pressure phenomena not appreciated using conventional ARM [5], the clinical diagnostic benefits of HR-ARM/3D-ARM in FI patients have been limited. Given the high costs associated with the technique compared with conventional ARM, some have questioned its role in the routine clinical evaluation of the FI patient [123].

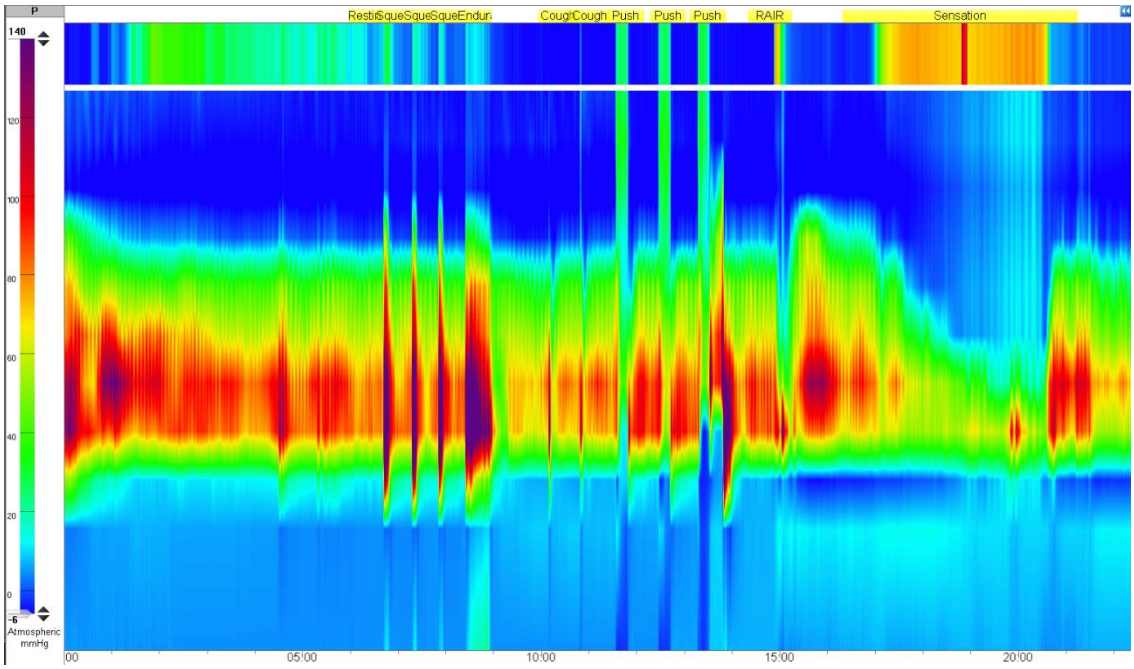


Figure 1.6 HR-ARM pressure trace in a healthy volunteer.

As with most clinical tests, interpretation of ARM is dependent on knowledge of normative ranges observed in HV; reference values have been described in adults using HR-ARM [5,149-152] and 3D-ARM [153-155] as well as conventional methods (e.g. in first pregnancy [156]; for summary see Felt-Bersma 1990 [157]). However, pressure measurements tend to vary by the technique, catheter-type, patient positioning, and type of interaction while performing the test [151,158-166]. Therefore, great care must be taken when choosing normative ranges by which to differentiate between patients and controls, or if comparing data between centres [167]. However, generation of normal values based on sufficient numbers of HV to account for demographic differences are often not feasible within individual trials or GI physiology services wishing to interpret patient findings.

Anal sphincter pressures

In general, FI patients have lower pressures compared with healthy volunteers [71-75,90,168], however there is considerable overlap in the range of pressures seen in health and disease [7,90,166,169,170] limiting sensitivity and specificity of the technique. Anorectal pressures in men tend to be higher than in women [5,154,171,172], with some studies suggesting a further influence of parity on anorectal function in women [5,173]. A negative impact of ageing on anorectal pressures has been associated with a decline in both IAS

and EAS function in some studies [154,174,175], but not in others[5]. More recently, a significant effect of BMI on resting and squeeze pressures has been observed [171].

Functional anal canal length

Functional anal canal length (FACL) using conventional manometry, was defined as the length over which anal pressure exceeds rectal pressure by >5 mmHg [176,177] or the length of the anal canal over which pressures are greater than half of the maximal pressure at rest [61]. A short FACL has been associated with FI [178], particularly in women [30,179]. Using a 20 mmHg pressure contour to define FACL using HR-ARM, Vollebregt et al [30] demonstrated a significantly shorter FACL in women with FI compared to healthy volunteers. However, the observation of a short FACL provided little additional information on the pathophysiology of FI, since it was never an isolated finding (i.e patients with short FACL also had either hypotonia or hypocontractility). Similarly, no significant benefit of measuring FACL has been observed in other studies [153,180]. While measurement of FACL is not advocated by recent guidance for performing manometry [146], it is worth noting that a shorter FACL in older persons with anal weakness may be related to IAS atrophy [30]

Cough reflex

An impaired cough reflex or cough response has been previously observed in cauda equina and patients with spinal cord defects [181]. Thus, an abnormal cough reflex has been suggested to indicate evidence of a peripheral neuropathy or a subclinical pathophysiology in FI patients with otherwise normal sphincter pressures [62]. Meanwhile, a normal cough response in the presence of weak squeeze may indicate poor voluntary squeeze effort, as opposed to impaired EAS function [145]. Conversely, anecdotal evidence by members of the International Anorectal Working Party Group (IAWPG) members suggests that the cough response may be used for describing a more severe phenotype of anal hypocontractility [146]. Measures used to define an abnormal/normal cough response are discussed in detail in Chapter 3. However, given the ambiguity of purpose, it comes as no surprise that despite most centres responding to an international survey of manometry practices indicating that they routinely performed cough as part of the manometry protocol, as with other measures, there was limited coherance between respondents regarding analysis[148]. According to

accepted guidelines[62,145,146], both rectal and anal maximum pressures during cough should be reported. However due to there being virtually no evidence to support its use as a specific measure of anorectal function, interpretation of the cough response does not form part of the recently published diagnostic classification, known as The London classification of anorectal physiological dysfunction[146].

Attempted and simulated defaecation

Historically, ARM has also been used to identify impaired patterns of recto-anal coordination during attempted defaecation ('bearing down', 'pushdown', or 'Valsalva' manouvres)[182]. Previously, it has been erroneously assumed that obstructed defaecation is rarely observed in FI patients [4,183] recommending that evaluation of attempted defaecation was not required in FI patients [145]. The theory at the time was that the low sphincter pressures seen in patients with FI would protect against dyssynergia. In patients with functional constipation, dyssynergia, or the inability to coordinate the abdominal, rectoanal, and pelvic floor muscles during defaecation [182,184], has been observed in up to 94% of patients [142]. Due to increased recognition of co-existent constipation symptoms in FI patients [68,69], these manouvres combined with an assessment of simulated defaecation (balloon expulsion test [2,185]), remain a part of the recommended manometry protocol published by the IAWPG [146]. For example, Carter et al [186], observed dyssynergia in just over half (51%) of patients with both constipation and FI symptoms. In another study [183], 32% of patients with FI undergoing ARM had abnormal BET (defined as >2 min). In these patients, abnormal BET was related to a sensation of incomplete emptying and abdominal pain, significantly lower rectal propulsive pressure on manometry, and a higher threshold for first sensation.

Impaired rectoanal co-ordination during defaecation may result in incomplete evacuation and subsequent FI due to overflow of post-defaecation residual stool[4]. Indeed, Rao et al [4] demonstrated a dyssynergic pattern of defaecation in 72% of patients with faecal seepage. However, in the same study, a proportion of both FI patients (30%) and HV (16%) also exhibited an abnormal relationship between rectal pressure during straining and anal residual pressure. In another study by the same author [187], 36% of HV demonstrated a dyssynergic pattern on traditional manometry, while attempting to expel a balloon in a recumbent, left-lateral position. More recently, Grossi et al [166]

demonstrated that 87% of normal volunteers who underwent HR-ARM as part of a research protocol, exhibited an abnormal pressure pattern during attempted defaecation refuting the concept that either a failure of anal relaxation or paradoxical anal contraction are of pathophysiological significance.

Anal slow wave and ultraslow wave activity

At rest, rhythmic undulations in pressure can be observed with both conventional and high-resolution methods. Ultraslow waves, defined as repeated pressure oscillations occuring at a frequency of 1-2 cycles per minute [188], are mainly associated with anal fissures [189]. Meanwhile, anal slow wave activity [189-191] is defined as undulations with pressure peaks occuring 6 to 20 times per minute at amplitudes between 5 to 25 mm Hg [192]. Anal slow waves reflect myogenic activity of the IAS [193] and may be important for tone generation and the maintenance of high anal resting pressure. Using HR-ARM, Carrington et al [190] identified anal slow waves at frequencies of 9-19 cpm in 44% of HV. In another study, qualitative and quantitative assessment of anal slow waves in men with low anterior resection syndrom (LARS: a syndrome post cancer surgery that is typified by incontinence and other defaecatory symptoms) showed altered anal slow wave pressure activity compared with health [191]. These studies suggest that anal slow wave analysis may be clinically useful in some patient groups, but further validation of novel analytical approaches and relative contributions of sex and human baseline variance are required[191].

RAIR

Distension of the rectum with air induces a reduction in anal pressure, associated with relaxation of the IAS (RAIR)[9]. A normal response is indicated by a reduction in maximum anal pressure in response to rapid rectal distension[146]. Absent RAIR (termed anorectal areflexia[146]) may be seen in Hirschsprungs disease[194], while differences in reflex parameters have been observed in some patients with FI compared with controls[59,195,196]. Quantifiable parameters of RAIR include the degree of relaxation and duration of the response which correlate with distending volume; the volume required to elicit a response may vary[9].

Balloon distension

The principle of distending the rectum to evoke perception of filling was first introduced by Goligher and Hughes in 1951[22]. Today, rectal distension performed using a simple syringe and balloon catheter assembly is considered an effective, economical, and convenient technique for evaluating rectal sensory thresholds in everyday clinical practice[9]. Evaluation of sensory thresholds may reveal rectal hypersensitivity (heightened sensation) or rectal hyposensitivity (diminished sensation) in comparison to the range of values typically seen in health[146 62]. Typically, threshold volumes (or pressures) for first sensation, desire to defaecate, and maximum tolerable volume (MTV) are recorded[158,197,198]. Distension may be continuous (ramp) or intermittent (phasic[199] or stepwise[200]) and performed manually (using a hand-held syringe) or pump-assisted using air or water[158,198].

The type of distension, distending medium, speed of inflation, distance of balloon from the anal verge, and position of the patient are known to affect distension[9]. Furthermore, structural or biomechanical properties, such as size or elasticity (compliance) of the rectum, may influence sensory thresholds[201,202]. Thus, "rectal perception of distension" may not be reflective of afferent nerve function[203], which might occur after nerve or spinal injury[204]. For example, in a large or compliant rectum, elevated sensory thresholds may reflect increased rectal size and inadequate stimulation rather than dysfunction of the rectal afferent pathway itself[205].

Nevertheless, rectal distension by a balloon or bag shows good reproducibility, particularly at higher volumes[11,199,206], and may have the potential to assist with clinical management of patients[2,126,145,158].

Balloon distension, especially when performed as part of anorectal manometry using an integrated balloon, may include a pressure sensor inside the balloon and recording of intra-balloon (rectal) pressure changes in response to distension. This enables the evaluation of rectal compliance, provided the inherent elasticity of the latex balloon is corrected for [9]. However, errors caused by elongation of the balloon and geometric assumptions of the rectum may also influence results and derivation of data regarding tone, tension and distensibility leading to further inaccuracy [207]. Hence, compliance

measurement is not routinely recommended during sensory testing using balloon distension[146].

Electromyography

Anal electromyography (EMG) is less commonly performed as part of routine assesment of anorectal function[148], except in patients with known or suspected neurological disorders[2]. Anal EMG can be performed using needle, surface, or anal plug electrodes to map out sphincter defects, determine striated muscle function, and assess for neural injury[146]. Assessment of denervation–reinnervation potentials (indicative of neural injury [208]) is subject to specialist expertise in neurophysiology for correct data interpretation and requires multiple needle insertions that may be uncomfortable for the patient. On the other hand, the use of surface electrodes is relatively straight forward for obtaining evidence of muscle contraction (for example during squeeze), however motor potentials measured are not specific to a particular muscle [209]. When used in conjuction with pressure measurement, surface EMG can provide verification of anal sphincter activity during squeeze, cough, or Valsalva.

Anal electromucosal sensitivity

Assessment of mucosal electrosensitivity provides quantitative measurements of anal epithelial sensitivity, which may be reduced in FI [105,210,211]. A catheter-mounted bipolar ring electrode (**Figure 1.7)** is inserted into the anal canal and steadily increasing current is passed through the electrode until sensation (typically prickling or tingling) is noted by the patient [9,212]. Threshold sensations increase with age [9,105] and differ between the upper and lower anal canal with the distal anal canal being more sensitive [105,156]. Anal electromucosal sensitivity has been shown to be an accurate and repeatable quantitative test of the anal sensation [213] and is the preferred quantitative test of anal sensation [9,214]. Clinically however, the test has fallen out of favour in routine practice due to limited clinical value [9,158].

Figure 1.7 Bipolar ring electrode mounted onto a flexible catheter (Gaeltech, Isle of Skye, UK). White, horizontal markings indicate 1 cm intervals from the centre of the two electrodes.

Endoanal ultrasonography

Endoanal ultrasonography [215] is the most important form of imaging in FI [216]. Anal imaging studies are routinely performed in FI patients to evaluate morphological integrity of the anal sphincters, to guide primary/secondary sphincter repair, and/or to inform planning of subsequent deliveries [217-219]. Endoanal ultrasound (EAUS), performed using a rigid endoscope with a rotating crystal (3-20MHz) to provide 360 degree axial views of the anal sphincters, is simple to perform and generally well tolerated by patients. Continous image capture as the crystal is withdrawn from the anal canal can be digitally stiched together to create multiplanar 3D images on some systems [2].

IAS and EAS structural integrity are assessed by visual inspection of the integrity of hypo- and hyper-echoic structures respectively[215] (**Figure 1.8**). While sphincter defects (which may or may not be associated with functional impairment) are common in FI patients [90], discontinuity of the IAS or EAS in isolation may not be conclusive, since up to 10% of assymptomatic women show occult sphincter defects [220,221]. Subepithelial tissues, the conjoined longitudinal muscle, transverse perineii and puboanalis may also be visualised. Anal sphincter thickness may be measured using 2D or 3D images, although the latter may provide better distinction from adjoining structures especially of the EAS [221].

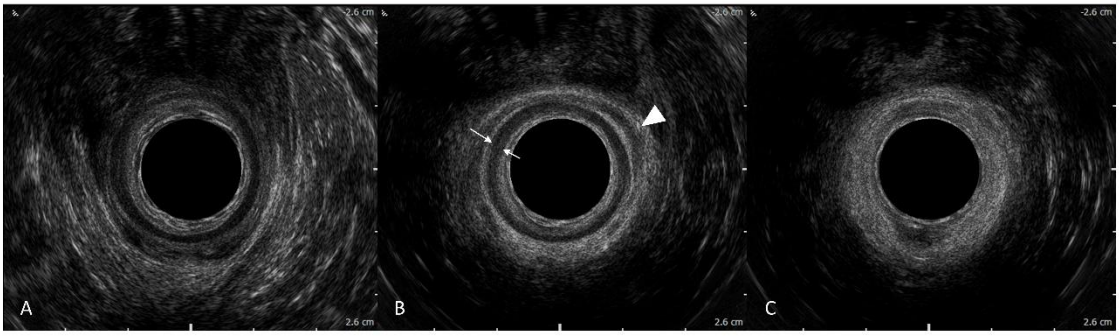


Figure 1.8 Representative normal endoanal ultrasound at high (A), mid (B), and (C) low levels. The IAS (white arrows) appears as a hypoechoic ring in high and mid-anal canal. The EAS (arrowhead) is shown as a hyperechoic ring surrounding the IAS. At low level (C) only the EAS is visible. The puborectalis sling is shown (C).

According to a recent consensus statement by the IAWPG [2], the IAS can be classified as normal or have a defect [90], atrophied (identified by diffuse thinning of the sphincter (≤1mm thick) and/or degenerate[222] or hypertrophied [223,224]. Defects and atrophy are common in FI patients [72,73,90] while hypertrophy is associated with intussusception or prolapse [223,225]. Similarly, the EAS can be characterised as normal or diseased based on continuity or intrerruption of its fibrillar echotexture manifesting as focal thinning, scarring or atrophy [215]. EAS disruption is a characteristic feature of 3rd and 4th degree tears [226,227] and is associated with anal hypocontractility [96,228,229]. EAS defects were present in up to 68% of individuals presenting with FI in one study [96].

Transperineal or trans-vaginal ultrasound may also be used to detect anal sphincter defects or to assess urinary incontinence, voiding difficulties and pelvic organ prolapse symptoms [230]. These tests, together performed together with endoanal ultrasound (total pelvic ultrasonography) may help to assess symptoms related to evacuatory dysfunction. In addition, pubovisceral avulsion (abnormal insertion of the levator ani on the inferior pubic ramus [231], an important pathophysiological mechanism for pelvic organ prolapse and FI, may be identified [2]. Endoanal and/or pelvic MRI are less widely available, but provide an alternative imaging modality in specialist centers [230].

Rectal barostat

Rectal resevoir function is dependent on adequate rectal capacity, relaxation of the rectal wall and appropriate sensitivity to inform the individual when the continence mechanism is threatened [232] and is best evaluated by barostat techniques, which

measure digestive tone and sensory thresholds triggered by mechanical, luminal distension [233].

The electromechanical barostat (refered to as 'standard barostat' hereforth) is a computer driven device that consists of a pressure transducer coupled through an electronic relay servomechanism and electric motor to a pneumatic pump. An oversized polyethylene bag is connected to a barostat by means of a closed tip double-lumen polyvinyl tube rectal catheter. One lumen is used for filling while the other is used to measure intrabag pressure making simultaneous acquisition of volume and pressure data possible [9]. The barostat bag, secured at both ends (to avoid migration into the rectosigmoid and promote circumferential distension) can be regarded as infinitely compliant meaning its intrinsic properties do not influence internal pressure [234]. The principle of the barostat is to maintain a constant pressure within the air-filled flaccid bag positioned in the lumen of the organ to be studied providing an indirect measurement of physiological variation in tone and is the best device for measuring rectal sensorimotor function [9].

While the barostat is considered the gold standard technique for measuring rectal sensation, it is not widely available. Further, study protocols are lengthy and poorly standardised. Hence its use is often reserved for those with abnormal sensation thresholds to balloon distension or high suspicion of abnormal rectal capacity or compliance.

Sensory evaluation

The distension rate and pattern of filling are controlled by computer software and may involve phasic or stepwise isobaric paradigms [235]. The "ascending method of limits" protocol is most commonly used to measure sensory thresholds during distension and involves presentation of an increasing mechanical stimulus until it is perceived by the subject. However, the predictable protocol and repetitive questioning at each step of pressure distension may produce an element of fatigue or perceptual bias [203,233], which may be reduced by random order phasic distensions [236,237]. Alternatively, a conditioning distension (from 0 to 36 mmHg or MTV) prior to the ascending method of limits protocol has been shown to improve reproducibility [235]. As with simple balloon distension, conscious perception is measured by recording the volumes or pressures required to

trigger rectal sensations (first sensation, urge to defaecate, MTV, and pain). Alternatively, Likert or visual analog scales might be used [234].

Abnormalities of rectal sensitivity may be defined with reference to normal values reported in health. Both hyper- (reduced pressure or volumes thresholds) and hyposensitivity (elevated thresholds) have been recognised in FI patients [7,238].

Capacity and compliance

The standard barostat is the only commonly available technique for accurately measuring rectal compliance [234]. Compliance refers to the ability of the rectum to distend and accommodate to the arrival of stool made possible by its viscoelastic properties. It is defined as the "volume response to an imposed pressure" [214] and expressed numerically as the change in volume divided by the change in pressure [62,158]. However, describing compliance as a single value (based on a linear measurement of compliance) may be simplistic and imprecise [145,158,239] since plotting the volume response (y-axis) to an imposed pressure (x-axis) results in a sigmoid curve [145,158], where compliance is best defined by an exponential function [240]. The triphasic curve reflects initial reflex relaxation of the rectum, followed by a linear section that reflects partly the elasticity of the viscous wall, and a final plateau phase [203]. Therefore, a more accurate "linear" measure of compliance may be given by calculating the slope of the pressure-volume curve [203,234].

Reduced compliance values indicate an abnormally 'stiff' rectum while increased compliance indicates a lax or floppy rectum. However, there is currently no consensus for the best, clinically useful method of assessment (calculation of compliance) and knowledge of normal values in health are limited [7,150], particularly with regard to stratification by age and gender. While knowledge of rectal wall accomodation in itself may be of use in some patient groups (e.g. patients with inflammation, pouch patients, following radiotherapy), there is currently no clear consensus regarding the clinical value of compliance measurement or impact on clinical management [239]. The main purpose of compliance measurements may be to elucidate the influence of rectal biomechanical properties on sensitivity [62,203]. The ability to measure sensory thresholds and compliance routinely or on a selective basis may be useful for clinical evaluation [47,241], however lengthy investigation time, poor patient acceptability, specialist

equipment, and complexity of protocols (ramp vs phasic distension, methods of levels or limits) make standard barostat of limited use. The newly developed 'rapid' barostat [150] (Chapter 7) overcomes some of these challenges representing a viable new addition to routine clinical practice.

Functional lumen imaging probe

The Functional Lumen Imaging Probe (EndoFLIP®, Medtronic) is an emerging technology to describe anorectal function. It utilises the principles of high-resolution impedance planimetry to measure lumenal cross-sectional area (CSA) and pressure within hollow, tubular organs [242]. From these data, the distensibility index (DI), which describes the CSA-pressure relationship can be determined. Patients with FI have been shown to have higher distensibility, indicating a less resistant anal canal, when compared with healthy volunteers [141,243,244], although only a proportion of FI patients exhibited abnormal values [245]. While EndoFLIP has shown a large diagnostic overlap with 3D-HRAM[141,245], these methods are not complementary [246]. Evaluation of anal canal function using EndoFLIP likely reveals yet another pathophysiological mechanism contributing to continence[167]. At present, application of the EndoFLIP technology in the anorectum is limited to research due to the limited number of studies available, including only one study establishing normative values [141]. However, as was the case with high resolution manometry to begin with, the assessment of distensibility is gaining headway in upper GI disorders [247-249]. The principles and literature on the use of EndoFLIP in the anal canal are described in greater detail in Chapter 8; the results of our own investigations in healthy volunteers are also described.

Chapter 1b Knowledge gaps

Heterogeneity and lack of standardisation (of definitions, study protocols, measurement parameters etc.) affects everything from patient choice and relevant investigations to interpretation of results and perceived clinical utility of diagnostic tests. Well-controlled, large, prospective studies in health and FI are needed to evaluate the impact of phenotypic variation on health and disease.

Normal values for novel (and some established) tests of anorectal function lack completely or have been established in limited numbers, especially in men and in nulliparous women. This may be partly due to the perception that FI is a condition which mainly impacts women after childbirth and the (institutionalised) elderly population. However, epidemiological studies suggest that FI symptoms are equally prevalent in men and nulliparous women when compared with (parous) women.

While the pathophysiology in these distinct patient groups is likely to be different, current metrics may not be sufficiently sensitive for revealing these differences fully. The role of subclinical pathophysiology in “less severe” forms of FI and asymptomatic individuals with underlying risk factor (including parous women) also needs elucidating as these may serve as useful predictors of developing FI. Finally, the type of FI (urge/passive) is historically attributed to particular pathophysiology (EAS/IAS weakness), though these simplistic associations have been questioned more recently, with advanced knowledge of the multifactorial nature of FI. The pathophysiological mechanism(s) in other forms of FI (e.g. stress) is currently unknown.

Chapter 1c Aims and objectives

The aims of this thesis are:

- to investigate the importance of traditional manometric variables/diagnoses in different patient groups with particular focus on gender, parity, and less recognised forms of FI;
- to further understanding of anorectal function by contemporary and novel investigation tools through expansion of normal ranges and development of novel metrics;
- to develop understanding of (the role of) parity on anorectal function in health and FI;
- to consider the interaction between components of continence in health, with a view of furthering understanding of the multifactorial pathophysiological nature of FI.

The specific objectives of this thesis are:

- to determine the relative prevalence of major disorders of anorectal function (hypotonia and hypocontractility, hyper- and hyposensitivity) in men and women;
- to describe the prevalence, symptoms, and pathophysiology of stress FI;
- to evaluate the role of the cough response in health and FI using HR-ARM;
- to generate novel measures of function and assess the impact of parity on previously under-reported measures of anal sphincter function (cough and anal slow waves);
- to generate or expand knowledge of normal ranges in health with regards to gender, age and parity using novel and established investigation methods, with view of adopting them into clinical practice;
- to investigate the interactions between tests of anorectal function in health.

Chapter 2 Systematic review of the prevalence of major disorders of anorectal dysfunction in FI patients

Introduction

Faecal incontinence (FI) is a debilitating condition with a prevalence of 8.4% in the general population [65]. Symptoms may be related to a sense of urgent need for bowel opening (urge incontinence), occur in the absence of a conscious need to defecate (passive incontinence), or follow defecation (post-defecation leakage/soiling). Many patients suffer from mixed symptoms. While the nature of FI may suggest pathoaetiology [79], investigation of the physiological and structural mechanisms that help maintain continence is often necessary to establish pathophysiological factors that may be amenable to treatment [125,126,128,250].

Anal sphincter dysfunction is regarded as the most important pathophysiological mechanism in FI [6,112]. Meanwhile, factors including rectal reservoir function, stool form, defecatory efficiency, and cognitive or physical ability may be as important [85], as discussed in the previous chapter. This is especially so in men and in women who do not have evidence of obstetric anal sphincter injury [96,251].

Anorectal manometry is the best-established diagnostic tool to assess whether an individual's resting tone (considered reflective of internal anal sphincter function) and squeeze pressure (reflective of external anal sphincter function) are within or without a normal range [2]. Such normal ranges should preferably comprise values seen in healthy volunteers with similar demographics [252] using the same manometry equipment and set-up [162]. Ideally, manometry results should be interpreted uniformly with other centrs [145].

To facilitate comparison of diagnostic findings between centres, the International Anorectal Physiology Working Group (IAPWG) recently published a consensus for the performance, terminology, and interpretation of anorectal manometry [146]. The London Classification now provides standardised terminology for diagnosis/reporting of anal and rectal dysfunction; pathological terms (hypo, hyper) are based on a deviation from

normal ranges rather than mean pressure. Reduced anal resting pressure, termed hypotonia, and reduced voluntary squeeze pressure, termed hypocontractility, are classed as major disorders of anal tone and contractility diagnosable by anorectal manometry. Likewise, routine determination of rectal sensitivity is also recommended in the consensus statement [146]. Rectal hypersensitivity (meaning a heightened sensory awareness) and hyposensitivity (diminished sensory awareness) are both classed as major disorders of sensation, as their potential to adversely affect continence is recognised [23,205].

Nevertheless, the prevalence of anal motor and rectal sensory dysfunction as diagnosed by the above approaches in patients with FI is currently uncertain. The specific aims of this review were to: a) determine the number of adequately controlled studies reporting on the prevalence of major classes of anal and rectal dysfunction; and b) calculate the pooled prevalence of anal hypotonia/hypocontractility and rectal hyper-/hyposensitivity for males and females.

Materials and methods

Registration

The protocol for this systematic review was registered on PROSPERO (www.crd.york.ac.uk/PROSPERO: registration number CRD42020146507). The subsequent review was conducted in line with the protocol and is reported according to Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines [253].

Eligibility

Original English language articles investigating adult patients (≥18 years old) with FI as the primary complaint using anorectal manometry and / or rectal balloon distension were considered. A minimum sample size of 50 FI subjects and 20 healthy volunteers (HV) was imposed for eligibility. Crucially, control subjects had to be investigated using the same investigative technique; however these data could be historical (referenced within the main article) or current. Eligible studies had to report the prevalence of at least one primary outcome: anal hypotonia, anal hypocontractility, rectal hypersensitivity and/or rectal hyposensitivity. Exclusion criteria were studies of children

(age <18 years) due the differences in aetiology of adult and children's FI [254] and the lack of normative manometry data in children [255] and studies on homogeneous groups of adult patients with specific conditions that are known to impact anorectal function (e.g. Parkinson's, multiple sclerosis, spinal cord injury, and diabetic neuropathy). Studies in which prevalence data could not be segregated by sex (i.e. the results in men and women were combined) were also excluded.

Information sources

Medline (via OVID) and EMBASE libraries were searched for eligible studies published between 1966-2020. Searches were not restricted by language, but non-English language articles were subsequently removed. The final search was performed on 06 July 2020. The reference lists of included articles were reviewed for any additional studies.

Search

Studies were searched by using the term "fecal incontinence" with synonymous variants [as medical subject headings (MeSH) and free text terms]. These were combined using the set operator AND with studies identified with the terms: "resting pressure" OR "squeeze" both with synonymous variants (both as MeSH terms and free text terms). Results were then further combined with the operator AND "anorectal manometry" OR "rectal sensation" both with synonymous variants (both as MeSH terms and free text terms). The detailed search strategy can be found in **Appendix 1**. All records identified through database searches were downloaded and duplicate records were removed.

Study selection

All citations were imported into a bibliographic database. The title and abstract of all identified articles were screened against inclusion criteria independently by two authors (AR and KG). Subsequently, the full text of any title or abstract deemed potentially eligible by either investigator was retrieved. The two reviewers independently assessed the eligibility of each full-text article and disagreements were resolved by consultation with the senior author (SMS). Where necessary, the referenced article detailing a historical control group was also retrieved. In case of

inadequate information to assess eligibility, the corresponding author was contacted for relevant data.

Data collection process

Data were extracted into a spreadsheet (Microsoft Excel 365, 2012) by KG and verified by AR. Any disagreements were resolved by consultation with the senior author (SMS). Outcome data included: numbers of patients (male and female) and proportions with anal hypotonia and/or hypocontractility and/or rectal hypersensitivity and/or hyposensitivity. One study [74] provided individual data points for patients and controls. From these data, the reviewers calculated the lower limit of normal in health (5th percentile) and applied this cut-off to the disease group to obtain prevalence in the absence of a defined normal range by the authors.

Two articles [30,256] included overlapping patient cohorts. As only one of these articles [30] included data on rectal sensitivity both studies were included, however anal motor function data was only extracted from the article with a greater number of patients [256].

Where the prevalence of rectal hyper- or hyposensitivity were reported using multiple sensory thresholds, data were extracted for the first sensation volume [257] or maximum tolerated volume [7,30,96,258,259], as these were presented most consistently in eligible studies. In one study [7], rectal hypersensitivity was based on either first sensation or urge volume. Study authors were contacted by email for missing data.

Data items and summary measures

Outcome data were selected to reflect the specifc aims of the review, namely to determine the pooled prevalence of anal hypotonia/ hypocontractility and rectal hyper-/hyposensitivity in male and female FI patients. These data (hereafter denoted primary outcomes) were extracted as the proportion of the patient population studied whose measures of anal motor function or rectal sensory function fell below the lower limit of normal defined in referenced healthy control subjects. In addition, data were collected on publication year, country of origin, study design, study period, mean or median age of study participants, types of FI, definitions of FI, total number of HV, and cut-off values and definitions used to determine ‘abnormal’ for each outcome measure. Type of

equipment and method used to perform anorectal manometry and/or rectal sensitivity testing were also recorded.

Assessment of risk of bias

Study quality was assessed using a modified version of the NIH Quality Assessment Tool for Case-Control Studies (https://www.nhlbi.nih.gov/health-topics/study-quality-assessment-tools). Two reviewers independently scored included studies out of a maximum of 12 points, with one point gained for each 'yes' answer, indicating that the study met the quality condition being assessed. Disagreements were resolved through discussion and a third reviewer (SMS) consulted if required for resolution. Parameters assessed were: research question, methods of randomisation, study population, sample size justification, random selection of study participants, concurrent controls, case and control definitions, statistical analysis, blinding of exposure/assessors. For clarity, studies were classed as having high (0-33%), moderate (>33-66%), or low (>66-100%) risk of bias based on the percentage of points attained. However, study quality did not influence the 'weight' or 'worth' given to any individual study.

Data synthesis and statistical analysis

For each of the primary outcomes (hypotension, hypocontractility, hypersensitivity, hyposensitivity) meta-analysis was performed using random effects models with a binomial distribution to model within-study variability. Results were accompanied by pooled estimates of the fixed (common) effect model [260] for transparency. Study-specific confidence intervals (95%) were calculated using the score method. Heterogeneity (based on the Chi-square) and proportion of variability attributable to heterogeneity rather than chance (I^2) were assessed. All statistical analyses were performed using Stata 16 (StataCorp LLC, College Station, TX, USA) with the metaprop function to calculate pooled prevalences [260].

Results

Study selection

The study selection process is summarized in **Figure 2.1**. Electronic and manual searches generated a total of 2116 records. Of these, 52 were duplicates leaving 2064 screened records, of which 300 were reviewed in fulltext, and 287 did not meet study criteria.

Five of these studies [72,78,261-263] included male and female patients, but prevalence data could not be segregated by sex and were subsequently excluded from the review. Two additional studies[264,265] were excluded as it was not possible to obtain required information relating to size of the control group. In total, 13 studies fulfilled the inclusion criteria; 12 were identified from database searches and one [177] was identified from screening of references.

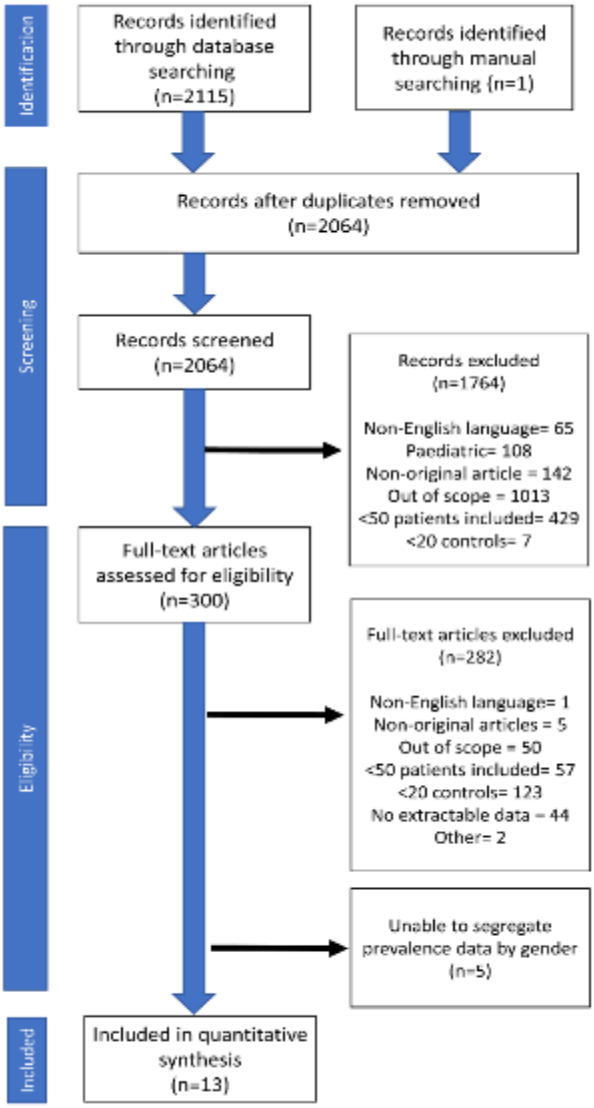


Figure 2.1 PRISMA flow diagram

Study characteristics

The features of the included studies are detailed in **Table 2.1**. Included studies were published between 1987 and 2019. A total of nine studies originated from European centers, one from the USA [90], two from Canada [177,257], and one from Australia [266]. Of the nine European studies, five originated from a single unit [30,96,256,258,259].

Study designs

The majority (7/13) of studies were cross-sectional, involving a retrospective review of data gathered into a patient database [30,245,256,258,259,266,267]. The remaining six studies were classed as prospective (n=4) [7,74,90,177] or retrospective (n=2) [96,257] case-control studies.

Nine studies [30,96,245,256-259,266,267] utilised normal values from previously investigated healthy controls; four studies recruited their own control group as part of the study design [7,74,90,177]. The normal cut-off or range in each study is shown in **Table 2.2**. Sources of funding were acknowledged in five studies [90,96,177,257,266]. Ethical approval was discussed in all but five studies [74,96,258,259,267]

Authors	Publication year	Country	Study design	All patients (n=)	Female (n=)	FI assessment	Controls (n=)	Control Type	Manometry technique	Balloon distension technique
McHugh & Diamant	1987	Canada	Prospective case-control	143	97	Referral	157	Current	**1**	5
Felt-Bersma	1990	The Netherlands	Prospective case-control	178	122	Medical history / referral	80	Current	**1**	8
Delechenaut	1992	France	Cross-sectional study	332	257	Unvalidated questionnaire	114	Historical published	**1**	nr
Sun	1992	UK	Prospective case-control	302	235	Medical history / referral	65	Current	**2**	**6**
Bharucha	2005	USA	Prospective case-control	52	52	FICA scale	21	Current	**1**	na
Burgell	2012	UK	Cross-sectional study	160	0	Vaizey score	24 41[ab]	Historical published and unpublished	**1**	**8**
Hotouras	2012	UK	Cross-sectional study	88	88	Unvalidated questionnaire	92	Historical published	1	**7**
Paramor	2014	Canada	Retrospective case-control	310	235	Vaizey score	50	Historical unpublished	**1**	**8**
Townsend	2016	UK	Retrospective case-control	200	100	Vaizey score	WP 82 SS 115 91[ab]	Historical published	**1,3**	**8**
Carrington	2018	UK	Cross-sectional study	403	403	Vaizey score	85	Historical published	**3**	8
Leroi	2018	France	Cross-sectional study	83	83	Jorge-Wexner score	40	Historical published	**4**	6
Vollebregt	2019	UK	Cross-sectional study	192	154	Vaizey score	134	Historical published	3	**8**
Heitmann	2019	Australia	Cross-sectional study	538	423	Jorge-Wexner score	34	Historical published	**1**	7

1= conventional, water-perfused, stationary or rapid pull-through
2= conventional, water-perfused, stationary
3= high-resolution, solid state
4= high-definition, solid state
5= incorprated balloon, nr
6= incorporated balloon, stepwise distension
7= non-incorporated balloon, stepwise distension
8= non-incorporated balloon, ramp/continous distension

Bold indicates prevalence data are reported; nr = not reported; na = not applicable; *manometry; [a] = hypersensitivity; [b] = hyposensitivity; WP = water-perfused; SS = solid state; FICA = Fecal incontinence and constipation assessment scale

Table 2.1 Features of included studies

Authors	Publication year	Measures (Normative ranges or cut-off values)											
		Resting (mmHg)			Squeeze (mmHg)			Hypersensitivity (ml)			Hyposensitivity (ml)		
		Males	Female	Combined	Males	Female	Combined	Males	Female	Combined	Males	Female	Combined
McHugh & Diamant	1987	26-142	9-142		113-399	38-261		nr	nr	nr	nr	nr	nr
Felt-Bersma	1990	>22	>31		>88	>38		nr	nr	nr	nr	nr	nr
Delechenaut	1992			Upper part >24 Lower part >2.2			>63	nr	nr	nr	nr	nr	nr
Sun	1992	nr	nr	nr	123-250	66-177		DDV 60-150 Pain 150-200	DDV 50-100 Pain 100-200		FCS 10-20	FCS 10-20	
Bharucha	2005			>25			>87	na	na	na	na	na	na
Burgell	2012			>37			>37	MTV >80			FCS <150 DDV <190 MTV <320		
Hotouras	2012		>37			>37		MTV >75	MTV >75		MTV <290	MTV <290	
Paramor	2014			>44			>131			FCS 38-58 DD 103-123			FCS 38-58 DD 103-123
Townsend	2016	WP >24 SS >37	WP >24 SS >30		WP >26 SS >60*	WP >26 SS >42*		MTV >75	MTV >75		FCS <150 DDV <190 MTV <325	FCS <110 DDV <200 MTV <290	
Carrington	2018		>41			>29*		MTV >75			FCS <150 DDV <190 MTV <320		
Leroi	2018		>67			>139			150-340			150-340	
Vollebregt	2019	>38	>33		>61*	>45*		MTV >75	MTV >75		FCS <150 DDV <190 MTV <325	FCS <110 DDV <200 MTV <290	
Heitmann	2019			>40			>132			FCS 10-80 MTV 200			FCS 10-80 MTV 200

Table 2.2 Table of Normal values used in studies

Participants

A total of 2981 (75.1% female) FI patients were included across eligible studies. The number of participants included ranged from 52 to 538 (median 192). The number of control subjects included was 21-157 (median 80).

Four studies [90,245,256,258] included only female patients and one included only men [259], while the remaining eight studies included both men and women (**Figure 2.2**). In these studies, most patients were female with women making up 50-80% of the total.

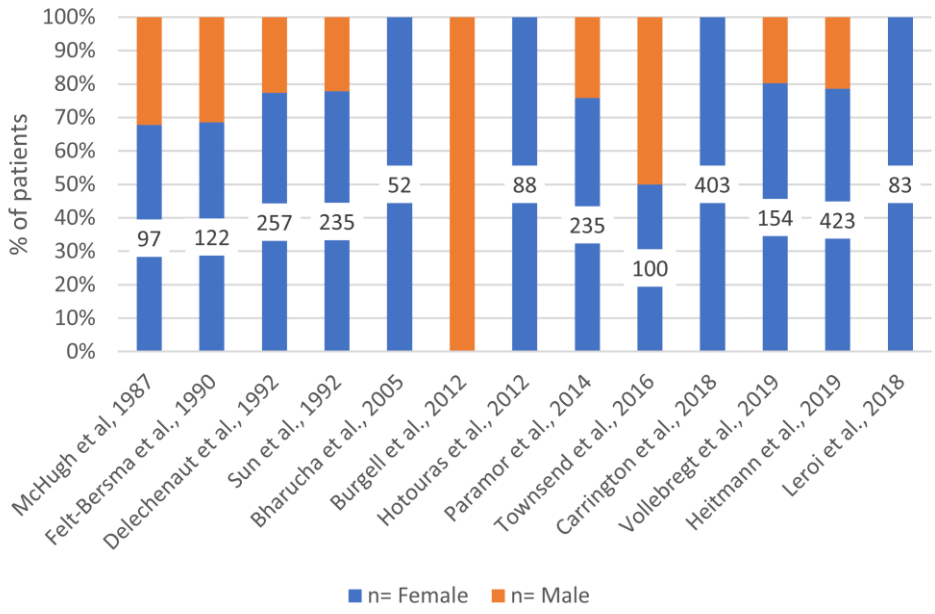


Figure 2.2 Distribution of male and female participants in selected studies.

The mean age reported across studies ranged between 52 and 67 years. Overall, the age of participants ranged from 13 to 97. Three studies [7,74,267] included a minority of patients under the age of 18 and were included in the review. Although it was not possible to ascertain the exact number of paediatric patients included, based on the information available their presence was deemed unlikely to have had any significant impact on overall prevalence data.

Severity of FI was evaluated in 10 studies using validated (n=8) or unvalidated (n=2) questionnaires. In the remaining studies, the evaluation of FI was based on clinical interview. Patients were described as having either isolated urge or isolated passive FI, or a mixed type of leakage in 5/13 studies [30,90,245,259,266]. In these studies, the proportion

of patients with urge FI was 14% to 44%, passive incontinence was 6% to 52%, and mixed FI was 19% to 53%.

Intervention characteristics

Details of the manometry set-up and rectal sensitivity protocol are presented in **Table 2.1**. Anorectal manometry was performed using a (conventional) water-perfused, station pull-through (n=6), stationary (n=1), or rapid pull-through (n=2) technique in nine studies, and a solid-state high-resolution/high-definition technique in three studies. The catheter type and technique used was mixed in one study.

Rectal sensitivity was evaluated by balloon distension in 12 studies. Most studies used a balloon assembly made up of a balloon (latex n=5, undefined n=3) tied to a urinary catheter (n=6) or other type of tubing (n=2). In other studies, the balloon was attached to the tip of the manometry catheter (n=2) or formed within the sheath covering the manometry catheter (n=1). No details on balloon assembly or filling method were available in two studies [177,267]. All studies used air to distend the rectal balloon; four followed a stepwise filling protocol and the remaining six used continuous (ramp) distension. Most studies asked patients to report the following sensory thresholds: "first sensation" (8/12 studies), "desire to defaecate" (8/12) and/or "maximum tolerable volume" (9/12 studies). Other sensory thresholds described included sensations of 'gas/wind' and 'pain' [7].

Outcomes

Anal tone and contractility

Overall, 11 studies reported prevalence data on anal tone and/or contractility. The prevalence of hypotonia and hypocontractility were each described in 10 studies [74,90,96,177,245,256,257,259,266,267]; one additional study described the prevalence of hypocontractility alone [7].

Resting pressure (hypotonia) and squeeze pressure (hypocontractility) were evaluated against the lower limit of normal defined by the 5th percentile in healthy volunteers in three studies [74,90,256], mean-2SD in three studies [7,96,177] and the ROC cut-off defining the best sensitivity and specificity against health for a given measure in one study[245]. The

remaining 6/15 studies with prevalence data on anal tone and contractility did not describe the cut-off definition used.

Rectal sensitivity

The prevalence of rectal hypersensitivity and hyposensitivity were each reported in only 5/12 studies that performed balloon distension, respectively. Only one study [96] stated the definition used to define the cut-off volume for hyper/hyposensitivity (mean-2SD) in health. Communication with the senior author of 3/4 of the remaining papers [30,258,259] revealed that all studies performed in the same unit used mean-2SD to define the normal cut-off.

Risk of bias in studies

Scores for each study are summarised in **Figure 2.3** (for further details of individual scoring see **Appendix 2**). Of the 13 studies, three achieved ≤33% of attainable points and were considered as low quality, with high risk of bias, while seven studies scored between 34-66% of available points (medium quality studies). Three studies[90,177,256] scored 67-100% and were classed as good quality (low risk of bias). The median score was 55% (range 17-75%).

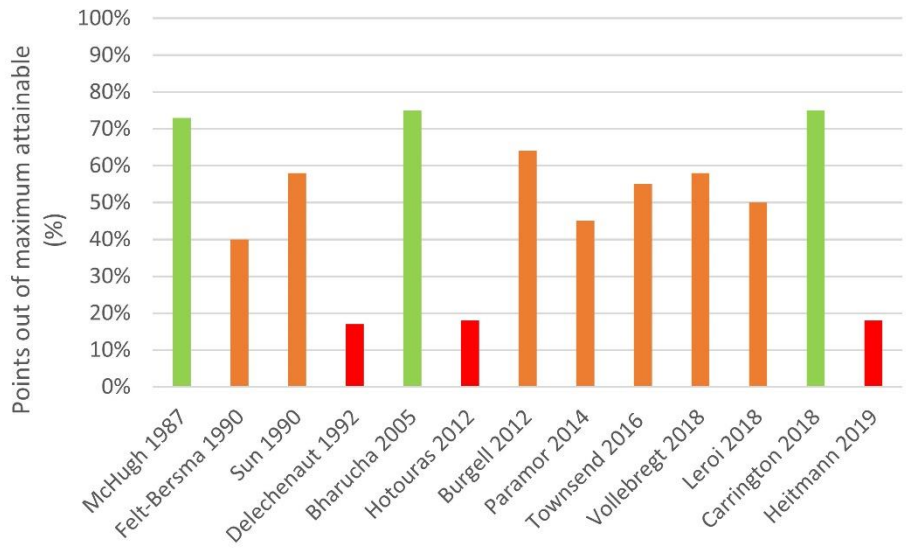


Figure 2.3 Summary scores for risk of bias assessment in individual studies.

Results of individual studies and data synthesis

Anal hypotonia

The pooled prevalence of anal hypotonia (**Figure 2.4**) was 44% (95% CI: 32-56%, I^2= 96.35%) in women (n=1767) and 27% (95% CI: 14-40%, I^2=94.12%) in men (n=624). Within study prevalence of hypotonia was always less in males (range: 9-58%) than in females (range: 21-80%), except for Heitmann et al [266] who reported a similar prevalence between men (58%) and women (59%), and McHugh and Diamant [177] who reported a higher prevalence of hypotonia in males (45%) than females (36%). Leroi [245] used the ROC cut-off for normal and reported the highest prevalence rate for hypotonia. While anal hypotonia was always the least prevalent form of sphincter dysfunction in women, anal hypotonia in men was most common almost half of the time [177,259,266].

Anal hypocontractility

The pooled prevalence of anal hypocontractility (**Figure 2.5**) was 69% (95% CI: 57-81%, I^2= 98.17%) in women (n=2007) and 36% (95% CI: 18-53%, I^2=96.77%) in men (n=696). Within study prevalence of hypocontractility was nearly always significantly greater in women than in men (over double that in men in 4/6 studies). It also generally exceeded hypotonia prevalence. Hypocontracility prevalence was highest in the paper by Sun et al [7] at 94% in women and 87% in men.

Rectal hypersensitivity

The pooled prevalence of rectal hypersensitivity (**Figure 2.6**) was 10% (95% CI: 4-15%, I^2= 80.09%) in women (n=577) and 4% (95% CI: 1-7%, I^2=51.25%) in men (n=373). In women, the prevalence of hypersensitivity was highest (17% vs 5-10% in other studies) in the study by Paramor et al [257]. This study, unlike others, based the diagnosis on volume at first sensation or urge sensation rather than MTV.

Rectal hyposensitivity

The pooled prevalence of rectal hyposensitivity (**Figure 2.7**) was 7% (95% CI: 5-9%;) in women (n=577; I^2 0%, p=0.88) and 19% (95% CI: 15-23%) in men (n=373; I^2 0%, p=0.46). The prevalence of hyposensitivity was higher in men than women in all studies. The

majority of data (3/4 studies in men and 3/4 studies in women) came from a single institution at different timepoints [30,96,258,259].

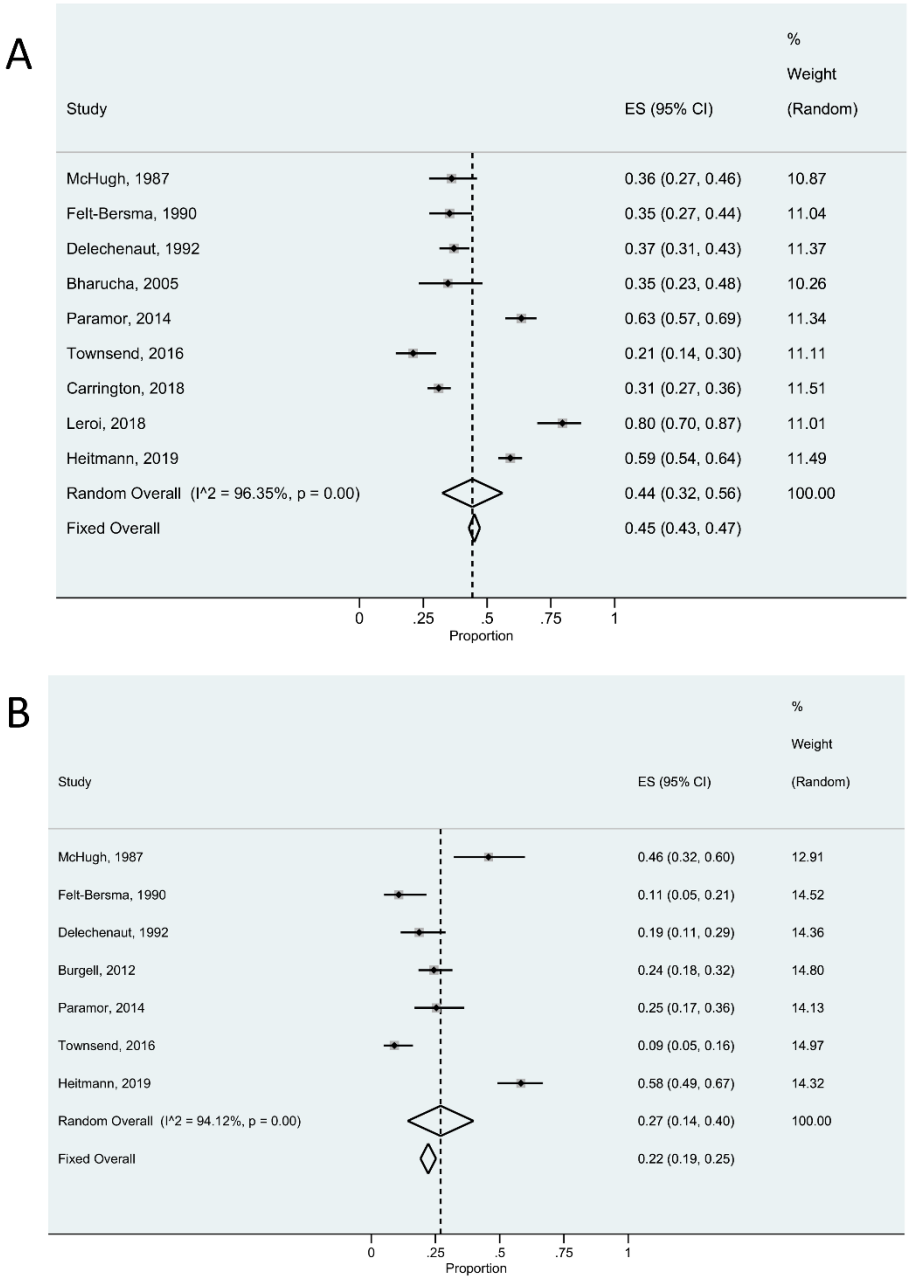


Figure 2.4 Pooled prevalence of anal hypotonia in (A) women (44%) and (B) men (27%).

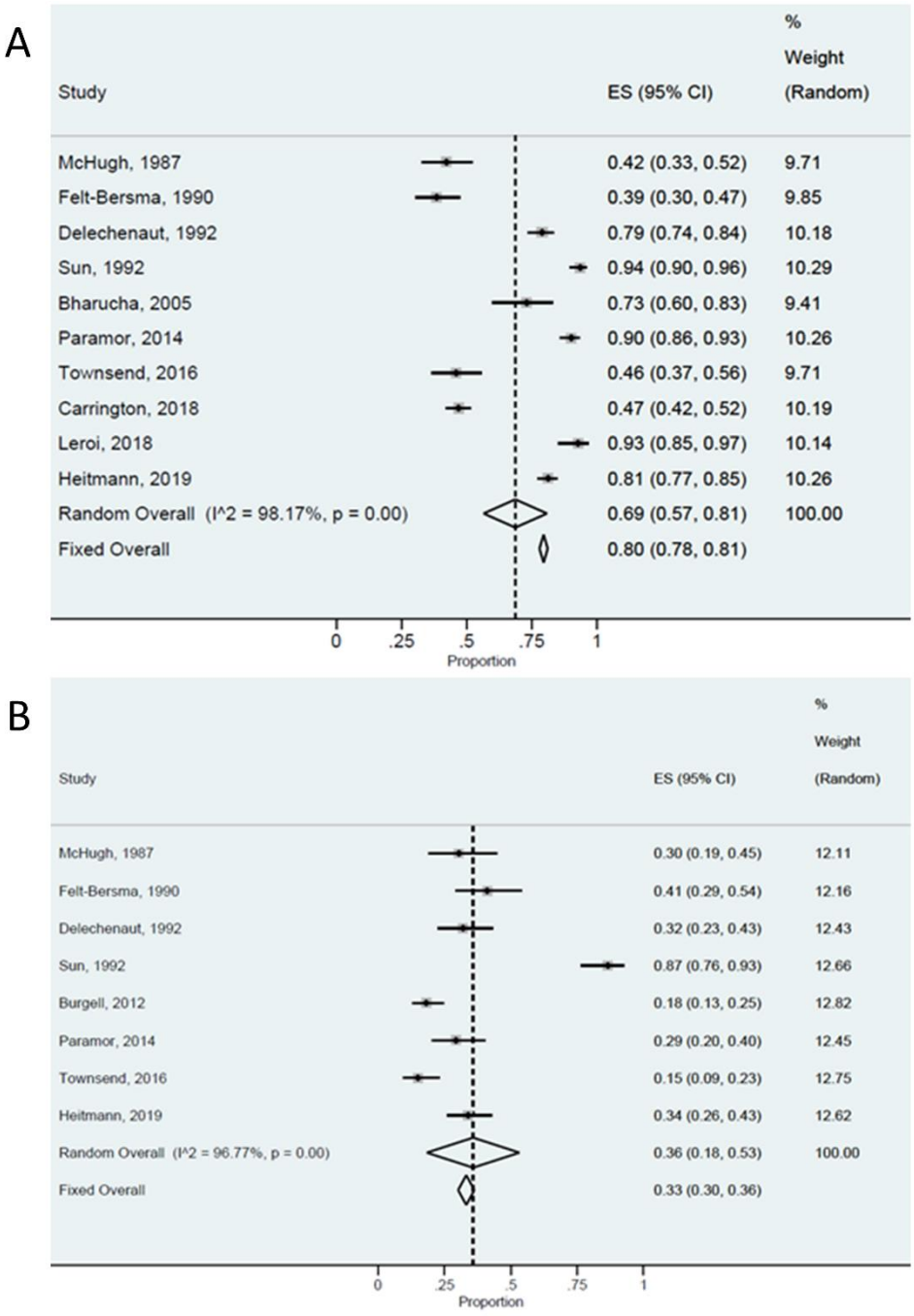


Figure 2.5 Pooled prevalence of anal hypocontractility in (A) women (69%) and (B) men (36%).

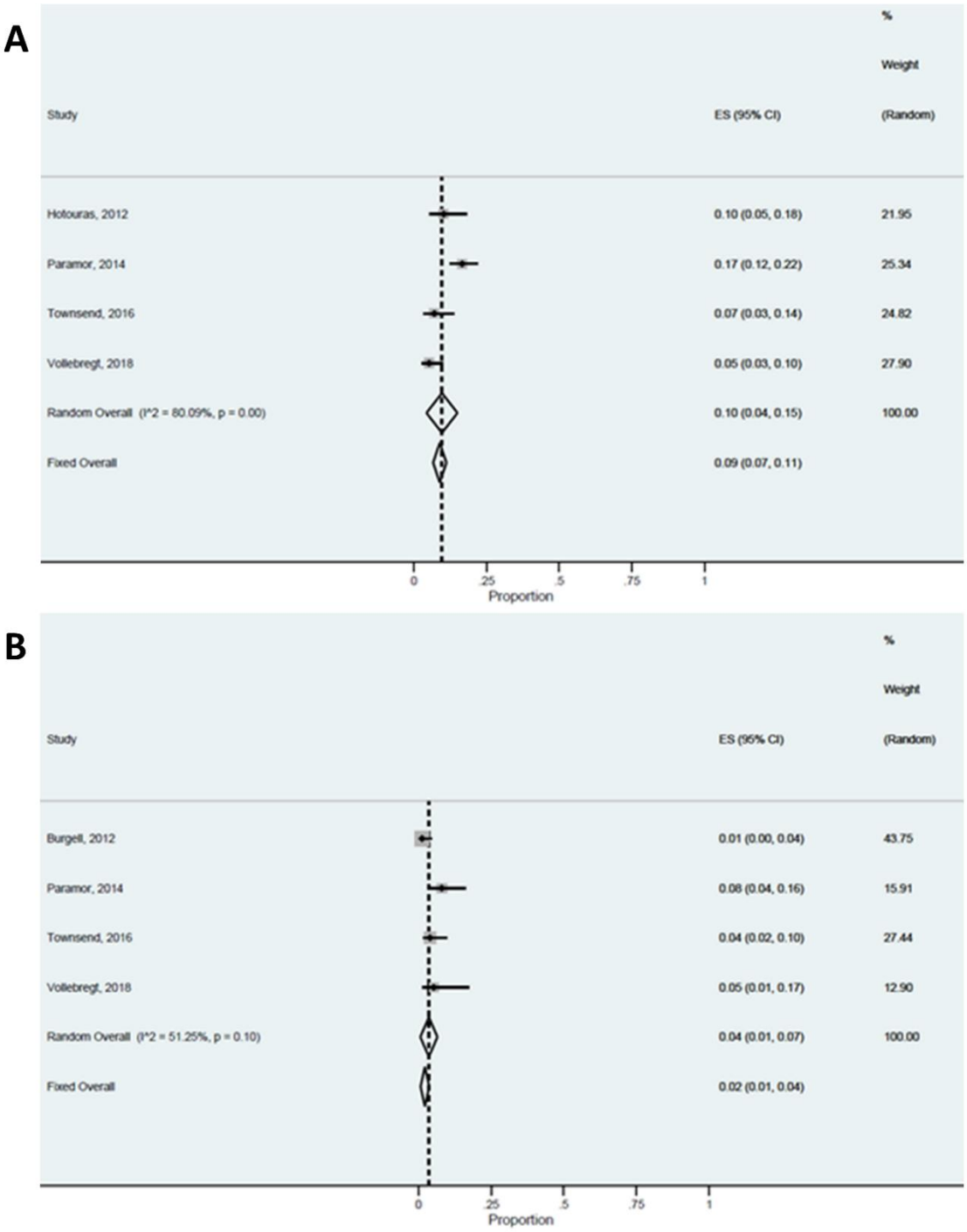


Figure 2.6 Pooled prevalence of rectal hypersensitivity in (A) women (10%) and (B) men (4%).

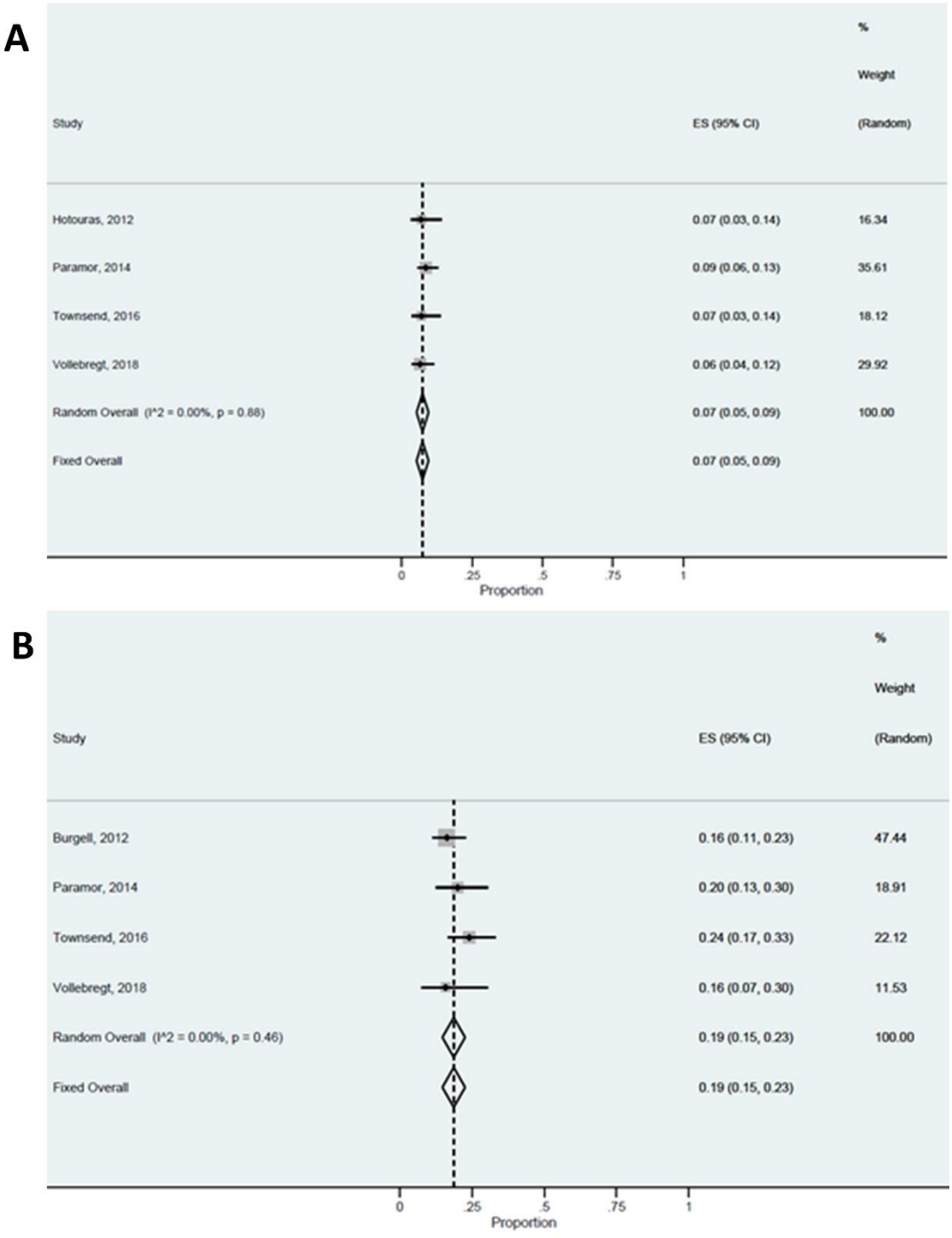


Figure 2.7 Pooled prevalence of rectal hyposensitivity in (A) women (7%) and (B) men (19%).

Discussion

Summary of findings

This systematic review and meta-analysis on the prevalence of major disorders of anal motor and rectal sensory function found only 13 original, English-language studies in patients with FI which met inclusion criteria. This illustrates both the paucity of FI studies with large sample sizes, consideration of gender differences, and highlights the limited numbers of included healthy volunteers upon which generation of prevalence data depend.

Anal sphincter dysfunction was the most prevalent pathophysiological finding. According to pooled results, 44% percent of women and 27% of men had anal hypotonia and 69% of women and 36% of men had anal hypocontractility. In women, these results support the popular notion that inadequate barrier function (whether of neurological/functional or structural origin) is the leading cause of FI. In contrast, only a minority of men present with attenuated anal sphincter function; other (suprasphincteric) mechanisms warrant consideration especially in this group. Nevertheless, it may be that measures of resting tone and squeeze pressure lack sensitivity to convey all degrees of anal sphincter dysfunction.

Rectal sensory dysfunction was present in up to one fifth of men (hyposensitivity) and a tenth of women (hypersensitivity). This highlights the need to evaluate rectal capacity and afferent pathway function in at least a proportion of individuals to determine the pathoaetiology of impaired sensation.

Comment on major findings

Overall, anal hypocontractility was the most common abnormality both in women and in men. In individual studies, prevalence of anal hypocontractility generally exceeded that of anal hypotonia, and rates of hypocontractility were nearly always greater in women than in men. The risk for anal sphincter barrier dysfunction is greatest in women due to obstetric injuries (including structural defects and pudendal neuropathy), whilst in men sphincter barrier dysfunction is generally considered to be iatrogenic [6]. However, even in the absence of structural sphincter defects, chronic straining at stool and subsequent pelvic floor denervation may also lead to hypocontractility in both

males and females [114]. Constipation and FI are known to co-exist in a sizeable proportion of patients with FI [68].

While the prevalence of male hypotonia was reduced in more recent studies (perhaps due to more sphincter sparing surgery [96]), the prevalence of anal hypotonia and hypocontractility in women remained consistent over the duration of included studies (>30 years). This is likely because the lag between sphincter injury and symptom occurrence in women is usually several decades [80] meaning that the benefits of any change in obstetric practice (e.g. move from posterior to mediolateral episiotomy) has yet to translate to observed changes in physiology. Notably, excluding patients with sphincter defects did not reduce the prevalence of hypocontractility in relation to other studies [267]. Bharucha et al [90] was the only other study to specifically exclude patients with sphincter defects, although they did include patients with history of forceps delivery, stitches and sphincter repairs.

Of the major disorders, rectal hyposensitivity was the only condition to have higher prevalance in males compared to females. Altered rectal sensitivity, especially in the presence of weak sphincters [7] may lead to incontinence due to reflex inhibition of the internal anal sphincter before the patient perceives the presence of stool in the rectum [238]. This may be of particular importance for some patients who complain of mainly passive leakage, which is more likely to be associated with a weak internal anal sphincter [72]. Meanwhile, hypersensitivity may result from altered rectal compliance, sensitisation of extrinsic peripheral pathways and/ or central afferent mechanisms, or abnormalities in perceptual and behavioural processes causing hypervigilance [92]. Often this leads to urgency and urge incontinence associated with an inability to defer defaecation [92]. Rectal hypersensitivity was more prevalent in women than men.

Comment on heterogeneity

The prevalence rates for anal hypotonia, hypocontractility, rectal hypersensitivity and hyposensitivity varied greatly. To reduce clinical heterogeneity resulting from choice of manometric or balloon distension technique, we considered only those studies with institution-derived or -identified control values to ensure appropriate normal ranges were included. However, we did not set criteria for the method or definition of normality used between studies, which may explain some of the variability observed.

For example, studies that define abnormal cut-offs based on the sensitivity and specificity of each test report generally higher prevalence rates, since these cut-offs may be different from normal values in a larger population of healthy volunteers [245]. Indeed, it was much more common that a value depicting only extreme outcomes in health (typically the 5th percentile or mean-2SD) was used. Reassuringly, when studies used the same method and definition for the lower limit of normal, as demonstrated in the pooled analysis of rectal hyposensitivity, findings between studies remained consistent over time and statistical heterogeneity disappeared [30,96,257-259]. This observation calls for international standardization of not only the parameters studied (as per the IAPWG protocol[146]) but also the definition of normal cut-offs.

Limitations

Our review has several limitations. In choosing search terms, we did not consider 'anal incontinence' due to its association with more minor forms of leakage including soiling and flatus incontinence[268]. Meanwhile, some eligible studies included a proportion of patients with lesser forms of FI [257]. One study [258] included only FI patients eligible for percutaneous tibial nerve stimulation, which may be offered to patients with more minor FI, dependent on hospital policy. For consistency, we did not impose set criteria for FI, relying on the authors' definition of FI. One exception was the study by Paramor et al [257] who included a FI and faecal leakage (FL) group (defined as leakage up to 2 tablespoons). For this study, we chose to combine the two groups as it was felt that their definition of FL was comparable to FI in other studies. However, it should be noted that Paramor et al [257] themselves concluded that pathophysiology in males with FL is different from that in males with FI and in females with FI and/or FL.

A high degree of co-existent symptoms amongst patients may influence rates of pathophysiology. Several of the included studies had large proportions of patients with co-existent constipation symptoms, IBS, rectal prolapse, etc. Although it was our intention to study patients with 'idiopathic' FI (thus excluding studies in homogenous groups of patients with conditions known to impact anorectal function), many studies included a proportion of patients with neurological or surgical risk factors, which could have influenced the results. For example, in considering the 40% of included patients with 'idiopathic' FI, Burgell [259] observed that normal sensation was more likely than

hyposensitivity (i.e. prevalence of hyposensitivity was lower in patients with idiopathic FI) compared to other causes of FI. One of the main problems in limiting any study of FI to 'idiopathic' FI is that only very rarely do patients present clinically with no precipitating factors to FI. Our results therefore, are generally reflective of patients attending tertiary sector care for the investigation of FI.

Regarding the primary outcomes, we did not impose the definition used for hypo/hyper within the selected studies and relied upon the definition used in individual studies. On occasion, the direction of results were difficult to interpret, especially for sensation. Having standardised definitions for pathological terms "hyper" and "hypo" will help future studies communicate these results [146]. To include the study by Felt-Bersma [74], we applied the 5th percentile in health to data presented based on this being the most widely employed definition for the lower limit. Although an alternative cut-off could have been chosen, using the 5th percentile resulted in a small percentage of FI men and women with sphincter dysfunction in line with the authors observation of "near complete overlap between incontinent and control subjects".

Application of minimum eligibility criteria regarding the sample and control group size was intended to ensure that studies included came from departments with sufficient experience in techniques and knowledge of normal ranges. Five included studies originated in a single unit while a large proportion of studies were excluded due to a small sample size; inclusion of such smaller studies may have yielded different results. On the other hand, while we imposed a criteria for the overall number of participants, we did not consider the numbers of male and female patients individually. In one study [30], the number of included males was <50, meaning that this study met inclusion criteria for the systematic review solely based on the number of women in the study.

A total of 5 studies [72,78,261-263] comprising both male and female patients were excluded from the review because prevalence data were reported as a single result (rather than specific values by sex). These studies represent some of the largest conducted, so the data loss is considerable. However, our aim was to specifically compare prevalence by gender (a decision justified by the widely differing results between males and females) and such data could not be accurately extracted from excluded studies. Overall, the

number of male patients included in the meta-analysis was considerably less than females, reflective of the clinical population typically investigated for FI.

Conclusion

These results convey clear gender disparity in the rates of sphincter barrier dysfunction and rectal sensory dysfunction. Poor voluntary sphincter control remains the most prevalent abnormality observed, especially in women. However, the number of appropriately controlled studies was small and few were judged as having low risk of bias. Consistent technique and definition of normal improved certainty of diagnosis (e.g. hyposensitivity), but overall wide confidence intervals and high levels of heterogeneity were observed. This indicates the need for large-scale prospective studies to be performed using a standardised protocol (e.g. the IAPWG protocol [146]) and call for a collective effort to harmonise practice.

Chapter 3a Minireview of Stress Faecal Incontinence

Introduction

Faecal incontinence (FI) is generally classified as being either 'urge' or 'passive' in nature based on the temporal association of incontinent episodes with the presence or absence of the need to defaecate. Although other types of incontinence have been recognised (for example, soiling, post-defaecatory leakage/faecal seepage, mixed FI) [4,72,73,269], these are rarely assesed in the literature. In particular, stress incontinence on coughing, bending, or walking [77] has received little attention in FI literature. However, based on clinical experience, at least a proportion of patients with FI complain of leakage associated with coughing, sneezing, or exercise (i.e. "stress" activities involving sudden increases in intra-abdominal pressure).

In urological literature, incontinence phenotypes including stress urinary incontinence (SUI) are well defined [268]. Involuntary loss of urine on effort or physical exertion, or on sneezing or coughing (i.e. SUI) is considered the *predominant* form of incontinence and was projected to affect >166 million individuals globally in 2018, the vast majority of whom were women [270,271]. The prevelance of SUI in women according to international, population-based surveys is between 9-29% [271]. The pathophysiology of SUI is related to weakness of pelvic floor and sphincteric structures [272]. Electrophysiological studies in FI and double incontinent (FI and SUI) women have implied similar pathophysiology between the two conditions [273]. Delayed terminal motor latencies in the pudendal nerve and perineal branches arising from sacral nerves in group of women with FI or double incontinence (FI and SUI) were observed compared with healthy controls. In the FI only group, the perineal branch was relatively spared. FI is common in women with SUI [274-276] and conversely, SUI, followed by urge incontinence, is the most commonly reported urinary symptom in double incontinence [277].

Recently, the International Continence Society (ICS) defined stress faecal incontinence as the "complaint of involuntary loss of faeces on effort or physical exertion including sporting activities, or on sneezing or coughing" [278]. The anal contractile reflex (or 'cough reflex') is an augmented guarding reflex which preserves the positive anal-to-rectal pressure gradient during intra-abdominal/intra-rectal pressure increase [9,61,279]. It

involves reflex contraction of the EAS muscle mediated by a spinal reflex. Anorectal manometry can be used to demonstrate the cough reflex by asking the patient to perform a single cough, while monitoring anal sphincter and rectal pressure changes [146].

Aims

The aims of this chapter are to a) describe the number and type of studies on stress FI available in the literature; b) to explore the definition and prevalence of stress FI reported in previous studies; c) to describe risk factors and pathophysiology associated with stress FI; and lastly d) to explore the relationship between stress FI and the cough response.

Methods

Unrestricted searches of Medline (via OVID) and Scopus libraries were performed using the terms "fecal (OR faecal) stress incontinence", "stress fecal (OR faecal) incontinence" and "cough incontinence". Additional searches for related articles were in performed in Google Scholar and through a global search for literature pertaining to the topic using the Queen Mary University library research tool. Titles and abstracts were reviewed for articles relating to the search terms and full texts were accessed to ascertain relevance. Reference lists of relevant articles were searched for further resources. Articles related to the topic were scrutinised and relevant data extracted. Findings related to stress FI/cough were summarised and considered in relation to other studies.

Results

Studies on stress FI

After removing duplicates, 15 publications on stress FI published between 1990 to 2020 remained. These included one book chapter [280], two abstracts [281,282] and 13 full text articles (**Figure 3a.1**). Six of these were review articles [280,283-286], two were either bench [287,288] or animal studies, and three were classed as surveys [289,290], one of which also included retrospective review of patients' medical records [281]. The remaining five studies were classed as experimental studies [277,282,291-293] (**Table 3a.1**). Literature searches produced several other publications featuring cough or the cough response in relation to FI (these will be discussed separately in a later section).

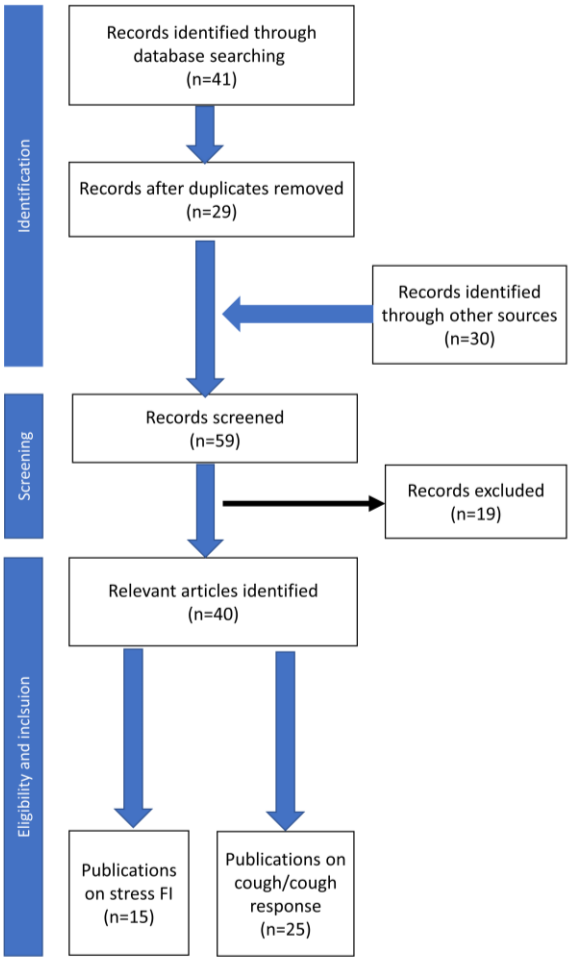


Figure 3a.1 PRISMA-style flow diagram

Author	Year	Publication type	Article type/ study design	Participants (% female)	Age (mean ± SD)	Population	Stress FI Prevalence
Swash	1990	Book chapter	Review	na	na	na	na
White	2000	Full text	Survey	71 (41)	F: 24.1 (range 18-38) M: 25.0 (range 18-44)	Cystic fibrosis patients	0%
Ross	2001	Full text	Experimental	46 (100)	nr	Double incontinent patients	na
Lacima	2002	Full text	Experimental	65 (100)	F: 58.5 ± 11.2 M: 59.6 ± 13.0	Double incontinent and FI patients	21.1%
Lacima	2003	Full text	Review	na	na	na	na
Ash	2005	Full text	Review	na	na	na	na
Schwabeggar	2007	Full text	Animal study	na	na	Dogs	na
Shafik	2007	Full text	Experimental	163 (57)	39.6 ± 11.3	FI patients	33.7%
Kumar	2008	Full text	Review	na	na	na	na
Marecki	2010	Full text	Review	na	na	na	na
Dessie	2014	Abstract	Survey[a]	nr	nr	FI patients	67.9%
Elgendy	2014	Abstract	Experimental	nr	nr	Stress FI patients and healthy volunteers	na
Benezech	2017	Full text	Survey	155 (59)	30.5 ± 11.0	Cystic fibrosis patients	25.8%
Hoke	2020	Full text	Experimental	247 (100)	57.7 0 ± 12.94	FI patients	11.7%
Marziale	2020	Full text	Bench study	na	na	na	na

[a] with retrospective review of patient medical records

na not applicable

nr not reported

Table 3a.1 Publications in stress FI

Participants

Considering only experimental studies and surveys, two of the articles were performed in patients with cystic fibrosis [289,290]. All other studies included patients with FI in isolation [277,281,282,292,293] and/or in the context of double incontinence (SUI and FI) [277,291]. Only one study specifically sought to recruit only stress faecally incontinent patients and included a healthy control group [282]; however this study was only published in abstract form.

Of eight studies including FI or double incontinent patients, three included only women [276,277,293], five included both men and women [276,277,289,292,293]. No information pertaining to the gender distribution of participants was given in the final two publications [281,282], both of which were only available in abstract form. The average age of included patients in six studies [276,277,289,290,292,293] ranged from around 25 years [289] to approximately 59 years [277]. Overall, studies included patients between ages 18-86 years old (**Table 3a.1**). Studies performed in CF patients [289,290] included participants who were younger than participants in other studies. Not all studies reported age [276,281,282].

Definition of stress FI

Of the 15 articles identified as being 'about stress FI', only 6 defined stress FI [281,290,292-295]. Of these 6 definitions, four included reference to faecal leakage occuring during or after coughing [281,290,292,293]. Two of these extended the definition of stress FI to leakage occuring with "stress" or " stress-like" activities (specifically coughing or sneezing [293] and/or laughing [281]). The definition of stress FI used by Shafik et al [292] was restricted to leakage of flatus or fluid stool after coughing.

Both Kumar [285] and Swash [294] defined stress FI as leakage related to loss of EAS control. According to Swash [294], stress FI is the loss of faeces occuring when the pressure in the rectum exceeds that generated by the anal sphincter musculature. Interestingly, the distinction between stress FI and urge incontinence made by Swash [294] was made on the basis of pathophysiology where the former was related to the (in)ability to produce or maintain a sphincter (pressure) barrier while the latter related to a "heightened sensory stimulation" caused, for example, by inflammation or irritable bowel syndrom (IBS). In contrast to Swash's [294] definition of urge FI, most epidemiological studies

associate urge FI with the inability to defer defaecation implying an inability to control bowel opening (due to poor sphincter function) until a toilet is reached [269,296]. This highlights potential for overlap between definitions of stress and urge FI in studies of FI.

Prevalence of stress FI

In four studies which included only FI patients [277,281,292,293], the average prevalence of stress FI was 33.6% (range 11.7-67.9%). In the study by Dessie et al [281], stress FI occurred in 67.9% (36/53) of subjects. However, among these patients more reported leaking gas (52.8%) with coughing, sneezing, or laughing than solid stool (39.6%). Lacima et al [277] reported prevalences of stress FI in 21.1% of patients with isolated FI and 34.4% of patients with double incontinence (SUI and FI). In the study by Hoke et al [293], 29 (11.7%) subjects reported isolated stress FI (compared with 179 subjects with isolated urge FI and 39 subjects with isolated passive FI). However, over a 14 year period (2003-2017) of 848 patients presenting for evaluation of FI, only 247 were included in the analysis (those reporting mutually exclusive symptoms). The majority of patients reported stress FI to both solid and liquid stool. Finally in the study by Shafik [292], 163 patients with partial FI (defined as occasional leakage of flatus or watery, loose stools) were asked to indicate if their FI occurred after coughing (stress FI), urgency (urge FI), or both (mixed FI); 33.7% (n=55) patients responded to having stress FI; 44.1% (n=72) patients had urge FI and 22% (n=36) patients had mixed FI.

Two additional studies investigated stress FI specifically in patients with cystic fibrosis (CF) in whom repeated coughing is thought to impose additional stress on the pelvic floor increasing susceptibility to leakage. The first study by Benezech et al [289] (2017) looked at population based prevalence of stress FI in patients with CF. The study found that 25.8% (40/155) of respondents reported FI (solid, stool or gas) and there was no difference in prevalence between men and women. FI episodes mainly occurred when patients coughed (77.5%), sneezed (50%), laughed (40%), or during sport (32.5%). Conversely, in the second study of CF patients [290], which was designed to interrogate the impact of CF on bowel function (including if continence prevented effective coughing), none of the 29 females and only 1/42 males interviewed described any leaking of faeces. In one man, a single episode of incontinence was reported related to

a panic attack. Conversely, SUI was common in women affecting 37.9% of female patients. The authors suggested that the low prevalence of stress FI in this study may have been related to constipation, common in CF [297], since the need to push or strain during bowel movements was found in 37.9% of females and 21.4% of males included in the study.

Risk factors and other associations with stress FI

Of all publications of stress FI (including reviews), seven studies mentioned directly or alluded to risk factors for stress FI [277,283,286,289,292-294]. Current smokers and patients with urinary incontinence were significantly more likely to have stress FI thas some other types of FI [293]. Benezech et al [289] studied stress FI in CF patients and showed that FI was significantly more frequent in older patients and in patients with associated UI. Furthermore, a logistic regression model showed that lung transplantation (OR [95% IC]: 2.5 [1.0–5.9], p = 0.04) and urinary incontinence (OR [95% IC]: 4.9 [2.0–11.9], p = 0.001) were independently linked to FI in CF patients. Lacima et al [277] found a greater proportion of rectoceles in double incontinent patients than patients with FI in isolation suggesting difficult defaecation and/or pelvic floor weakness in these patients. Logistic regrassion analysis also revealed that women with an abnormal anal-cough response were at greater risk of double incontinence (OR, 3.11; P =0.02; 95% CI, 1.24, 7.81). Stress FI was attributed to neurological dysfunction of the EAS muscle reflex response owing to diabetes mellitus by Shafik et al [292]. Meanwhile according to Swash's [294] neurogenic theory of origin of stress urinary and faecal incontinence, the greatest risk factor for pudendal neuropathy (the leading pathophysiology of stress FI) was childbirth.

Mechanisms of injury other than sphincter defects, such as SCI [286], damage to suspensory ligaments or cauda equina disease in the development of stress FI were also acknowledged [294]. Dessie et al [281] did not find any association between anorectal angle (measured during MRI) in stress FI vs. patients without stress FI despite patients who had underwear staining after a bowel movement having a more acute anorectal angle (105.7 degrees) versus patients without staining (122.0 degrees, p=0.048). Reduced EAS EMG activity was found in patients with stress FI and mixed (urge and stress) FI [292]. In contrast, only diminished IAS EMG activity was found in patients with urge FI (diminished activity in the mixed group was registered for both the EAS and the IAS).

Hoke et al [293] aimed to characterise symptom distress and symptom specific impact on quality of life (QoL) in women with stress FI. The authors reported no differences in symptom distress of overall or significantly negative impact of QoL among women with stress FI compared to urge and passive FI (if anything, physical and relationship stress were less in stress FI).

Anal sphincter function in stress FI patients

Anal sphincter function in relation to stress FI was assessed in 6 studies [276,277,281,282,292,293] using anorectal manometry (ARM).

Three of these studies compared results with those from healthy volunteers [281,282,292]. In a retrospective review of information available in patient's medical records, Dessie et al [281] found anorectal manometry results for 24/53 patients with FI (all types). The vast majority of patients (87.5%) had abnormal squeeze pressure (defined as <100 mmHg) and just over half (54.2%) had low resting tone (defined as <40 mmHg). Threshold volume to first sensation was abnormal (>30ml) in 22/24 of patients. Although the authors note that the type of FI was not correlated with results of anorectal manometry, no specific pressures for patients with stress FI were reported specifically. Similarly, Shafik et al [292] noted that anal pressure measured using water-perfused open-tip catheters was significantly below that of healthy volunteers in all three groups of FI patients (stress FI, urge, and mixed). Anal pressure in stress FI patients was 46.2 cm H_2O (SD 3.7 cmH_2O, range 42-50 cmH_2O) and 72.3 cmH_2O (SD 3.6 cmH_2O, range 68-77 cmH_2O) in healthy volunteers. Compared with urge incontinent patients (34.8 cm H_2O (SD 5.3 cmH_2O, range 28-41 cmH_2O), anal pressures were higher in stress FI patients. Curiously, those with mixed urge and stress incontinence had the lowest pressures (24.4 cm H_2O (SD 2.4 cmH_2O, range 20-27 cmH_2O). Elgendy et al [282] compared squeeze and cough responses between stress FI patients and healthy controls using an 8-channel, water-perfused manometry system (MMS/Laborie). Findings in stress FI patients were described as "preliminary" and showed either weak sphincters, or a problem with timing of anal contraction during cough (in relation to rectal pressure rise) either with or without weakness. No further information (quantitative or qualitative) in stress FI patients was given in the abstract.

Hoke et al [293] compared anal pressures in stress FI patients with those in patients who identified as having only urge or passive FI. The authors found no differences in anal sphincter pressures (at rest or during squeeze) using a disposable water-perfused catheter system (Medtronic, Inc.). However, rectal capacity (defined as the maximum tolerable volume measured by simple balloon distension during manometry) was lower in the urge FI group compared with other types of incontinence.

Two studies included groups of double incontinent (SUI and FI) patients. In the first study, Lacima et al [277] demonstrated that abnormal response to cough (i.e. when increase in anal pressure was lower than the increase in intrarectal pressure) on ARM (performed with a low compliance, four-channel, water-filled catheter) was significantly more frequent in patients with double incontinence (45.3%) compared with FI (all types) in isolation (21.2%). Anal and urinary sphincter function in double incontinent patients were assumed to have common underlying pathophysiology related to partial denervation of the pelvic floor striated sphincter musculature caused by vaginal delivery, difficult defaecation and chronic straining. Pudendal neuropathy in double incontinence was also evaluated in the study by Ross et al [276] who evaluated surgical success in double incontinent patients (genuine stress and faecal incontinence). They assessed the role of normal/unilateral pudendal nerve terminal motor latencies (PNTML) on the outcome of laparoscopic Burch colposuspension and overlapping sphincteroplasty. Patients with normal PNTML prior to treatment had a significant improvement in anal resting tone and squeeze pressure, while no signifinicant changes were seen in patients with unilateral pudendal neuropathy. Cough response was not assessed during manometry. 'The Neurogenic Hypothesis of Stress Incontinence' [280] discusses the pathophysiological relationship between urinary and faecal stress incontinence in greater detail.

Anal sphincter integrity in stress FI

Four of 14 publications on stress FI discussed assessment of sphincter integrity in relation to symptoms [276,281,289,293]. The study by Ross et al [276] only included FI women with sphincter defects. Dessie et al [281], found that sphincter integrity did not correlate with stress FI symptoms, although overall, only 5 of 31 patients who underwent imaging to determine sphincter integrity (MRI or EAUS) had evidence of sphincter disruption.

Similarly, Benezech et al [289] noted that despite a relatively high prevalence of stress FI in CF patients, obstetric history was not associated with FI and overall very few participants (13% of total participants) were parous, therefore limiting the potential impact of obstetric injuries in the development of FI in this cohort. Finally, no differences in anal sphincter integrity between patients with urge, passive, and stress FI were observed by Hoke et al [293]; EAS defects were found in 16%, 24% and 15% in each group, respectively. IAS defects were observed in 22% of urge FI, 24% of passiveFI, and 19% of stress FI patients.

Studies on cough or cough response in relation to FI

Five studies used survey or interview based methods to identify risk factors for FI or pelvic floor disorders including FI [298-302] **(Table 3a.2**). Two studies examined the risk of FI in chronic cough patients [299,300] or women attending primary care [298]. The remaining two surveys assessed risk factors (including chronic cough) in relation to FI [301,302]. In addition, Badalian [298] identified an (unadjusted) OR of 0.64 (CI 0.21-1.74) for chronic cough in patients with AI, Varma et al [301] reported associations of certain conditions with increased abdominal pressure (e.g. coughing related to COPD and obesity) to contribute to FI pathogenesis, and Yuan et al [302] found chronic cough to be a risk factor for FI in Chinese women.

Cough response was evaluated in thirteen studies (**Table 3a.1**). Eight studies evaluated the cough response in healthy or continent volunteers [30,282,303-308]. Four of these [30,282,305,308] also included groups of FI patients. Groups of only anal or faecal incontinent patients were studied in three articles [273,309,310]. Snooks et al [273] also included double incontinent patients. The cough response was additionally studied in women with voiding difficulties [311] and patients with spinal cord injury [312].

Author	Year	Participants (% female)	Age (mean ± SD)	Population	Summary
Varma	2006	2,106 (100)	55.9 ± 8.6	Community-dwelling women	Risk factors for FI (including chronic cough)
Kuzniar	2007	139 (65)	63.0 (IQR 53.9-72.2)	Women with chronic cough	Prevalence of incontinence (faecal and urinary)
Polley	2008	147 (54)	Asthma 51.6 ± 17.5 Bronchiectasis 57.5 ± 11.8 COPD 64.4 ± 9.7 Chronic cough 53.9 ± 13.3	Chronic respiratory disease patients	Prevalence of incontinence (faecal and urinary)
Badalian	2020	540 (100)	nr	Women attending primary care clinics in Armenia	Prevalence and risk factors for incontinence (faecal and urinary) including chronic cough
Yuan	2020	28,196	44.6 ± 16.24	Community-dwelling women	Risk factors for FI (including chronic cough)

[a] Abstract [b] Full text in Korean
na not applicable
nr not reported

Table 3a.2 Studies on risk factors for stress FI

Anal pressure response to coughing

In total, 9 studies (including the study by Elgendy et al [282], Table 3a.3) used anorectal manometry to assess anal sphincter pressure response to coughing. The anal cough test is simple and quick to perform [313]. Papachrysostomou hypothesised that since the cough anorectal response is not dependent on voluntary effort, it may be a more useful marker of treatment success than other parameters of sphincter function [309]. The authors noted improved cough response in all but three subjects after treatment of FI with pudendo-anal electrical stimulation. However, others have demonstrated variability in cough effort (which is voluntary or dependent on abdominal muscle function [312]) with consequent impact on reflex rise in anal pressures [282,310]. Intra-rectal pressure during cough ranged from 67-321 cmH2O in one study [310]. Anal response to an attempt to increase intra-abdominal pressure also differed greatly between subgroups of spinal cord injury (SCI)[312]. The response was present in most patients with motor incomplete lesions or motor complete lesions below T7, in-keeping with observations that cough response depends on the capacity to contract the abdominal wall and increase abdominal pressure; the reflex is variable in motor complete SCI with neurologic level above T7. The proportional relationship between perineal muscle contractions (quantified by anal sphincter EMG activity) the intensity of the cough (indicated by intravesical pressure) has been systematically studied by Amarenco et al [311]. They concluded that muscle responses were dependent on intravesical pressure, demonstrating that the cough response is not a binary (absent/present) response, but a modulated reflex. The authors hypothesised, that non-graded pelvic floor response (i.e. where increasing cough effort does not show a proportional increase in pressure) could be one of the pathophysiological mechanisms in faecal incontinence in women.

Author	Year	Participants (% female)	Age (mean ± SD)	Population	Summary
Snooks	1984	FI 20 (100) Double 20 (100)	FI 52 ± 18 Double 54 ± 16	Double incontinent and FI patients	Electrophysiological study of common aetiology
Meagher	1993	75 (71)	57 (range 22-82)	FI patients	Manometric response to cough
Papachrysostomou	1994	24 (88)	66 (range 51-70)	FI patients	Manometric response to cough
Arumagam	2004	39 (100)	43 (range 31-65)	Continent women awaiting hysterectomy	Cough incontinence was noted in a proportion of patients with intact sphincters
Amarenco	2005	16 (100)	52 ± 12	Women with history of voiding dysfunction	Pressure (intra-vesical) and EMG activity in response to cough
Deffieux	2006	15 (100)	53 (range 34-78)	Continent and urinary incontinent patients	Pressure (intra-vesical) and EAS/external intercostal EMGi activity in response to cough
Tantiphlachiva[a]	2008	FI 43 HV 46	nr	FI patients and Healthy volunteers	Manometric response to cough
Seong[b]	2009	FI 44 HV 42	nr	AI patients and Healthy volunteers	Manometric response to cough
Valles	2009	44 (41)	43 (range 17-71)	Spinal cord injury	Manometric response to cough
Alqudah	2012	21 (52)	36.5 ± 2.5	Healthy volunteers	Geometric changes to the anal canal (EndoFLIP) during cough
Mion	2018	FI 35 (91) CC 79 (82)	FI 62 (range 20-83) CC 52 (range 18-82)	FI and chronic constipation patients	Manometric response to cough
Mazor	2019	44 (100)	56 ± 12	Healthy volunteers	Manometric response to cough
Vollebregt	2019	FI 192 (80) CC 204 (86) HV 134 (75)	FI 61 (IQR 51-71) CC 46 (IQR 35-57) HV 42 (IQR 31-53)	FI and chronic constipation patients, Healthy volunteers	Manometric response to cough

[a] Abstract [b] Full text in Korean na not applicable nr not reported

Table 3a.3 Experimental studies evaluating cough response

Mion et al [313] measured maximum anal pressure during cough using high-definition ARM in 35 FI patients and 79 patients with chronic constipation. Maximal cough pressure was lower in the FI group and correlated with severity of FI (Jorge-Wexner score) and anal resting pressure. The authors noted that incremental (squeeze) pressure from rest (as opposed to absolute pressure) may be a better indicator of the vigour of EAS contraction. A similar observation may be applicable to cough [310], however in both studies, incremental pressures correlated poorly with FI symptom severity [310,313].

In the studies by Mion et al [313] and Meagher et al [310], anal cough pressures were superior to mean squeeze pressure. Mazor et al [306] evaluated cough response based on the anal cough-squeeze difference. This resulted in a negative average difference (-24 mmHg, 95% CI -45 to -3 mmHg) for the group overall (i.e. anal pressures were below squeeze pressures). However, comparison between nulliparous and parous women showed that parity was associated with a smaller difference between cough and squeeze pressures (-5 vs -54 mmHg, p=0.03). However, squeeze pressures were lower in parous women (173 vs 238 mmHg) which may explain these differences.

Several other ways to report cough were identified from studies. Seong et al [314] used a "cough index" to compare cough pressure response in 44 patients with AI and 42 healthy controls. Although mean resting pressure, maximum squeeze pressure were significantly different between groups, cough index (possibly expressed as a ratio of intra-rectal to intra-anal pressure [176]) was the same between groups. Meagher et al [310] also studied the gradient between rectal and anal pressure and found this correlated with the clinical severity of incontinence compared to maximum cough pressure alone. A positive recto-anal gradient (anal pressure > rectal pressure) had 100% specificity for FI although sensitivity was only 43%. Finally, Elgendy et al [282] examined the temporal differences between anal and rectal pressure increase. They found that in healthy volunteers, anal pressure increase preceded rectal pressure increase by 0.2 msec (0.16-0.3 msec). There was no significant difference in timing between peak of rectal pressure and anal pressure. In total, the rectal pressure increase was 0.6 msec and the anal pressure increase 0.8 msec with the authors concluding that the anal contraction starts before and ends after the rectal pressure rise. These observations are consistent with

those by Deffieux [305] who hypothesised, based on the measurement of EAS and external intercostal EMG latencies, that EAS activity during cough is part of a pre-programmed central nervous system process and occurs in preparation for (rather than in response to) the increase in intra-abdominal pressure to maintain continence. However, these results were only demonstrated in three healthy volunteers and variation in measurements was considerable. The average rectal pressure increase during cough reached 38 mmHg and the average anal pressure was 87 mmHg [282].

Several other studies were identified by the search, but these did not provide meaningful results regarding cough response specifically with regards to the chapter aims [30,304]. Tantiphlachiva et al [308] studied the cough response in 43 patients with FI noting only that the reflex was impaired in 21%. Many other publications in health (including two by members of our unit [5,151]) and disease [30,188,315-319] assessed the cough response using manometry. However, none of these studies were included in search results, which was designed to highlight studies of stress FI in the first instance. However, the fact that some studies on the cough response were identified while others were not suggests there are certain limitations in the search strategy and/or chosen search terms. The cough response in health and in FI will be considered in greater detail in Chapter 4 (Retrospective review of cough response in health and disease) and Chapter 6 (prospective study in health).

Pathophysiological findings in stress FI

In addition to the experimental and survey-based studies already discussed, three guidelines on the performance of cough using anorectal manometry were identified [62,145,146]. In addition, the cough response (in relation to FI) or stress FI was discussed in 7 review articles [29,283,284,295,320-322] and 1 book chapter [280]; the main findings relating to stress FI from these reviews are outlined below.

Stress FI suggests loss of EAS control and results from pudendal nerve or S2-4 lesions [280,285]. Reflex contraction of the external anal sphincter is important in maintaining continence during transient increases in intra-abdominal pressure, as in coughing, laughing, or sneezing [323]. Intra-abdominal pressure rise may also be related to exercise, although data pertaining to FI and exercise generally is very limited [321]. While exercise may be related to increased stress leakage episodes in at least some populations (for

example in CF [289]), *lower* levels of physical activity are generally associated with increased odds of FI, independent of BMI and functional limitations [321,324].

The cough reflex represents the increase in anal sphincter pressure during an abrupt changes in intra-abdominal pressure [322] and is impaired in subjects with cauda equina [145]. In FI, rectal pressure exceeds anal pressure for long enough for faeces to enter the anal canal and pass through the sphincter zone [29,325]. Childbirth, and more specifically obstetric injury, is a clearly recognised risk factors for FI, once thought to be responsible for most cases of FI [29] and iatrogenic factors related to delivery may play a role [29,283]. However, childbirth and obstetric injury cannot be the only cause of FI, clearly, since more recent studies have shown that almost as many men as women have FI [65]. Nevertheless, stress FI may result from damage to the pudendal nerves and direct pelvic branches of the sacral nerve roots (S3-5) during elongation of the birth canal or from direct trauma during passage of the foetal head [111]. Subsequent vaginal deliveries and recurrent stretch injury as a result of perineal descent induced by straining at stool [114] underpin the development of FI [29,280].

In addition to anorectal manometry, impaired anal canal response to abdominal pressure rise may be demonstrated during defaecography [322] by observing for leakage of barium (or other stool substitute) from the rectum under fluroscopy after the asking the patient to cough forcefully. Endoanal ultrasound, manometry, defaecatory proctogram and transit studies were completed in patients without bowel complaints awaiting abdominal hysterectomy or radical prostatectomy [304]. Stress incontinence was demonstrated in 2/45 males and 7/34 females with intact anal sphincters, suggesting that stress FI may occur even in the absence of FI symptoms (asymptomatic or “normal” individuals), presumably during proctography. When cough response was assessed using EndoFLIP [303], changes in the geometric pattern of the anal canal were observed, albeit more subtle when compared to squeeze. Coughing reduced cross-sectional area of the anal canal at both proximal and distal measurement points for the duration of the cough (as indicated by intra-bag pressure increase) and the narrow zone length increased in all healthy volunteers providing evidence of anal canal closure during cough, at least in health.

Summary

To summarise, based on existing literature the prevalence of stress FI amongst patients with FI ranged from 11.7-67.9%. The study of stress FI, usually refered to as leakage which occurs during coughing, sneezing, laughing or exercise, was limited to few studies in FI patients or populations identified to be at increased risk due to frequent coughing (for example cystic fibrosis patients). For example, this review did not find any population based studies of prevalence in the general population. The most commonly reported risk factors for stress FI included smoking [293], urinary incontinence [289,293], abnormal anal-cough response [277] and reduced pelvic floor function [277,292].

Anal sphincter function was generally lower in (stress) FI patients compared to healthy controls, but there were no significant differences in anal canal pressure or sphincter integrity between patients with stress FI and other types of FI (e.g. urge or passive)[292,293]. The anal-cough pressure response tests reflex EAS sphincter response to a sudden rise in intra-abdominal pressure. Although stress FI was generally related to coughing, the cough response was rarely studied in relation to stress FI (except in studies by Lacima et al [277] and Elgendy et al [282]). This may be due in part because there is a lack of consensus on how the cough response should be measured [310]. Nevertheless, studies alluded several 'opportunities' for stress leakage to occur during coughing including a timing issue between rectal and anal pressure responses, a non-modulated anal pressure increment relative to rectal pressure, and the impact of increasing or repeated cough effort on leakage, but also progressive pelvic floor weakness.

There was limited evidence comparing the severity of symptoms in stress FI vs other types of incontinence. Dessie et al [281] noted that stress FI patients were more likely to report leakage of gas than solid stools during coughing, sneezing or laughing, suggesting a less severe form of FI. The canal canal creates a barrier to flow, which Hoke et al [293] found that certain aspects of QoL were less affected in stress FI patients than urge or passive FI patients. However, stress FI was often occurred in conjunction with urge incontinence [292,293]; it may be that patients with mixed symptoms fare worse than those with isolated symptoms.

Conclusion

Contrary to the body of literature on SUI, there is a marked paucity of information available pertaining to stress FI. Further research is needed to understand the relationship between anal canal closure, cough response and stress FI. Although there is sound theoretical basis for the occurrence of stress FI in men and women, few studies have addressed the prevalence and pathophysiology of the condition. In studies which have assessed these, the number of stress FI patients was small [281,292,293], and patient selection was based on unstandardised definitions. Physiological assessment methods were often poorly described. Investigation of phenotypes, such as stress FI, is important for understanding FI as the pathophysiology may be different from other types of incontinence [293]. Furthermore, optimising characterisation of different types of FI could improve existing treatment outcomes and develop novel treatment modalities tailored specifically to FI phenotypes [293].

Chapter 3b Prevalence, symptomatology, and pathophysiology in patients with stress FI: a case control study

Introduction

Faecal incontinence (FI) is typically described as urge, passive, or of mixed nature and signifies a common and significant health problem which impairs quality of life [81,326,327]. Shared and often multifactorial pathophysiology underlies FI irrespective of the amount or type of leakage [72,79,257]. Often, leakage is presumed consequential to sphincter barrier dysfunction and structural defects, especially amongst parous women [112,227] following an assessment of putatitive risk factors including obstetric injuries [6]. However, as demonstrated in Chapter 2 (Systematic review), the relationship between FI and sphincter barrier dysfunction is not always present with many FI patients having normal sphincter structure and function. Further large-scale studies are needed to relate pathophysiology to symptom presentation. Recent evidence also shows that co-existent constipation symptoms (such as incomplete emptying and rectal evacuatory dysfunction) amongst adults complaining of FI are common and often unreported [68,326,328].

Stress urinary incontinence (SUI) is a well-recognised and regularly addressed symptom in urological and urogynaecological fields affecting between 4% to 14% of younger women and 12% to 35% of older women [329]. It is defined by the International Continence Society (ICS) as the complaint of any involuntary loss of urine on effort or physical exertion (e.g sporting activities) or on sneezing or coughing [268]. The pathophysiology of SUI commonly relates to a weakened pelvic floor and is shared between other types of incontinence and pelvic organ prolapse. However, unlike FI which is related to the arrival of stool in the rectum, the bladder fills at a constant rate until emptying occurs.

Unlike its urinary counterpart and as described in Chapter 4a, stress FI is not well described in literature. The ICS only recently defined stress FI as the "complaint of involuntary loss of feces on effort or physical exertion including sporting activities, or on sneezing or coughing" [278]. Nevertheless it is our longterm clinical experience that a proportion of patients describe faecal leakage occuring in instances where abdominal

pressure increases rapidly (coughing or sneezing) or in relation to exercise. Because these complaints commonly exist in conjuction with other FI symptoms, stress FI is rarely the "focus" or primary reason for investigation. However, it is possible that the pathophysiology of stress FI is shared between other types of FI, as is the case in SUI and that learning about it may reveal important targets for investigation and treatment.

The prevalence, symptoms and pathophysiology in patients complaining of stress FI have not been explored in previous literature. Therefore the aims of this chapter are to a) determine prevalence of stress FI symptoms in a large population of patients with FI who have been referred for specialist tests of anorectal physiology in a single tertiary clinic, b) to examine differences in symptoms between patients with and without stress FI, and c) to determine differences in pathophysiology between these groups.

Methods

Study population

A dataset of consecutive patients (aged 18-80 years) who underwent anorectal physiology at the Royal London Hospital Gastrointestinal Physiology Unit (Barts Health NHS Trust) between January 2004 and March 2016 for investigation of refractory symptoms of FI and/or constipation was used for analysis. Other studies on patients included in this dataset have been published [68,103,330].

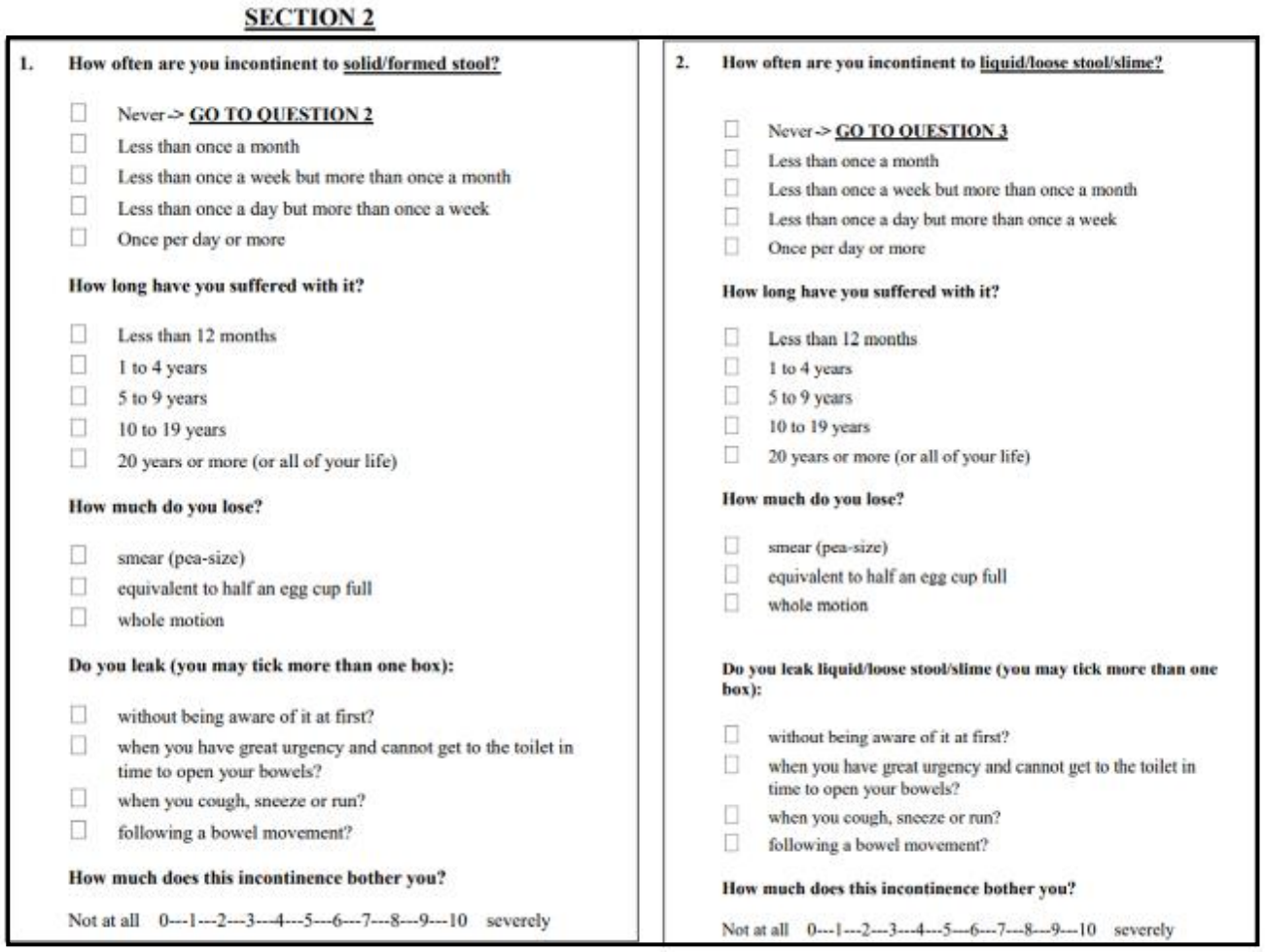
Prior to investigation, all patients completed a comprehensive bowel symptom questionnaire (for detailed description see Mohammed et al 2010 [331]) incorporating previously validated symptom questionnaires and symptom scoring systems for FI (St Mark's incontinence score, range 0-24) [332] and constipation (Cleveland clinic constipation score, range 0-30 [333]. All questionnaires were self-reported and completed at home, and then collected from the patient on the day of the appointment. Patients completed multiple choice questions related to current and past bowel habit, specifically: stool consistency, bowel frequency, duration of symptoms, difficult, incomplete or prolonged evacuation, excessive straining, sensation of blockage, passage of hard/lumpy stools, the use of manual assistance to evacuate, the need to strain, unsuccessful evacuatory attempts, bloating with or without nausea and vomiting, abdominal pain, and laxative use to evaluate 'constipation-type' symptoms.

Symptoms related to FI were assessed separately for solid and liquid leakage detailing the amount, type and situation in which leakage occurred (**Figure 3b.1**); symptoms of bowel prolapse were also systematically questioned. Symptoms were scored according to their frequency of occurrence: 0, never (symptoms do not exist); 1, rarely (less than quarter of the time); 2, occasionally (a quarter to half of the time); 3, usually (more than half of the time); and 4, always.

The questionnaire also documented past medical, surgical, obstetric and gynaecological histories, and details of current medication; clarification of these events was obtained during a structured history. Deliveries involving episiotomy/perineal tear were defined as traumatic and forceps/ventouse-assisted deliveries were defined as instrumental[68]. All patients then provided informed consent for anorectal physiological testing, including anorectal manometry, rectal sensation to balloon distension, and defaecography where indicated. Whole-gut transit studies (radio-opaque marker technique) [334] were performed in patients with <3 bowel movements/week. The principles and measures of these tests have been discussed in the introduction to this thesis and will be further detailed as part of study methods described in Chapters 4-6.

Anorectal manometry

Studies prior to 2013 were performed using a water-perfused, station pull-through technique (Medical Measurement Systems [MMS], Enschede, The Netherlands). Normal values were based on 82 healthy asymptomatic volunteers assessed previously within the GI physiology unit (Vasudevan 2014 phd). Between 2013-2016, studies were performed using a high-resolution manometry system (SolarGI HRM v9.1; MMS) and 12 sensor solid-state catheter (UniTip: Unisensor AG, Attikon, Switzerland). Normal values were published previously in 115 healthy volunteers (HV) [190]. Anal resting pressure below the 5th percentile in HV was termed hypotonia; reduced squeeze increment was termed hypocontractility.



SECTION 2

1. How often are you incontinent to solid/formed stool?

- ☐ Never -> **GO TO QUESTION 2**
- ☐ Less than once a month
- ☐ Less than once a week but more than once a month
- ☐ Less than once a day but more than once a week
- ☐ Once per day or more

How long have you suffered with it?

- ☐ Less than 12 months
- ☐ 1 to 4 years
- ☐ 5 to 9 years
- ☐ 10 to 19 years
- ☐ 20 years or more (or all of your life)

How much do you lose?

- ☐ smear (pea-size)
- ☐ equivalent to half an egg cup full
- ☐ whole motion

Do you leak (you may tick more than one box):

- ☐ without being aware of it at first?
- ☐ when you have great urgency and cannot get to the toilet in time to open your bowels?
- ☐ when you cough, sneeze or run?
- ☐ following a bowel movement?

How much does this incontinence bother you?

Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

2. How often are you incontinent to liquid/loose stool/slime?

- ☐ Never -> **GO TO QUESTION 3**
- ☐ Less than once a month
- ☐ Less than once a week but more than once a month
- ☐ Less than once a day but more than once a week
- ☐ Once per day or more

How long have you suffered with it?

- ☐ Less than 12 months
- ☐ 1 to 4 years
- ☐ 5 to 9 years
- ☐ 10 to 19 years
- ☐ 20 years or more (or all of your life)

How much do you lose?

- ☐ smear (pea-size)
- ☐ equivalent to half an egg cup full
- ☐ whole motion

Do you leak liquid/loose stool/slime (you may tick more than one box):

- ☐ without being aware of it at first?
- ☐ when you have great urgency and cannot get to the toilet in time to open your bowels?
- ☐ when you cough, sneeze or run?
- ☐ following a bowel movement?

How much does this incontinence bother you?

Not at all 0---1---2---3---4---5---6---7---8---9---10 severely

Figure 3b.1 Questions on FI from ADIS questionnaire.

Balloon distension

Balloon distension was performed using a distensible balloon tied to a 14 F Foley catheter and placed in the rectum 10 cm from the anal verge. During gradual and continous distension with air (rate of inflation: 1 ml/s), the patient was asked to report on three sensory thresholds: first sensation, urge, and maximum tolerable volume [9]. Rectal hypersensitivity was defined as a maximum tolerable volume <75 ml [96]. Hyposensitivity was defined as ≥2 rectal sensory thresholds above normal limits [146]. Normal values were based on 91 HV assessed in the GI Physiology Unit.

Endo anal ultrasound

Two dimensional cross-sectional axial images of the anal canal from the level of the puborectalis muscle until the anal verge were acquired using a 13 MHz transducer (BK Medical 2101, Berkshire, United Kingdom). Both the internal and external anal sphincter were classified as intact or abnormal (disrupted, degenerate/atrophic, or focally abnormal). Sphincter disruption was defined by a discontinuity of the muscle ring or by loss of muscle architecture. Degeneration/atrophy was diagnosed if the anal sphincter was thin or poorly defined, often with increased echogenicity. Focal abnormalities were defined by scarring, thinning or an area of mixed echogenicity [68].

Defaecography

Evacuation proctography was performed in all patients according to the departmental protocol [335] without prior bowel preparation. Enough neostool was instilled using a large bore syringe to stimulate a sustained desire to defaecate. Functional abnormality was assessed in terms of speed (time taken for evacuation) and effectiveness (percentage of neostool evacuated) [336]. Incomplete evacuation was defined as <60% of neostool expelled and protracted evacuation was defined as duration >150 seconds [142]. Evacuation was allied to poor opening of the anorectal angle, poor relaxation of the anal canal or poor expulsive force generated [335].

The classification of structural abnormalities has been described previously [68]. These comprised significant intussusception (obstructing recto-rectal [Oxford grade I – II] or recto-anal [Oxford grade III–IV])[337], rectocoele (depth >4cm or 2-4 cm allied to symptoms of obstructed defaecation) [142,335], enterocoele [142], megarectum (mid rectal

diameter >8·1 cm in men and >6·9 cm in women; routinely measured from 2013 onwards) [335] and external rectal prolapse (Oxford grade V)[337].

Whole-gut transit

Transit studies were performed in patients who reported fewer than 3 bowel movements/ week. Delayed whole-gut transit time was diagnosed if 20% or more of 50 ingested markers were retained at 100 hours after ingestion, as visualised on a plain abdominal radiograph [334,338].

Inclusion and exclusion criteria

The study sample was restricted to patients with a minimum dataset of: (i) primary reason for referral documented in the referral letter; (ii) complete self-reported questionnaires; (iii) anorectal physiology and defaecography performed. Exclusion criteria were: age <18 or ≥ 80 years, patients examined outside of the study period, patients with incomplete questionnaires (defined as ≥2 missing values for SMIS, CCCS, or no details on parity/referral), patients with stoma *in-situ*, and patients without self-reported FI.

Definition of cases and controls

Patients with self-reported solid and/or liquid FI at least 1/month were identified based on their answers to questions the following questions: “how often are you incontinent to solid/formed stool?” and “how often are you incontinent to liquid/loose stool/slime?” (**Figure 3b.1**). Of patients with FI, stress FI ‘cases’ were identified as those FI patients who ticked ‘yes’ for the follow-up question “Do you leak when you cough, sneeze or run?” for either type of leakage (solid/liquid).

Identified cases were matched for gender, age, and parity (nulliparous/parous) on a 1:2 basis with FI controls who did not admit to leaking during coughing, sneezing, or running (controls). First, the number of cases in each 5-year age group for gender and parity was identified. Then a computer-based algorithm was used to randomly select the required number of ‘controls’ in each gender/age/parity group (**Appendix 3**).

Clinical characteristics

Occurrence of risk factors [81,326,327,339] were derived from the structured history and questionnaires and their prevalence compared between groups (cases/controls).

Detailed symptomatology and differences in anorectal physiological testing were also compared between groups.

Data analysis

Descriptive statistics were performed to describe cases and controls. Differences in risk factors, symptoms and proportions of patients with abnormal anorectal physiological measurements in cases and controls were compared using chi-square tests/ Fishers' exact test for categorical variables and parametric and non-parametric ANOVA methods for continous variables. To account for multiple tests, a p-value of <0.01 was considered significant.

Results

Prevalence of FI

In total 3353 patients met inclusion and exclusion criteria, of whom 2136 (63.7%) reported symptoms of FI (**Figure 3b.2**). Of these patients, 595 patients (509 females [85.5%]) had FI during coughing, sneezing or running as were labelled as stress FI 'CASES'. This represents an overall prevalence rate of 27.9% for stress FI amongst patients with FI.

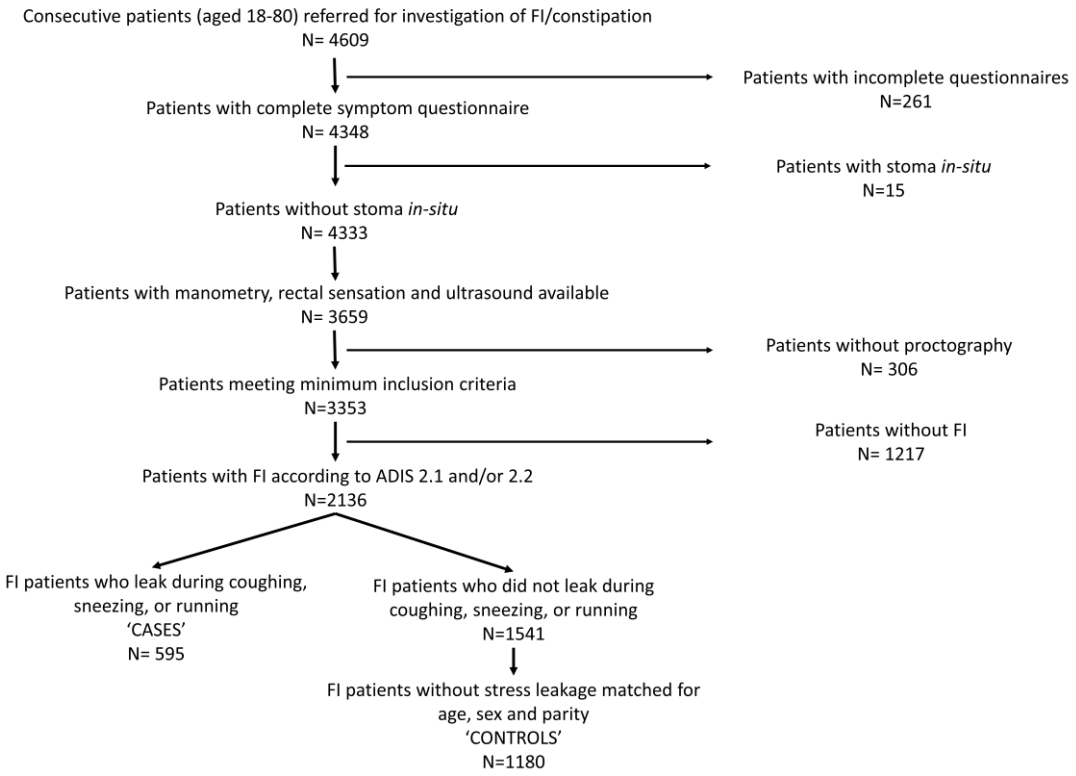


Figure 3b.2. Flow diagram showing the selection of the study population from patients referred to the Gastrointestinal Physiology Unit for investigation of symptoms of refractory faecal incontinence and/or constipation

Stress FI was reported by both sexes, in all age groups, and irrespective of parity. A quarter of FI males (25.1% [86/343]) and 28.4% of FI females (509/1793; p= 0.2095 vs males) had stress FI. The proportion of nulliparous women with stress FI (58/218; 26.6%) was similar to the proportion of parous women with stress FI (451/1575; 28.6%, p=0.5334). The majority of stress FI individuals were parous women (21.1%) reflective of the clinical population typically referred for GI physiology at the Royal London Hospital (**Figure 3b.3A**). The majority of parous women with stress FI were aged 51-60 years. Similarly the majority of males with stress FI were between ages 51-60 years. The majority of nulliparous stress FI patients were under age 30, but overall they represented a small proportion of the population (**Figure 3b.3B**).

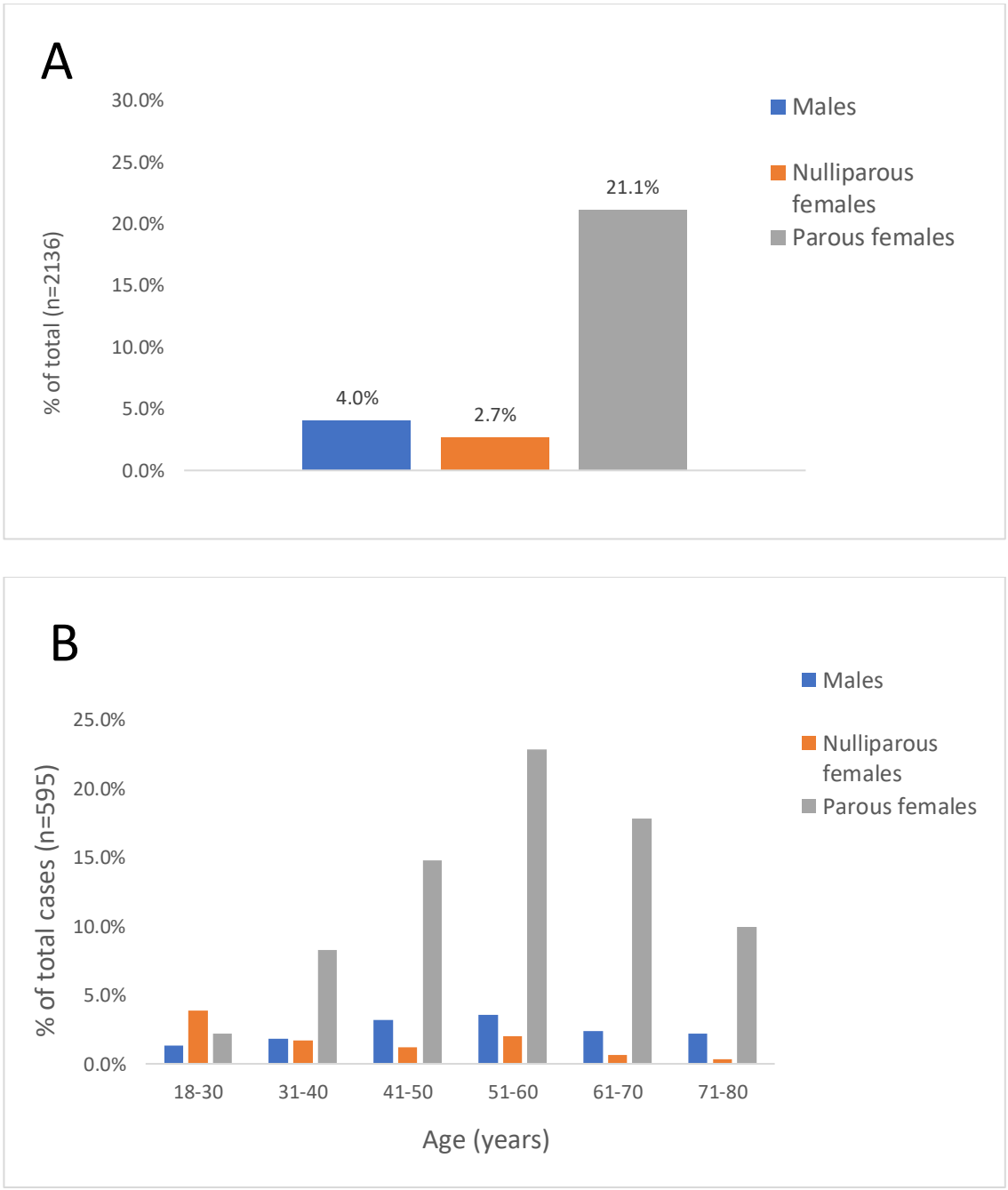


Figure 3b.3. Prevalence of stress FI cases (A) as a proportion of all FI patients categorised by gender and parity; (B) and age group.

Demographics and putative risk factors amongst cases and controls

Following random case-matching for age, sex, and parity, 1180 'CONTROLS' were identified from a pool of 1541 FI patients without stress FI. Details of the numbers required for matching in each group and any 'incomplete' groups are shown in Appendix 3. The demographic details for cases and controls are shown in **Table 3b.1**.

	Stress FI ***n*** **(%)**	**No stress FI** ***n*** **(%)**	***P*-value**
Sex (*n*, %)	595	1180	>0.9999
Female	509 (85.5)	1009 (85.5)	
Male	86 (14.5)	171 (14.5)	
Age (median, min-max)	55 (18-84)	55 (18-80)	0.6338
Obstetric history (*n*, %)			
Nulliparous	58 (9.7)	107 (9.1)	-
Parous	451 (75.8)	902 (76.4)	0.6626
Number of deliveries[1]			
1	68 (15.1)	120 (13.3)	-
2	170 (37.7)	451 (50.0)	-
3	144 (31.9)	199 (22.1)	-
≥4	69 (15.3)	132 (14.6)	**<0.0001**
Traumatic vaginal delivery[1]	356 (78.9)	715 (79.3)	0.8873
Instrumental delivery[1]	128 (28.4)	232 (25.7)	0.2973
Cesarean section[1]	63 (14.0)	145 (16.1)	0.3377
Surgical history (*n*, %)			
Abdominal/bowel surgery	162 (27.2)	336 (28.5)	0.6146
Pelvic surgery, including hysterectomy	243 (40.8)	471 (39.9)	0.7198
Anal/perineal surgery	106 (17.8)	248 (21.0)	0.1160
Rectal			
Comorbidities			
Diabetes	78/465 (16.5)	110/945 (11.6)	**0.0077**
Opioids	94 (15.8)	181 (15.3)	0.8007
Antidepressants	164 (27.6)	289 (24.5)	0.1612
Childhood bowel problems	144/572 (25.2)	274/1142 (24.0)	0.1443

Table 3b.1. Demographics, obstetric, surgical, and medical history of case matched FI patients with and without stress induced leakage.

There were no significant differences between groups for gender, age, or number of parous and nulliparous women in each group. However, there was a significant difference in the number of deliveries amongst parous women in those with and without stress FI; the mean number of deliveries was also higher for cases (2.6, SD= 1.262) compared to controls (2.5, SD= 1.110), p =0.039). There were no significant differences between rates of traumatic vaginal deliveries (78.9% vs 79.3%, p=0.8873), instrumental deliveries (28.4% vs 25.7%, p=0.2973), and Caesarean sections (14.0% vs 16.1%, p=0.3377) between groups. History of diabetes was significantly greater in cases (16.5%) compared with controls (11.6%, p=0.0077). Meanwhile, surgical history and rates of other comorbidities did not differ between groups.

Symptom profiles in stress FI

Faecal incontinence symptoms

Patients with stress FI (cases) had significantly higher median St Marks Incontinence Score (Table 3b.2); median SMIS score 15 (IQR 11-18) vs 12 (IQR 9-16) in controls ($p<0.0001$). Cases were also more likely (90.8% vs 81.2%, $p<0.0001$) to have SMIS score ≥6.

In both groups, the majority of subjects were incontinent to solid and liquid faeces. The type of incontinence was significantly different between groups ($p<0.0001$). A greater number of subjects experienced liquid leakage (559/595 in cases; 1059/1180 in controls, $p=0.0032$ vs no liquid leakage) than solid leakage (433/595 in cases, 766/1180 of controls, $p=0.0008$).

A greater proportion of patients described urge (67.1% vs 59.7%, $p=0.0027$) and passive (67.2% vs 58.2%, $p= 0.0002$) type of incontinence compared with controls. A larger proportion of cases (47.6%) described mixed urge and passive symptoms compared to controls (30.5%, $p<0.0001$). There were no differences in the amount of post-defaecatory leakage/soiling ($p=0.1084$). Amongst cases, 8.2% (49/595) experienced stress FI in isolation.

Flatus incontinence (more than 1/month) was experienced by 80.5% of cases vs 65.8% of controls ($p<0.0001$). Symptoms had been ongoing for more than 5 years in 38.7% of cases and 27.6% of controls ($p<0.0001$). Cases (78% vs 71%, $p=0.0018$) were also more likely to experience faecal urgency. Overall, stress FI patients had a worse phenotype than controls.

	Cases *n* (%)	Controls *n* (%)	*P*-value
St Marks incontinence score Median (IQR)	15 (1-24)	12 (1-24)	**<0.0001**
Solid leakage	36 (6.1)	121 (10.3)	-
Liquid leakage	162 (27.2)	414 (35.1)	-
Solid and liquid leakage	397 (67.7)	645 (55.7)	**<0.0001**
Faecal urgency	464 (78.0)	838 (71.0)	**0.0018**
Frequency > monthly	536 (90.1)	953 (80.8)	**<0.0001**
SMIS ≥6	540 (90.8)	958 (81.2)	**<0.0001**
Duration of symptoms (>5 yrs)	238 (40.0)	373 (31.6)	**0.0004**
Type			
Urge	399 (67.1)	705 (59.7)	**0.0027**
Passive	400 (67.2)	687 (58.2)	**0.0002**
Post-defaecation	321 (53.9)	589 (49.9)	0.1084
Mixed (urge and passive)	283 (47.6)	360 (30.5)	**<0.0001**
Flatus incontinence			
Frequency > monthly	479 (80.5)	776 (65.8)	**<0.0001**
Duration of symptoms >5 yrs	230 (38.7)	326 (27.6)	**<0.0001**
Use of constipating medications	151 (25.4)	254 (21.5)	0.0632

Table 3b.2 FI symptoms in cases (patients with stress FI) and controls (FI patients without stress FI)

Constipation symptoms

Some constipation symptoms were experienced by a significantly higher proportion of cases than controls (**Table 3b.3**). Patients with stress FI experienced more frequent abdominal pain (68.7%) than the control group (62.2%, p= 0.0074). Evacuation was more likely to be painful (58.2% vs 52.7%, p= 0.0301) in cases. There was no difference in CCCS score [333] between groups (mean 13.1 in patients vs 12.5 in controls, p=0.0630). While a feeling of incomplete evacuation was not significant at 99% significance level, which was chosen to account for multiple tests, 88.1% of stress IF patients reported a feeling of incomplete emptying compared with 83.6%, of controls (p=0.0134).

Bloating (40% cases vs 35% of controls, p=0.0083), rectal bleeding (49% vs 42.6%, p=0.0112) and mucus discharge per rectum (72.4% and 65.6%, p=0.0042) were all more frequently reported in cases with stress FI. The feeling of a blockage leading to difficult

bowel emptying was considerable and similar between groups (circa 70%). Meanwhile the feeling of a bulge or prolapse was experienced by 50-55% of all subjects.

	Cases (*n,* %)	Controls *n* (%)	*P*-value
Cleveland Clinic constipation score			
Median (IQR)	13 (8-18)	12 (7-17)	0.0630
Bowel movements (≤ once per week)	72 (12.1)	141 (11.9)	0.9384
Painful evacuation effort (≥ sometimes)	346 (58.2)	622 (52.7)	0.0301
Feeling incomplete evacuation (≥ sometimes)	524 (88.1)	986 (83.6)	0.0134
Abdominal pain (≥ sometimes)	409 (68.7)	734 (62.2)	**0.0074**
Minutes in lavatory per attempt (≥ 10 minutes)	218 (36.6)	383 (32.4)	0.0799
Assistance for defaecation (digital assistance or enema)	268 (45.0)	565 (47.9)	0.2679
Unsuccessful attempts per 24 hr (≥ three attempts)	308 (51.8)	584 (49.5)	0.3927
Duration of constipation (≥ five years)	280 (47.1)	528 (44.7)	0.3638
Oral laxative use	200/558 (35.8)	443/1108 (40.0)	0.1014
Bloating (>25%)	240/586 (40.0)	401/1162 (35.0)	**0.0083**
Feeling of a blockage/difficult emptying	407/586 (70.8)	810/1148 (70.6)	0.6347
Feeling of bulge or prolapse	325 (54.6%)	589 (49.9)	0.0611
Blood loss per rectum	281/573 (49.0)	485/1139 (42.6)	0.0112
Mucous discharge per rectum	410/566 (72.4)	744/1135 (65.6)	**0.0042**

Table 3b.3 Constipation symptoms in cases (patients with stress FI) and controls (FI patients without stress FI)

Impact of symptoms on normal living

Moderate or greater interference with daily tasks due to symptoms was observed more often in controls (385/1180, 32.6%) than cases (136/595, 22.9%, p<0.0001).

Anorectal physiological measurements stress FI

Anal sphincter structure and function

On endoanal ultrasound, cases were more likely to have a disrupted internal anal sphincter (20.2% vs 15.5%, p=0.0138) compared to controls (**Table 3b.4**). Further, cases were more likely to have reduced squeeze increment (hypocontractility) on manometry (p=0.0094). Meanwhile controls were more likely to have normal pressures (p<0.0001).

Rectal sensory testing

The majority of cases (82.2%) and controls (83.2%) had normal rectal sensory function (p=0.5846). Hypersensitivity appeared more common than hyposensitivity in cases (11.3% vs 6.6%) and controls (11.7% vs 5.1%) but with no significant difference between groups.

Whole-gut transit time

In total, 550 individuals had a transit study; these were performed equally between cases (31.3% of all cases) and controls (30.8% of all controls). Whole-gut transit was delayed in 27.4% of cases and 35.2% of controls (p=0.0666).

Defaecography

Defaecography revealed an isolated functional abnormality in a similar proportion of cases and controls. In respect of structure, around half of cases (48.9%) and controls (50.7) showed rectal intussusception; these rates were comparable between groups (p=0.4813). Similarly, high rates of rectoceles were observed (around 61%) in each group; around 40% of rectoceles in each group were either significantly large (≥ 4 cm) or symptomatic (did not empty). Patients with stress FI (cases) showed higher rates of external prolapse (4.5% vs 2.7%, p=0.0428) on imaging.

Variable	Cases *n* (%)	Controls *n* (%)	*P*-value
Anorectal manometry			
Normal	216 (36.3)	565 (47.9)	**<0.000**
Anal hypotension + normal contractility	77 (12.9)	121 (10.3)	**1**
Anal normotension + hypocontractility	180 (30.3)	289 (24.5)	0.0896
Anal hypotension + hypocontractility	122 (20.5)	205 (17.4)	**0.0094**
			0.1082
Rectal sensory testing			
Normal	489 (82.2)	982 (83.2)	0.5846
Hyposensitive	39 (6.6)	60 (5.1)	0.2027
Hypersensitive	67 (11.3)	138 (11.7)	0.7869
Endoanal ultrasonography			
Internal anal sphincter			
Intact	335 (56.3)	717 (60.8)	0.0710
Disrupted	120 (20.2)	183 (15.5)	**0.0138**
Degenerate	138 (23.3)	254 (21.5)	0.4239
Abnormal, focal	36 (6.1)	75 (6.4)	0.8019
External anal sphincter			
Intact	288 (48.4)	594 (50.3)	0.4413
Disrupted	201 (33.8)	352 (29.8)	0.0898
Degenerate	66 (11.1)	101 (8.6)	0.0844
Abnormal, focal	84 (14.1)	200 (16.9)	0.1245
Whole-gut transit studies			
Performed	186 (31.3)	364 (30.8)	0.859
Delayed	51 (27.4)	128 (35.2)	0.0666
Evacuation proctography			
Functional abnormality	126 (21.2)	278 (23.6)	0.2584
Significant structural abnormality			
Intussusception	291 (48.9)	598 (50.7)	0.4813
Obstructing recto-rectal	69/291 (23.7)	147/598 (24.6)	0.7764
Recto-anal	94/291 (32.3)	212/598 (35.5)	0.3537
Rectocoele	365 (61.3)	729 (61.8)	0.8588
Depth ≥4cm	84/365 (23.0)	165/729 (22.6)	0.8876
Depth 2–4cm, symptomatic	57/365 (15.6)	130/729 (17.8)	0.3585
	25 (4.2)	68 (5.8)	0.1635
Enterocoele	37/259 (14.3)	67/497 (13.5)	0.7604
Megarectum	27 (4.5)	32 (2.7)	**0.0428**
Prolapse	46 (7.7)	104 (8.8)	0.4389
Functional + structural abnormality			

Table 3b.4 Outcomes of physiological testing in cases and controls

Discussion

The aims of this chapter were to identify the proportion of FI patients who experience leakage associated with coughing, sneezing, or jumping (stress FI) based on self-

reported questionnaire data collected in patients attending a tertiary level GI physiology service. We then sought to describe the symptom profile and physiology results in FI patients with and without stress FI symptoms using a matched case-control design.

The main findings were:

- Over a quarter of FI patients describe instances of faecal leakage associated with stress.
- Stress FI occurs equally in both sexes and regardless of parity, and across all ages.
- Overall, FI symptoms occur more frequently and appear worse in those with stress FI (i.e. stress FI is additive), but do not follow a distinctly different pattern to the FI symptom profile of patients without stress FI.
- Meanwhile, many constipation symptoms appear of greater burden in stress FI, namely those related to incomplete emptying, painful evacuation, and rectal bleeding. Abdominal pain and bloating are also more frequent as are feelings of prolapse and mucus discharge per rectum.
- Structurally, IAS disruption is more common in cases, as is anal hypocontractility, indicative of EAS dysfunction
- Symptoms suggestive of prolapse are supported by the higher rate of external prolapse in cases observed using defaecography and occurred in a small number of subjects; rates of other types of prolapse were high in both groups

Prevalence and symptom severity in stress FI

Over a quarter of patients with FI (27.9%) attending a tertiary sector GI physiology service associated at least part of their incontinence episodes with *stress* (coughing, sneezing or running). In the previous chapter, we showed that very few studies have assessed prevalence of stress FI in clinical populations and there is no data on prevalence in the general community. Meanwhile, one study showed that coughing, sneezing, laughing and sport were the most prevalent reasons for FI episodes amongst 40 FI patients with cystic fibrosis[289], with no significant difference in prevalence

between men and women. Similarly, the prevalence of stress FI in the current study was found to similar in men (25%) and women (28%, p>0.05).

In the literature, anal sphincter tears during vaginal and instrumental deliveries are the most frequent aetiological factor for FI[112,220]. Indeed, the majority of patients in the current study were multiparous women. However, similar proportions of nulliparous (27%) and parous (29%, p>0.05) women reported stress FI. Furthermore, the rates of traumatic, instrumental or C-section deliveries were similar amongst parous cases and parous controls suggesting that sphincter disruption is unlikely to be the major causative agent leading to stress incontinence in particular. Furthermore, only IAS disruption was found to be significantly more common in cases (p=0.0138). Reasons for stress incontinence other than those related to obstetric history have been proposed previously [289] with pelvic floor weakness being the most common reason for incontinence in cystic fibrosis patients[340].

Others have suggested that pregnancy and childbirth are generally held to be major risk factor for incontinence due to the potential for developing lesions to the peripheral nerves supplying the sphincters and the pelvic floor [341]. Stress FI was associated with more EAS dysfunction (hypocontractility) on anorectal manometry, however these differences were modest (30% vs 25%, p= 0.0094) compared with controls. Non-stress incontinent controls were also more likely to have normal sphincter function (normal tone and contractility; p<0.0001 vs cases). These results, supported by high rates of structural abnormalities on defaecography, suggest a degree of pelvic floor weakness amongst included patients, at least in response to voluntary contraction. However, continence (urinary and faecal) during stress is dependent on the synchronous *reflex* contraction of the striated urethral/anal sphincter and all the pelvic floor muscles[342-344]. The sphincter's involuntary contractile response may be measured manometrically in response to cough[310] or Valsalva[7] or monitored using electromyography (EMG)[311,343], neither of which was measured in the current study.

The importance of the guarding reflexes is well documented in urological literature. The guarding reflexes are a distinct set of neural reflexes which prevent involuntary emptying of the bladder during filling [345,346]. Under normal circumstances, the bladder fills progressively at low intravesical pressure. External urethral sphincter (EUS) activity

increases involuntarily to maintain continence. As the sensory threshold for bladder filling is reached, EUS activity is further increased voluntarily. Meanwhile, enhancement of the guarding reflex (EUS contraction) suppresses bladder contractility, until voiding is timely. Micturition is initiated by the suppression of the guarding reflex (EUS relaxation). Coughing prompts an augmented guarding reflex (EUS contraction) to prevent unwanted urine loss during situations of stress [347,348]. This increased reflex activity has been proposed as the primary mechanism to mitigate against unwanted stress incontinence [345].

In addition to pregnancy and childbirth, constipation and straining at stool may contribute to neuromuscular deterioration [349]. Overall, constipation related symptoms were highly prevalent in all patients, irrespective of stress FI status. In addition to intact neural pathways, correct anatomical position and pelvic floor support are integral for bladder and bowel function [350,351]. Pelvic floor impairment leads to increased stretching and work of the suspensory ligaments and fascia of the pelvic organs [352]. During coughing and under normal circumstances/in health, abdominal pressure is transmitted to the urethra closing it; this is known as the hammock theory[353]. In women with SUI pressure transmission is often reduced[354] due to urethral hypermobility [351,355-357], which is present in the majority of women with SUI [358]. Similarly, Parks[359] proposed that a flap-valve mechanism, dependent on an obtuse rectoanal angle, was responsible for maintaining continence during coughing. Later the authors, proposed pudendal neuropathy as the unifying theory between prolapse, perineal descent and FI[48]. Although the current study did not evaluate pudendal nerve terminal motor latency (PNTML), we did observe significantly higher rates of diabetes (a risk factor for peripheral neuropathy and incontinence)[360] amongst stress incontinent patients.

In addition to evidence of significant structural abnormalities on defaecography suggestive of rectal evacuatory disorder[361], stress incontinent patients reported incomplete emptying more frequently than controls (p=0.0134). Intuitively, for stress incontinence to occur, the bladder or rectal reservoir must contain urine/stool when intra-abdominal pressure suddenly increases. Defaecation [362-364] and bowel frequency [365] are temporally associated with high amplitude propagating contractions (HAPC) i.e. the manometric equivalent of mass colonic movements. In adults, HAPCs occur on

average 5-6 times a day (range 3-24) [366], though only a third of these were found to reach the anus according to one study[364]. This is consistent with a "normal" frequency of defaecation between three motions per day to three motions per week [367,368]. Since voluntary defaecation generally occurs shortly after the signalling of stool arrival in the rectum under normal circumstances, the chances of intra-abdominal presure increase coninciding with stool presence in the lower rectum are relatively small. This might be contrasted with SUI, which as a result of constant bladder filling, has the potential to occur whenever intra-abdominal pressure is raised [329,369].

As a result, stress FI episodes may be infrequent and perceived as mild at first despite presence of underlying pathophysiology. Our analysis showed that stress FI was associated with a longer duration of FI symptoms compared to controls. It is plausible that the underlying pathophysiology of stress FI develops slowly over time, as is typically the case in SUI[370]. Over time, or with further insult to the pelvic floor the well-compensated pelvic floor becomes functionally decompensated [371] and the development of more severe forms of incontinence ensues, prompting investigation.

Urge incontinence (associated with urgency, liquid leakage, and EAS dysfunction) and passive incontinence (associated with a lack of sensory awareness, solid stool, and IAS dysfunction) are usually considered to be more severe forms of incontinence[72]. Stress incontinence occurred in conjunction with other forms of incontinence in 92% of cases with a signficantly greater number of patients suffering from mixed FI compared to controls. Accordingly, stress FI was associated with higher SMIS, more frequent FI, and more flatus incontinence in modest, but significant, rates compared with controls, suggesting that stress FI may be a biomarker for more severe incontinence symptoms. Rather spuriously then, quality of life was less impacted in cases than controls. However, this finding could be explained by how impact on quality of life was evaluated, i.e. in terms of 'interference with daily tasks'. It is possible that patients had developed sufficient coping mechanisms over time such as avoiding predictable behaviours (like exercise or coughing) which they knew to be challenging for continence[289] and therefore symptoms were not perceived as 'interfering' even if they were distressing.

Limitations

Our choice to perform a case control study was based on the assumption that gender, parity, and age may have an impact on anorectal physiology. The main weakness of case control studies is the potential for introducing biases, including measurement bias and selection bias[372]. Although this chapter is based on analysis of retrospective data, patients completed symptom questionnaires prospectively and tests were performed in the same way in all patients (irrespective of presenting symptoms). In addition we used a random sampling algorithm to select controls from a pool of subjects. Admittedly, due to the specific age bins chosen, some of these pools were small.

Aside from the choice of design, this study has several limitations. Firstly, as alluded to previously, manometrically weak muscles were not investigated with EMG or PNMTL to determine the cause for the abnormality[373]. Thus the presence or absence of neural dysfunction has not been confirmed using neurophysiological techniques. However, since the analysis was based on retrospective data although this had been filled in prospectively by patients, prior to any physiological tests, we were limited to the procedures performed routinely at the time of data collection. Future studies should evaluate the manometric and EMG responses to coughing, and evaluate these response in relation to stress FI.

Secondly, although the data were collected over a number of years representing a relatively long study period, the cross-sectional nature of the study means these data represent a mere snapshot of findings from a single tertiary sector physiology unit. Furthermore, we limited analysis to only those individuals with complete physiology studies, including defaecography. This suggests a degree of selection bias toward patients with rectal evacuatory dysfunction by default, however, a number of previous studies have shown that co-existent constipation is a common feature of FI [68,374,375].

We used a computer based algorithm to select controls based on matching for age, gender and parity, which was known to impact anorectal physiological function[146]. However, due to the limited numbers of younger patients typically seen in our unit, 2:1 matching was not possible in a minority of age categories.

Finally, the presence/absence of stress FI was determined by asking patients if their FI is associated with coughing, sneezing or running. Although this question was designed to interrogate incontinence related to episodes of increased abdominal pressure, it does not differentiate between events that occur during periods of urge (i.e when the patient feels the need to empty their bowels anyway) and no urge. Furthermore, using 'running' as indicative of exercise may be confusing. So called 'runners diarrhoea' which is related to stool consistency and stimulation of colonic activity through exercise rather than intra-abdominal pressure, may occur in some patients. Furthermore, in patients with urge incontinence, it may be associated with 'running for the toilet' misinterpreting urge incontinence as stress FI. Despite this, our analysis has clearly shown that stress incontinence is a prevalent and important symptom and there may be the need to rephrase questions in the future.

Conclusion

Stress FI was present in over a quarter of FI patients and represented a more severe FI phenotype overall (higher SMIS, more frequent FI, longer duration of symptoms, more likely to have mixed symptoms, more flatus incontinence, and more co-existence) compared to non-stress incontinent controls). Although stress FI patients had more hypocontractility, pathophysiology was only modestly different to controls overall. Based on the importance of a neurologically intact pelvic floor in SUI, future studies should determine anal sphincter response to coughing by incorporating electrophysiological measurements with manometry.

Chapter 4 Retrospective analysis of the cough response in healthy volunteers and FI patients

Introduction

Anorectal manometry is the principal diagnostic tool to assess anal sphincter dysfunction in faecal incontinence (FI). Anal resting pressure, a primary indicator of internal anal sphincter (IAS) function, and voluntary squeeze increment pressure, a measure of external anal sphincter (EAS) and likely puborectalis contractility [376,377,153] are the most recognized and consistently reported measures of anal function [148]. However, although mean resting pressures and squeeze increments are generally regarded as being reduced in FI compared to healthy subjects, only approximately one-third of individual patients have anal hypotension, and only two-thirds of patients have voluntary anal hypocontractility [90,261,378]. Accordingly, for such a high proportion of symptomatic individuals to exhibit 'normal' function, either there are suprasphincteric factors of equal or greater importance for continence than anal barrier function (e.g. rectal sensation, compliance, stool form and volume etc.), or traditional measures (rest and squeeze) lack specificity to have dependable diagnostic value. Indeed, the limited utility of these measures to distinguish between health and disease has hindered their acceptability as clinically meaningful measures of anal function for decades [379,62,262,146].

High-resolution anorectal manometry (HR-ARM), with improved spatiotemporal resolution, may allow for assessment of other, or more subtle features of anorectal function including visualization of dynamic events and simultaneous pressure measurement at multiple levels. Novel measures may enhance diagnostic value and utility of the technique. For example, several guidelines and best practice documents advocate assessment of anorectal pressure responses to cough as part of the manometry protocol [380,62,145,146]. Indeed, 83% of 107 centers responding to an international survey of manometry practice [148] reported that they routinely performed a cough maneuver. However, despite its perceived simplicity, the method for analysis and reporting of cough varied widely, with most respondents (42%) reporting only qualitative impression of muscle recruitment. Of quantitative measures, maximum anal pressure was most common (28/89 centers or 31%).

The involuntary external anal sphincter contractile response to cough [381] is mediated by a spinal reflex [343] and may be observed during other activities which increase intra-abdominal pressure including sneezing and postural changes [382], or inflating a rectal balloon [181]. A 'normal' cough response on manometry has a measurable increase in anal pressure, the duration and amplitude of which is believed to exceed the increase in cough-generated rectal pressure, so that anal sphincter barrier function is maintained despite the intra-abdominal / intra-rectal pressure challenge [145,62,380,146]. A 'post-cough relaxation' or drop in anal resting pressure following a sudden increase in abdominal pressure by coughing[5] or by blowing up a balloon [181], may be seen in some individuals and is akin to the 'early relaxation' pattern observed by Gowers [383] in response to mucosal irritation during coughing.

While cough-anorectal pressure responses have been documented previously [5,151,306,384] no study has applied HR-ARM to qualitatively and quantitatively study changes with parity (in health) and with disease (fecal incontinence). This was the aim of the current study through systematic, retrospective analysis of HR-ARM recordings.

Methods

Study population

Healthy volunteers (HV)

Volunteers were recruited by advertisement between 2012-13 and had no history of significant gastrointestinal disease. All had a St Marks incontinence score [332] ≤5 and Cleveland Clinic Constipation Score [333] ≤8. Exclusion criteria were pregnancy or lactation, history of diabetes, cardiovascular, renal or hepatic disease. HR-ARM recordings were scrutinized, and only studies that incorporated at least one cough maneuver were considered for inclusion. Ethical approval was provided by Queen Mary University Research Ethics Committee (ref QMREC 2010/74; QMREC 2013/12) and written informed consent was provided by all volunteers. Results of other HR-ARM measurements from this cohort have been reported on previously.[5,166,30]

Patients

Consecutive parous, female patients attending The Royal London Hospital GI Physiology Unit between January 2018 and December 2018 for routine investigation of fecal

incontinence were considered for inclusion. Patients were included if they had a St Marks incontinence score [332] >10. Patients referred for a primary presenting symptom of constipation/evacuation disorder, or for symptoms of prolapse, anal fistula, or cancer were excluded. Further exclusion criteria were history of diabetes or inflammatory bowel disease, known neurological disease, and any anal or pelvic floor surgery (except vaginal hysterectomy and primary sphincter repair in the case of 3rd or 4th degree tears sustained during childbirth). For parous groups to be comparable for age, all patients over the age of 72 years were deselected (n=6) prior to further analysis.

All subjects (HVs and patients) underwent HR-ARM and assessment of rectal sensation to balloon distension. In addition, all patients with FI (but not HV) also underwent endo-anal ultrasonography and a proportion (36/57, 63%) underwent defecography. All tests were performed and interpreted in accordance with departmental protocols [96,335,142]. During defecography, as part of the standardised protocol, maintenance of continence was evaluated following insertion of barium contrast, both during transfer of the patient to the commode, and also under fluoroscopy on instruction to cough.

HR-ARM

Technical specifications and test procedure

All participants underwent investigation using a 12F solid-state catheter (UniTip: UniSensorAG, Attikon, Switzerland) incorporating 12 unidirectional pressure transducers each embedded within a silicone gel cuff. Prior to the study, the catheter was immersed in tepid water for at least 3 minutes to pre-wet sensors, which were then zeroed. Data acquisition and visualization was performed using a commercially available manometric system (Solar GI HRM V.9.1, Medical Measurement Systems, Enschede, The Netherlands). Data were generated at 10 Hz. Manometry was performed using a ratified protocol.[5]

Cough selection

Each HR-ARM trace (irrespective of health or disease status) was examined for trace quality and presence of single, discrete cough (as opposed to multiple, rapid coughs, which are frequently observed). A study was included in the final analysis if: a) at least one single cough had been performed; b) there were discernible and distinct anal and

rectal pressure areas; and c) 'traditional' resting and squeeze pressures could be measured. Traces with artefacts affecting the quality of the recording were excluded. When two coughs had been performed as per protocol, the first single, analyzable cough was used for analysis.

Development of measures

Qualitative and quantitative assessment of each cough were performed independently by two practitioners with previous experience in performing and analyzing HR-ARM (AR and KG). Measures were first developed in the healthy cohort and subsequently applied to the FI group.

For qualitative assessment of cough morphology, a 'standard view' of the cough was created by taking a 15 second window surrounding the cough and setting the pressure scale from -5 mmHg to 140 mmHg. Cough morphology was determined by the 'shape' of the pressure contour and the perceived temporal relationship between rectal and anal pressure changes. Images were reviewed offline and disputes resolved through discussion. During online analysis, the period immediately after each cough was inspected and a 'post-cough relaxation'[190] deemed present if there was a noticeable drop in pressure or shortening of anal canal length. The e-sleeve function was used to highlight areas of interest in the rectum or anal canal region. The following quantitative parameters were directly derived (**Figure 4.1**):

- automated, system-generated values for *pre- and post-cough anal resting pressure* and *pre-cough rectal resting pressure*, representing the mean of the highest pressure measured at any level within the e-sleeve area of interest;
- *anal and rectal pressure durations*, by adjusting vertical borders of the e-sleeve box to correspond to the start and end of the pressure peak produced by coughing using the composite line pressure graph for reference;
- *maximum pressure during cough* and *maximum pressure increment during cough* for rectal and anal peaks.

The following were derived offline:

- *absolute anal-rectal pressure difference* (maximum anal pressure during cough minus maximum rectal pressure during cough);
- *anal-rectal duration difference* (anal pressure duration minus rectal pressure duration);
- *incremental anal-rectal pressure difference* (maximum anal increment during cough minus maximum rectal increment during cough). This measure describes the 'excess' sphincteric pressure generated once the abdominal pressure rise ('cough effort') has been accounted for.

Traditional measures of anal function (resting pressure and squeeze increment) were also evaluated in all subjects, as previously described[5].

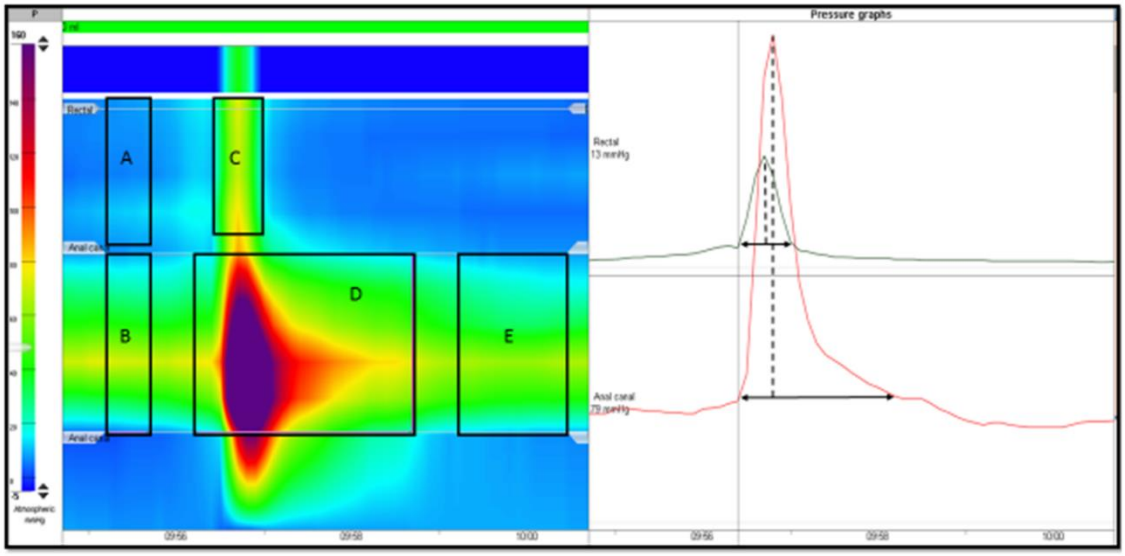


Figure 4.1 Quantitative measurements of the cough-anorectal response. A) rectal resting pressure; B) pre-cough resting pressure; C) maximum rectal pressure; D) Maximum anal pressure; E) post-cough pressure. Solid arrows: anal and rectal pressure duration; Dashed lines: anal and rectal increment from rest.

Statistical analysis

Values were expressed as means with 95% confidence intervals. The 5th and 95th percentiles in healthy parous and nulliparous women were calculated to define upper and lower limits of normality for resting pressure, squeeze increment and cough

parameters. Differences between groups were analyzed using ANOVA with Bonferroni post-hoc analysis for multiple comparisons. Independent Kruskal-Wallis with Bonferroni correction was used if homogeneity of variance was violated. A p-value <0.05 was considered significant. All statistical analyses were conducted using SPSS version 26 (IBM Corp, Armonk, NY, USA).

Results

Participants

Healthy volunteers

Of 66 healthy female volunteers, 50 subjects (median age: 42 years, range 18-64) had at least one interpretable cough. Numbers of parous and nulliparous women were equal. Nulliparous women were significantly younger that parous women ($F(1,48)=11.08$, $p=0.002$) (**Table 4.1).**

		Nulliparous	Parous	FI
	n	25	25	57
	Age (years)	36 (31-41)*	46 (42-50)	47 (43-50) †
1. Resting and squeeze (routine measures)	Resting pressure	67 (59-76)	69 (62-77)	58 (52-65)
	Squeeze increment	202 (170-234)	164 (132-197)	78 (61-95) †§
2. Pre cough	Pre-cough rectal resting pressure	15 (11-19)	11 (6-15)	12 (11-13)
	Pre-cough anal resting pressure	70 (62-79)	75 (64-86)	57 (50-63) ¶
3. Pressures during cough and their duration	Maximum rectal pressure during cough	100 (87-113)	105 (88-122)	82 (72-91) ¶
	Maximum rectal increment during cough	85 (73-97)	94 (78-110)	70 (61-79) ¶
	Rectal pressure duration (sec)	1.0 (0.9-1.1)	1.0 (0.9-1.2)	0.9 (0.8-1.1)
	Maximum anal pressure during cough	198 (171-224)	174 (152-197)	125 (113-136) † §
	Maximum anal increment during cough	127 (104-150)	100 (76-124)	68 (56-81) † ¶
	Anal pressure duration (sec)	2.3 (1.8-2.7)	2.0 (1.7-2.3)	1.5 (1.3-1.8) ‡
4. Post cough pressures (relaxation)	Post-cough anal pressure (n=56)	59 (53-66)	67 (58-76)	48 (41-54) §
5. Derived measures from above	Absolute anal-rectal pressure difference	98 (73-123)	70 (53-87)	43 (30-56) †
	Incremental anal-rectal pressure difference	42 (21-64) *	6 (-14-25)	-2 (-15-12) †
	Anal-rectal duration difference (sec)	1.2 (0.8-1.7)	1.0 (0.7-1.2)	0.6 (0.4-0.8) ‡
	Pre-post cough anal pressure difference (n=56)	11 (6-16)	8 (4-12)	9 (7-12)

*Nulliparous vs Parous p<0.05; †FI vs Nulliparous p≤0.001; ‡FI vs Nulliparous p≤0.01; §FI vs Parous p≤0.001; ¶ FI vs Parous p<0.05

Table 4.1 Cough parameters in nulliparous and parous healthy volunteers and FI patients

Patients

Of 137 incontinent parous women attending the department within the study period, 57 met inclusion and exclusion criteria. Median age was 43 years (range 28-72), with median number of births being 2 (range: 1 – 7). In total, 86% reported some form of insult to the perineal or sphincteric region during at least one delivery (this included perineal tears, episiotomies, and forceps). Overall, 51% had either a forceps-assisted

delivery or sustained a 3rd or 4th degree tear on at least one occasion. Three women (5.2%) had only given birth by Caesarean section, and only 7% (4/57) had had vaginal deliveries without complications. St Marks incontinence score ranged from 11 – 22 (median 16). With regard to presenting symptoms, 49% of patients had passive incontinence and 54% had urge incontinence (12% both passive and urge symptoms). Fifty-eight percent of patients also reported fecal urgency and 33% complained of other symptoms (such as evacuatory difficulties).

On endo-anal ultrasound, an isolated IAS abnormality was identified in 4/57 (7%), while 29/57 (51%) had an isolated EAS abnormality. Combined IAS and EAS abnormalities were found in 13/57 (23%). Sphincter morphology was normal in 11/57 patients (19%). Defecography was performed in 36/57 individuals. There was evidence of neostool leakage either passively on transfer to commode, or on instruction to cough in 23 patients (64%). At least one significant abnormality[142] was reported in 47% (large and/or retaining rectocele in 12, obstructing intussusception in 4, and non-relaxing pelvic floor in 1). Overall, 22% had both leakage and a structural abnormality; 8% had no leakage or structural abnormality (normal defecography or functional deficit only

HR-ARM

Cough-anorectal response: qualitative analysis

By examination of all cough-anorectal responses (i.e. both in health and in patients with FI), qualitative assessment identified six 'prototype' cough morphologies, designated: a) 'teardrop'; b) raindrop; c) staccato; d) diamond; e) spear; f) spear (upper) (**Figure 4.2**). The teardrop shape was the most common in health (54% overall; 60% in nulliparous and 48% in parous women) and in FI (37%). Spear or spear (upper) morphology was significantly more common in the FI group (16/57, 28%) than in healthy women (3/50, 6%; χ^2; p=0.0044). Spear (upper) was seen *only* in the fecal incontinence group (14%). Inspection of line plots from individual sensors in traces depicting a 'spear (upper)' morphology suggested attenuated or absent contraction in the distal part of the anal canal (**Figure 4.3**). This was observed despite an *overall* pressure rise within the anal canal. Post-cough relaxation was observed in 64.0% of HV (32/50) and 64.2% of patients (36/56) The presence/absence of relaxation could not be evaluated in one individual for technical reasons.

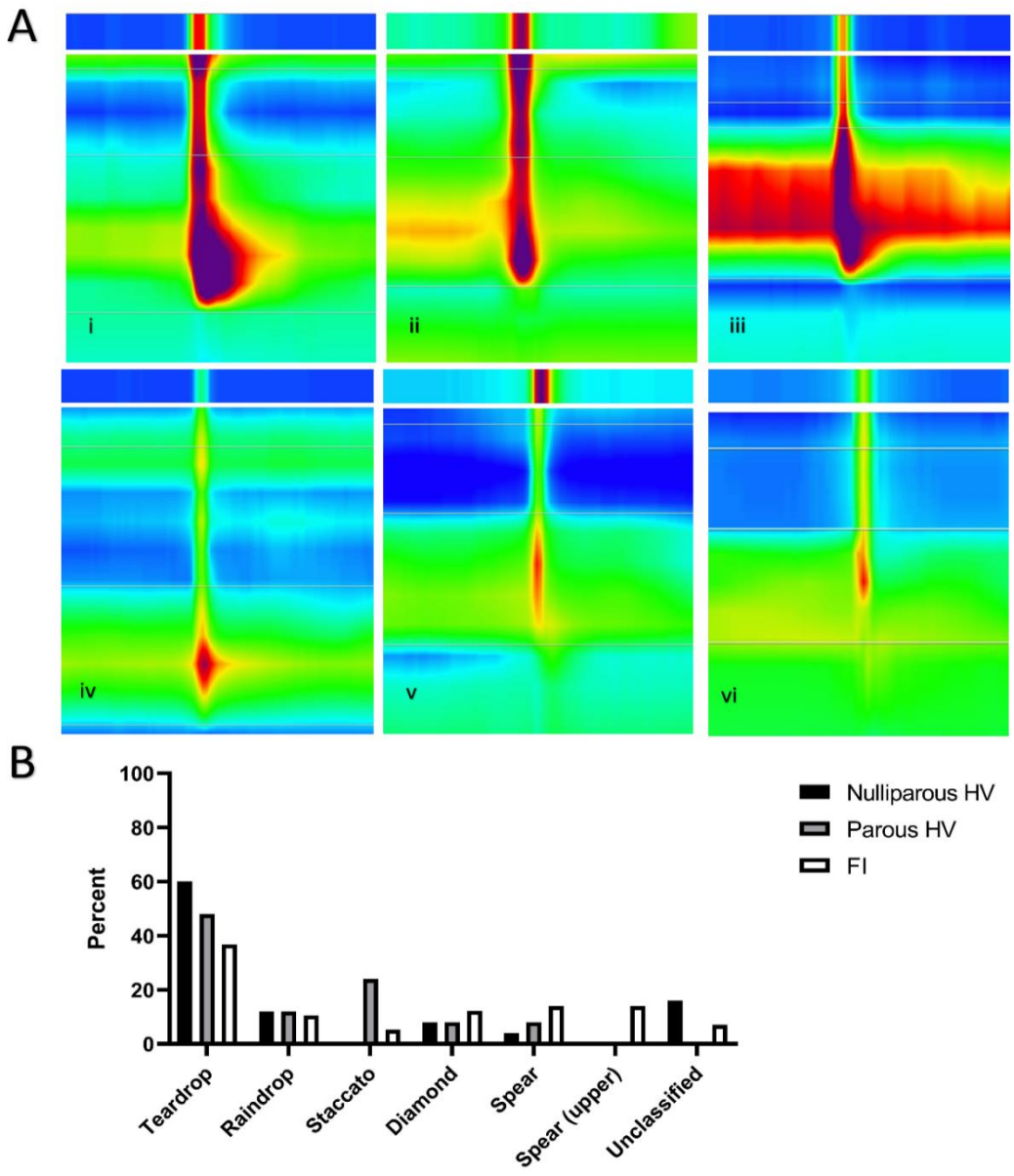


Figure 4.2 Qualitative assessment of the cough-anorectal response. A) Six 'prototype' cough morphologies, and B) distribution by group.

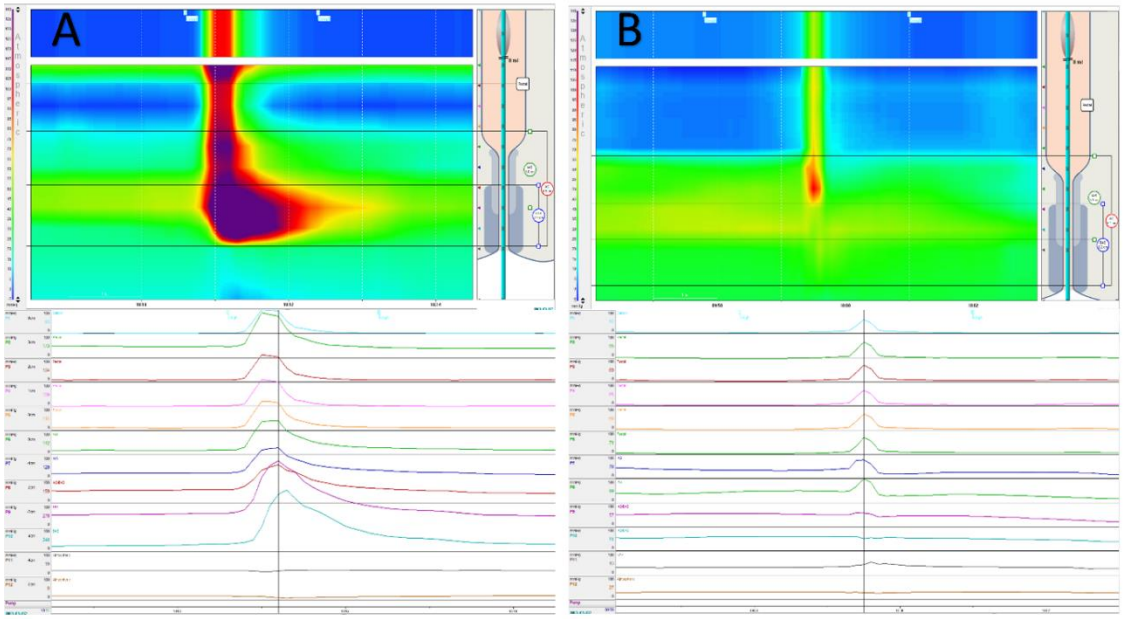


Figure 4.3 Colour-contour and line plot depiction of teardrop and spear (upper) morphology. A) representative 'teardrop' morphology observed in a healthy volunteer with corresponding line trace below. B) Spear (upper) trace with absent contraction in the distal part of the anal canal. Note that maximum pressure peaks (solid vertical line) in the anal canal and rectum are temporally dissociated from each other. This is not characteristic of all traces but occurs in both health and FI.

Quantitative analysis

Healthy subjects

Standard pressure measures

Mean resting pressure was 68 mmHg (SD: 19 mmHg; range: 34-112 mmHg) and mean squeeze increment was 183 mmHg (SD: 79; range: 43-387). Overall, the lower limit of normal (LLN) for resting pressure (5^{th} percentile) was 41 mmHg and LLN for squeeze increment was 51 mmHg.

Cough-anorectal responses

During coughing, a measurable increase in rectal pressure (102 mmHg, SD 36) occurred in all healthy subjects with a concomitant increase in anal pressure (186 mmHg, SD 60). Maximum anal pressure during cough was higher than maximum rectal pressure during cough in 49/50 subjects (98%). Anal pressure duration (2.13 sec, SD 0.94) was also longer than the rectal pressure duration (1.03 sec, SD 0.35) in 49/50 subjects. Maximum anal increment during cough (113 mmHg, SD 58) was greater than maximum rectal increment during cough (90 mmHg, 33) in 29/50 subjects.

Overall, the mean absolute anal-rectal pressure difference was 84 mmHg (SD= 54.4), mean anal-rectal duration difference was 1.1 sec (SD= 0.89), and mean incremental anal-rectal pressure difference was 24 mmHg (SD=52.2). Mean pre-post cough anal resting pressure difference was 9.7 mmHg (SD= 11.5).

Nulliparous vs parous healthy subjects (Table 4.1)

Standard pressure measures

Neither anal resting pressure (nulliparous vs parous: 67 vs 69 mmHg; $F(2,48)=0.131$, $p=0.719$) nor anal squeeze increment (202 vs 164 mmHg; $F(2,48)=2.926$, $p=0.094$) differed significantly between healthy nulliparous and parous women.

Cough-anorectal responses

No significant difference was found between nulliparous and parous HV groups for maximum rectal pressure during cough, maximum anal pressure during cough, maximum rectal increment during cough, maximum anal increment during cough, rectal pressure duration, or anal pressure duration ($p>0.05$), suggesting an equivalent cough effort and anal sphincter contractile response. However, incremental anal-rectal pressure difference was reduced (mean difference -36 mmHg (95% CI -1.8 to -71.2, $p=0.036$) in parous subjects reflecting greater magnitude of rectal incremental pressure and lower anal incremental pressure. Further, in nulliparous women, the incremental anal-rectal pressure difference was positive for 72% (18/25) of subjects (median incremental anal-rectal pressure difference: 31 mmHg, IQR: -3 – 76mmHg) compared to just 44% (11/25) of subjects in the parous group (median incremental anal-rectal pressure difference: -5 mmHg, IQR: -32 – 36; χ^2, $p=0.045$).

Patients

Standard measures

Mean resting pressure was 58 mmHg (SD: 25 mmHg; range: 13-115) and mean squeeze increment was 78 mmHg (SD: 65 mmHg; range: 5-339: Table 4.1). Group analysis showed that resting pressure did not differ between health and FI (**Figure 4.4**), but mean squeeze increment was significantly lower compared to both parous and nulliparous HVs (78 vs. 164 and 202 respectively, $p<0.001$). Overall, using the London

Classification[146], 10 patients (18%) had anal hypotension with normal contractility, 19 (33%) had anal normotension with hypocontractility, and three (5%) had combined anal hypotension with anal hypocontractility (**Figure 4.5**).

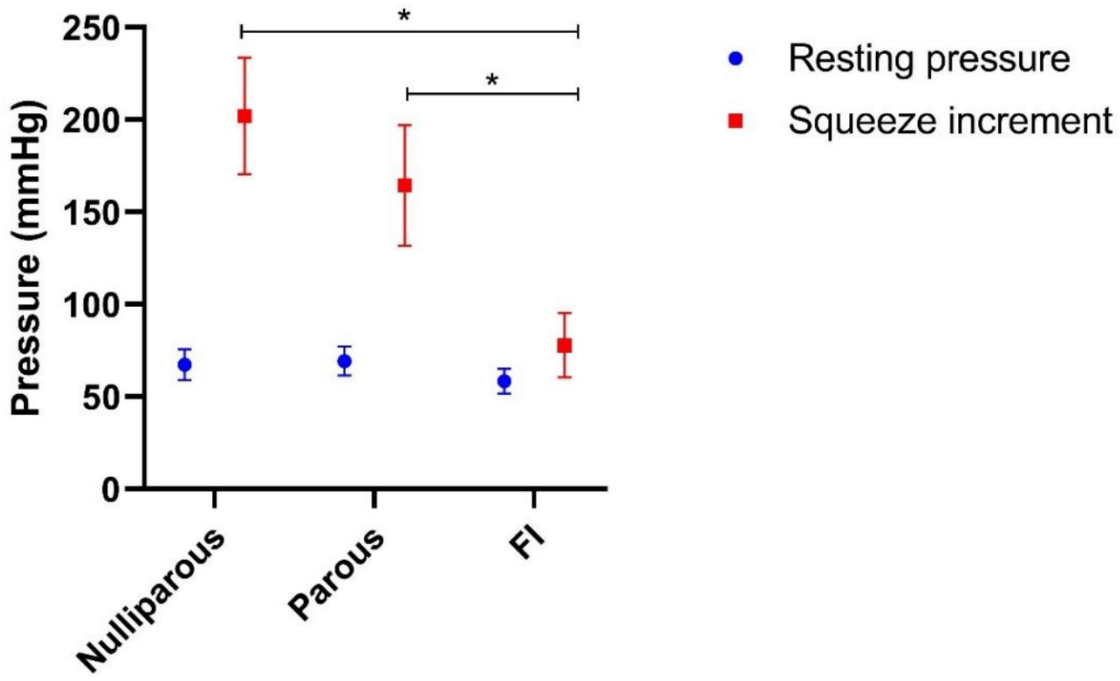


Figure 4.4 Anal resting and squeeze pressure differences between groups.

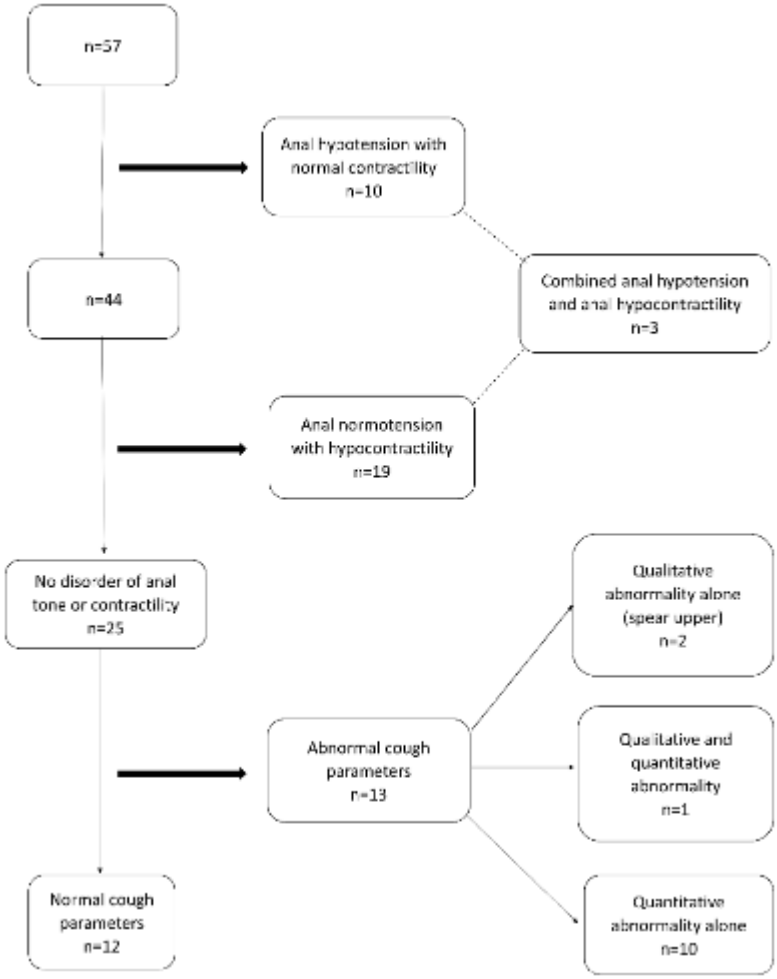


Figure 4.5 Findings according to the London manometric classification.

Cough-anorectal responses

Like healthy volunteers, all patients had measurable changes in rectal and anal pressures during coughing. However, maximum anal pressure during cough was higher than maximum rectal pressure during cough in only 45/57 patients (78.9%) (p=0.0025 vs. HV [98%]), and anal pressure duration was longer than the rectal pressure duration in 45/57 patients (78.9%) (p=0.0025 vs. HV [98%]). Group analysis showed that between nulliparous women and FI, there were no significant differences between rectal pressures before, during or after coughing (neither in absolute, increment nor duration measures). However, between parous HV and FI, incontinent women generated a lower maximum rectal pressure during cough (82 vs. 105, p=0.027) and lower maximum rectal increment during cough (70 vs. 94, p= 0.013).

Pre- and post-cough anal resting pressures were similar between groups. However, maximum anal pressure during cough (125 mmHg, SD= 44) and maximum anal increment during cough (68 mmHg, SD=47) were lower in FI than in health ($p<0.05$). Nulliparous women had greater maximum anal pressure during cough (198 vs. 125 mmHg, $p\leq0.001$) with anal pressurization being maintained for longer compared to that in incontinent women (anal pressure duration: 2.3 vs 1.5 sec $p\leq0.01$). Further, the mean anal-rectal duration difference in nulliparous women was twice that of incontinent women (1.2 vs. 0.6 sec, $p\leq0.01$). Maximum anal increment during cough was also greater in nulliparous women (127 vs. 68, $p\leq 0.001$) as was the incremental anal-rectal pressure difference (42 vs. -2 mmHg, $p\leq0.001$). The proportion of incontinent women with a positive increment difference (44%, 25/57) was less than seen in nulliparous women (□2, p=0.019). Between parous healthy women and incontinent patients, maximum anal pressure during cough (174 vs. 125 mmHg, $p\leq 0.001$) and maximum anal increment during cough (100 vs. 68 mmHg, $p\leq0.05$) were greater in health. In contrast, there were no differences in duration of rectal or anal pressure increases between these groups and incremental anal-rectal pressure changes were not significantly different (mean difference -7.399 (-36.8 – 22.0), p=1.000).

Clinical utility of cough measures

Twenty-five (43.9%) patients had no disorder of anal tone or contractility by 'traditional' measures (**Figure 4.5**). Of these 25 individuals, 52% had either abnormal qualitative (2/25) or, based on values outside of the normal range (Table 4.2), abnormal quantitative (10/25) cough parameters. One patient had both qualitative and quantitative abnormalities. This translated to 22.8% of the incontinent group as a whole, or just under 1 in 4, with 'isolated' sphincter dysfunction during coughing.

		All HV	Nulliparous	Parous	% FI patients below LLN
	n	50	25	25	
Routine measures	Resting pressure	41 – 105	42 – 111	36 – 101	22.8
	Squeeze increment	51 – 333	61 – 373	48 – 313	38.6
Cough-anorectal response measures	Maximum anal increment during cough	30 – 217	36 – 218	12 – 221	1.8
	Anal pressure duration (sec)	0.76 – 4.08	1.10 – 4.86	0.63 – 3.69	14.0
	Incremental anal-rectal pressure difference	(-52) – 126	(-47) – 145	(-53) –127	8.8
	Anal-rectal duration difference (sec)	0.00 – 3.06	0.03 – 3.85	(-0.07) – 2.4	21.1

Table 4.2 Proposed lower limits of normal (5th and 95th percentiles) for cough parameters in health.

Amongst patients with normal anal contractility, 40% (14/35) had a negative incremental anal-rectal pressure difference, whereas in patients with hypocontractility, 77% (17/22) had negative incremental anal-rectal pressure difference (χ^2 (1, 50), p=0.006). Overall, a negative incremental anal-rectal pressure difference was associated with a higher prevalence of post-cough relaxation (χ^2 p=0.038) and greater occurrence of involuntary leakage on defecography (i.e. 15 of 23 (65%) patients with observed leakage had a negative incremental anal-rectal pressure difference, χ^2 p=0.015). The proportion of subjects with abnormal cough response (31/57) did not differ by ultra-sound outcome (χ^2 (1, 57), 0.0001, p=0.991).

Discussion

To our knowledge, this study is the first to systematically compare anorectal pressure changes during coughing in healthy nulliparous and parous women and in patients with FI using HR-ARM.

The main findings of this study were:

1. considering the anal canal as a single functional unit, we were able to measure some degree of anal sphincter response to cough in all subjects;

2. qualitative identification of six 'prototype' morphologies of the cough-anorectal response. The most common in both health and disease was a 'teardrop' appearance, characterized by a longer duration of anal compared to rectal pressurization. In contrast, a 'spear' or 'spear (upper)' morphology were both more common in FI than health, manifest as a more simultaneous rectal and anal response; spear (upper) was unique to the FI group, and was characterized by attenuated or absent contraction in the distal part of the anal canal;
3. maximum anal pressure and duration of the pressure response were greater in the anal canal than in the rectum in 98% of HV, but in only 79% of patients (p=0.0025);
4. the maximum anal increment during cough exceeded the rectal increment in a proportion (58%) of healthy individuals, of whom the majority (72%) were nulliparous (compared to 44% of both parous HV and FI patients);
5. incremental anal-rectal pressure difference varied significantly between nulliparous and parous HV, but no such difference was found between parous HV and FI;
6. maximum anal pressure, maximum anal pressure increment and the duration of the anal pressure response on coughing were significantly lower in FI than in health;
7. in patients with FI, 25/57 (43.9%) had no disorder of anal tone or contractility using traditional measures (rest and squeeze), but 13 of these patients with apparently 'normal' anal function had qualitative or quantitative abnormalities (or both) using new cough measures (representing ~1 in 4 of the group as a whole).

These findings merit discussion with reference to previous literature. In healthy individuals and individuals with high spinal lesions, the anal response to a rise in abdominal pressure is increased anal sphincter EMG activity and, on manometry, greater maximum anal pressure compared to intra-rectal pressure[181]. Our results are consistent with these findings, since 98% of healthy volunteers maintained (theoretically) an efficient barrier during cough, based on the maximum anal and rectal pressure difference alone. Nevertheless, a significantly smaller proportion of FI patients demonstrated the same response (78.9%).

Early manometry studies identified reduced anal resting pressure in two-thirds of incontinent patients[385,386], however other studies have shown that a subject with low

resting pressure may also be perfectly continent [62], demonstrating the overlap between health and disease. In the current study, anal resting pressure was the same between all groups, despite nulliparous subjects being significantly younger compared to asymptomatic and symptomatic parous women. Voluntary squeeze increment discriminated between continent and incontinent subjects, but failed to show a difference between nulliparous and parous healthy volunteers. This is despite consideration of parity being reported as essential for correct interpretation of manometric results [387,5], though findings vary according to equipment used [388,174]. Overall, 43.9% (25/57) of incontinent patients showed no evidence of impaired sphincter function based on traditional measures alone. However, it may be that broad measures of rest and squeeze are too 'blunt' as tools to identify sphincter dysfunction in all patients, and that including analysis of the cough maneuver may enable identification of a, perhaps more subtle, functional deficit. Indeed, of these 25 individuals, cough metrics were abnormal in 52% (or ~1/4 of all FI patients studied).

Other studies have addressed the sphincteric pressure response to coughing using maximum anal pressure during cough as the main outcome [317,313,306] and this reflects clinical practice [148]. Most notably, Mazor et al [306] found that maximum anal pressures during cough were generally lower than maximum anal squeeze pressures in healthy individuals, and that parity was associated with a smaller difference between maximum cough pressure and maximum (absolute) squeeze pressure. Our study did not directly compare the differences between squeeze and cough pressure, however, abnormal cough parameters were common in individuals with hypocontractility based on squeeze increment, demonstrating the inherent relationship between voluntary and involuntary EAS contractility.

Although past manometry guidelines have emphasized the role of the cough response in the identification of patients with neural damage to the sacral reflex arc [62,145], we remain cautious regarding such interpretation based on the interaction between squeeze and cough pressure alone, without corresponding diagnostic neurophysiological information.

Rather, Meagher et al [310] suggested that analysis of cough may offer additional information on EAS contractility above squeeze pressure. Based on the LLNs detailed in

Table 4.2, we identified 22.8% of our incontinent population with isolated cough-related anorectal pressure dysfunction. The LLN for maximum incremental anal pressure in this study (30 mmHg) was slightly higher than that reported by Gosling [384], who included fewer parous than nulliparous healthy subjects, and lower compared to the 43 mmHg reported by Rasijeff [151]. The LLN for maximum anal pressure during cough (94 mmHg) was higher than in previous studies ranging between 82-86 mmHg [306,5,384]. However, studies by Mazor [306] and Gosling [384] used water-perfused catheters, which register lower pressures during rapid changes in pressure compared to solid-state catheters [151]. Mazor [306] also report the 10th, rather than 5th percentile.

Nevertheless, caution should be taken in using maximum pressure as a biomarker for reflex contractility due to variability in each of its component parts. Firstly, differences in anal resting tone shift the 'starting line' in favor of one group over another. Second, intra-abdominal pressure transmission to the pelvic floor may artificially increase anal pressures with more intense coughing. The positive relationship between a greater rise in intra-abdominal pressure and subsequent anal pressure increment first suggested by Meagher et al [310], was confirmed by Amarenco et al [311], who showed pelvic floor contraction was proportional to the rise in intra-vesical/intra-abdominal pressure (intensity) generated by cough. Early EMG observations by Melzak and Porter [389] also showed that the amplitude of electrical response to coughing was greatest in patients with more innervated trunk musculature, suggesting this was due to their ability to generate higher intra-abdominal pressure. Finally, the potential to respond reflexively may be dependent on parity.

Study limitations

There are several limitations to our study. Firstly, nulliparous women were younger than parous healthy and incontinent women. However, though ageing is thought to impact primarily internal anal sphincter tone, we saw no difference in anal resting pressure between groups and hence it is unlikely that ageing played a significant role in our findings. Secondly, maximum pressure responses to coughing were lowest in the incontinent group who also had the lowest intra-abdominal/rectal pressure increment indicating that they coughed with the least effort (likely for fear of incontinence). Given the retrospective nature of our study, we were unable to standardize cough effort to

maintain a consistent 'challenge' to sphincters of all individuals as was achieved in some previous studies [174]. Since lower intra-abdominal pressure rise may lead to smaller degrees of anal sphincter response [310], ensuring consistency of the challenge produced may be an important consideration in future studies. Thirdly, our observations are based on a small number of available single coughs in a limited number of healthy volunteers. Difficulties in recruitment of healthy volunteers may introduce bias in the selection of subjects and data thus obtained[379]. As far as we know, all asymptomatic volunteers included in this study met the appropriate criteria for healthy volunteers, however we cannot guarantee the results obtained are representative of all healthy females, particularly with regard to ethnicity and BMI [171]. We took a consistent approach to choosing eligible patients, manometry traces, and single, discrete coughs included in the study to reduce bias. We did not endeavor to compare intra-individual variation in cough response but recognize this to be an important focus of future work given the recommendation that the cough maneuver is repeated in standard manometry protocols [146].

Finally, our interpretation of qualitative cough morphologies suggests that pressure changes during coughing can vary between distal and proximal parts of the anal canal. Given their polar extremities, these differences may be related to the type of muscle tissue (smooth or striated) that predominates. EMG studies describe the cough response as an external anal sphincter reflex[381], so the true cough reflex may be expected to occur in the distal or mid anal canal. We observed an attenuated or absent response associated with spear (upper) morphology in some individuals, in whom reflex contraction could be truly absent. However, because high-resolution anorectal manometry is unable to reliably differentiate between puborectalis, internal and external anal sphincter contributions to pressure, we considered the anal canal as a single unit for quantitative measures. We also considered the maximum pressure measurement to be representative of anal response to cough irrespective of the level at which it occurred within the defined sphincteric or rectal area of interest.

Potential for clinical application

A key question is whether cough measurements could have future clinical utility, especially since anorectal manometry including the cough challenge already forms part

of routine assessment following traumatic childbirth in many parts of the world [148]. While we present evidence of an 'additional yield' of abnormal findings using detailed cough metrics in a proportion of patients with FI who had normal standard metrics, a further interesting finding is that we could also detect differences in sphincter function between asymptomatic nulliparous and parous women. Vaginal delivery is a risk factor for fecal incontinence due to mechanical or neurogenic damage imposed upon (predominantly) the striated sphincter muscle [111], however there is often a considerable time lag between injury and symptom onset [6]. During this lag period, further insult to pelvic floor function (menopause, subsequent vaginal delivery, persistent straining etc.) may occur. Evidence of subclinical predisposition to pelvic-floor weakness and consequent incontinence before symptoms present may allow for preventive measures (such as pelvic floor exercise or Caesarian section) to be taken in at risk individuals [390]. Subclinical neuropathy may also explain persistent anal dysfunction following sphincter repair in a proportion of patients [391]. Accordingly, detailed examination of cough-anorectal pressures suggests that the anal-rectal incremental pressure difference may be able to identify potential subclinical sphincter dysfunction in women following childbirth, even in the absence of symptoms. For a similar cough effort, the anal-rectal incremental pressure difference was significantly lower in parous continent and incontinent women compared to the nulliparous group. Conversely, no such significant difference was observed between parous continent and parous incontinent women. Whether this is a useful biomarker of subclinical injury and future risk would require a longitudinal study.

Conclusion

Undoubtedly, the role of HR-ARM in identifying disorders of tone and contractility remains, though the need for more intense stratification within normative datasets is recognized. We present a promising basis for interpreting cough clinically, though future prospective studies are needed to fully understand its potential. Furthermore, in-depth analysis of the cough-anorectal reflex, an under-utilized yet routinely performed maneuver, appears to have the potential to identify subclinical sphincter dysfunction in parous and in fecally incontinent women compared to asymptomatic nulliparous women. These results present the opportunity to reconsider HR-ARM not

only as an “expensive hobby”[392], but as an important tool for identification of at risk individuals in whom preventive measures may serve to halt progression of subclinical anal dysfunction into life-altering disease. Where FI symptoms are already established, evaluation of sphincter function with a dynamic maneuver like cough, which challenges the sphincter barrier response, may be more clinically valid than static measures.

Chapter 5 Retrospective analysis of anal slow waves in healthy volunteers and FI patients

Introduction

Anorectal manometry is the principal diagnostic tool to assess anal sphincter dysfunction in faecal incontinence (FI). Anal resting pressure is a primary indicator of internal anal sphincter (IAS) function and is one of the most recognised and consistently reported measures of anal function [393]. Anal resting tone is thought to reflect myogenic activity of the IAS [36], which is subject to nervous and hormonal modulation. As pointed out in previous chapters, anal resting pressure, a broad measure of IAS function, discriminates poorly between health and FI[146]. Mean resting pressure measured by HR-ARM, reflects the average of the highest pressures recorded at any level within the anal canal [146], but may not be sensitive enough for detecting small pressure changes, for example in early disease or following treatment (Chapter 2). The summation of IAS tone as a single average pressure measurement during manometry may be too simplistic i.e. it omits information which may be gained through inspection of the nuances of tone.

Visual inspection of a resting manometry trace typically reveals rhythmic pressure oscillations known as slow and ultra-slow waves [157,394]. Recent studies in animals suggest that interstitial cells of Cajal (ICC), located in the intramuscular space of the IAS, act as "pacemaker cells" [36]. The authors hypothesised that the resultant fluctuation in basal electrical activity observed in response to these cells causes the cyclical oscillations in pressure observed on a manometry trace.

Anal SW activity has been studied using conventional manometry and electrophysiological techniques in the past [157,394-399], but studies quantifying their amplitude and frequency using HR-ARM are limited [5,189,191]. These studies have however demonstrated that SW activity, seen as cyclical high-pressure spikes superimposed on the basal resting pressure (for representative image see Vollebregt *et al* [191]), was present in 15%-44% of healthy subjects at a frequency of 9–19 cycles/minute [5,189]. However, given the greater data yield and complexity of analysing multiple, overlapping frequencies across many sensors spanning the length of the anal canal using HR-ARM,

manual counting of pressure peaks over time to quantify oscillating behaviour is subject to bias [400]. Therefore novel, automated, analytical approaches are needed to capture data on SW activity using HR-ARM.

Wavelet transform is a computational method designed for converting data in the time-amplitude domain into a frequency spectrum, often revealing the most distinguished information hidden within the signal in the process (Polikar 2006). This form of mathematical analysis has been used previously to analyse colonic motility in response to a test meal measured using high-resolution manometry[400] and more recently, to demonstrate differences in SW frequency between low anterior resection patients (LARS) and healthy males [191]. The aim of this chapter is extend the use of this method to compare anal SW amplitude at various frequencies between healthy nulliparous and parous women and patients with FI and to determine the direction of propagation at each frequency and to determine whether it is feasible to distinguish between study groups using manometric measures of anal tone other than resting pressure.

Methods

Study population

Healthy volunteers (HV)

Nulliparous and parous women recruited by advertisement for assessment of anorectal function between 2012-18 were considered for inclusion. Volunteers had no history of significant gastrointestinal disease, all had a St Marks incontinence score (SMIS)[332] ≤5 and Cleveland Clinic Constipation Score (CCCS)[333] ≤8. Exclusion criteria were pregnancy or lactation, history of diabetes, cardiovascular, renal or hepatic disease. HR-ARM traces from women who qualified for assessment of cough response (Chapter 4) were considered. Data from some of these individuals has been published as part of other studies in health [5,30,166,188], however a detailed analysis of SW activity has not been performed in previous studies. Additional manometry data from 27 healthy volunteers (HV) collected prospectively as part of this thesis (see Chapter 6 for details) was also considered. In these individuals, assessment of rectal sensation to balloon distension, endo-anal ultrasonography, EndoFLIP, rapid barostat, and a balloon expulsion test (BET) were also performed following HR-ARM. The result of these other tests are reported in subsequent chapters. Ethical approval for HV studies was provided by Queen Mary

University Research Ethics Committee (ref QMREC 2010/74; QMREC 2013/12; QMERC2017/33) and written informed consent was provided by all volunteers.

Patients

The patient group consisted of those women previously selected for assessment of cough response (Chapter 5). These were consecutive parous female patients attending The Royal London Hospital GI Physiology Unit between January 2018 and December 2018 for routine investigation of FI. Patients were included if they had a St Marks incontinence score [332] >10 (SMIS). Patients referred for a primary presenting symptom of constipation/evacuation disorder, or for symptoms of prolapse, anal fistula, or cancer were excluded. Further exclusion criteria were history of diabetes or inflammatory bowel disease, known neurological disease, hysterectomy, and anal/pelvic floor surgery apart from primary sphincter repair following vaginal delivery.

All subjects (HVs and patients) underwent HR-ARM and assessment of rectal sensation to simple balloon distension. In addition, all patients underwent endo-anal ultrasonography and a proportion (36/57, 63%) underwent defaecography. All tests were performed and interpreted in accordance with departmental protocols [96,142,335].

HR-ARM

Technical specifications and test procedure

As described in Chapter 4, all participants underwent investigation using a 12F solid-state catheter (UniTip: UniSensorAG, Attikon, Switzerland) incorporating 12 unidirectional pressure transducers each embedded within a silicone gel cuff. Prior to the study, the catheter was immersed in tepid water for at least 3 minutes to pre-wet sensors, which were then zeroed. Data acquisition and visualization was performed using a commercially available manometric system (Solar GI HRM V.9.1, Medical Measurement Systems, Enschede, The Netherlands). Data were generated at 10 Hz. Manometry was performed using a ratified protocol [5].

Trace selection and extraction of pressure data

For each HR-ARM trace (irrespective of health or disease status) the stationary recording incorporating stabilisation and resting periods was reviewed for trace quality and artefacts. The maximum length of artefact free, stable recording was identified; at

least 120 seconds of continuous, artefact-free recording was required for inclusion. Anal and rectal regions of interest were identified using a 10-mmHg colour-contour on the topographic pressure plot. The total number of channels within the anal region was identified and the most distal (closest to the outer edge of the anal canal) and proximal (closest to the internal edge of the anal canal) channel number for each individual was noted in a spreadsheet. Channels had to be fully within the 10-mmHg colour contour to qualify (**Figure 5.1**). The "Quick-view" feature was then disabled and raw data for all recording channels for the duration of the stable period were extracted into a CSV file and de-identified.

Anal canal resting pressure and voluntary squeeze increment were measured as previously described [5].

Wavelet-analysis of slow-wave activity

For each recording channel, a continuous wavelet transform [401] at frequencies between 0.5 and 32 cycles per minute (cpm) was computed and the root-mean-square over time and channels calculated to obtain the average amplitude, or "power" per frequency. The cross-wavelet transform was calculated between adjacent channels, and the time and channel averages of the phase-difference computed to reveal the average direction of propagation per frequency. A Bayesian linear regression with Gaussian process responses [400] was used to compare amplitudes and directions between groups. The wavelet analysis was performed blinded to parity and FI status of each group.

Other statistical analyses

Descriptive characteristics for grouped analyses are expressed as means with 95% confidence intervals. Differences between groups were analyzed using ANOVA with Bonferroni post-hoc analysis for multiple comparisons. A p-value <0.05 was considered significant. All statistical analyses were conducted using SPSS version 26 (IBM Corp, Armonk, NY, USA).

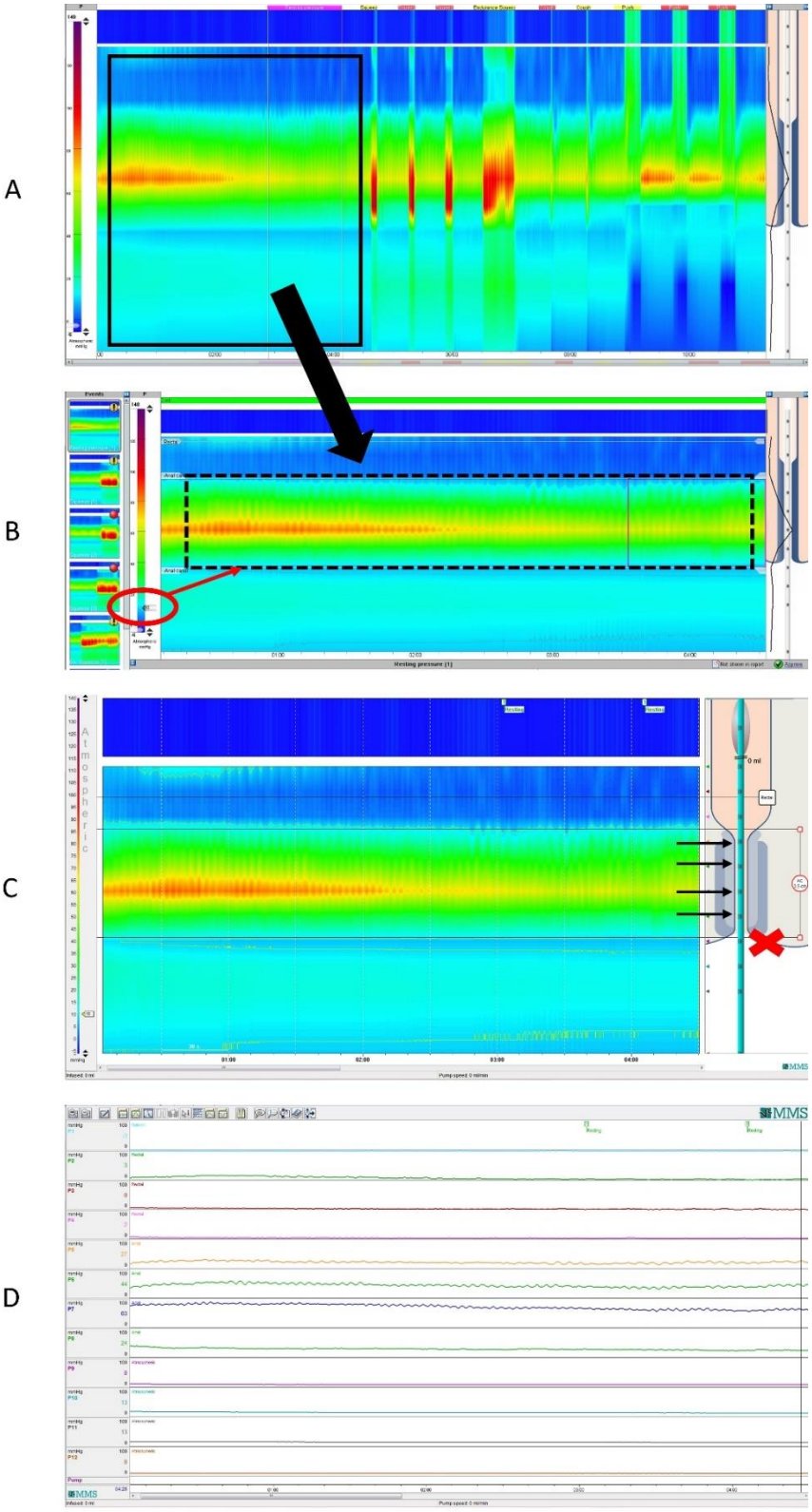


Figure 5.1 (A) HR-ARM trace. (B) The maximum possible stable recording time was identified, and anal canal region marked by a 10-mmHg colour contour. (C) the number of channels fully within the anal region was noted. (D) With the 'Quick-view' disabled, the raw data is viewed as line plots and available for data extraction.

Results

Participants

Healthy volunteers

Of 77 healthy female volunteers, 73 subjects (median age: 37, range 18-64) had at least 120 seconds of qualifying stable trace (mean 287 sec, SD 67.9; **Figure 5.2**). The final dataset included 41 nulliparous (median age: 32 range: 18-67) and 32 parous (median age: 45, range 31-64) women. Nulliparous women were significantly younger than parous women (F (2,120)= 11.25, p=0.003).

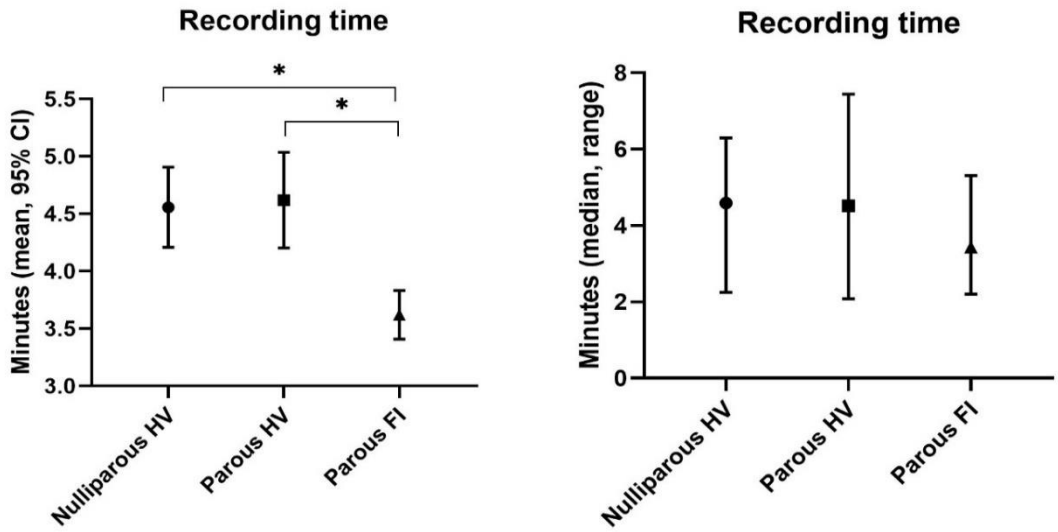


Figure 5.2 Mean duration of stable recording time analysed per group.

Patients

In total, of 137 incontinent parous women attending the department within the study period who met study criteria as described in the previous chapter, 57 patients qualified for assessment of cough response (Chapter 4). From these traces, 88% (n=50) of studies had at least 120 seconds of eligible stable recording. These 50 subjects formed the patient group in this study. The mean duration of stable recording was 228.5 sec (SD 44.7). Median age was 45 years (range 30-72), with median number of births being 2 (range: 1-7). Six (12%) women had only ever given birth by Caesarian section or uncomplicated vaginal delivery (including 3 women who reported giving birth only by Caesarian section). Of women who had vaginal births, 50% (25/50) reported having had

perineal tears (1st or 2nd degree); in 6 this was the only reported adverse outcome associated with childbirth. 44% (22/50) women had had an episiotomy; this was the only adverse outcome reported by 4 individuals, while episiotomy and perineal tear (1st or 2nd degree) was reported by 7 women. 48% of women reported having had at least one assisted vaginal birth (34% forceps, 14% ventouse). 18% of women reported a 3rd degree tear and 8% had sustained a 4th degree tear. The median St Mark's Incontinence Score (SMIS) [332] was 16 (range 11 – 22) and median Cleveland Clinic Constipation Score (CCCS) [333] was 7 (range 0-17). Presenting symptoms were as follows: 52.0% of patients had passive incontinence and 60% had urge incontinence (14% had both passive and urge symptoms). Faecal urgency was reported by 56% percent of patients and 32% had symptoms of evacuatory difficulties.

On endo-anal ultrasound, an isolated IAS abnormality was identified in 4/50 (8%), while 25/50 (50%) had an isolated EAS abnormality. Both IAS and EAS abnormalities were found in 10/50 (20%). Normal sphincter morphology was observed in 11/50 patients (22%). Of the 34 patients who had defecography, 32.4% had a large and/or retaining rectocele, and 11.8% had evidence of obstructing intussusception. Defecography revealed isolated functional abnormalities in 3 individuals (8.8%) and leakage upon transfer or coughing in 65.6%.

HR-ARM

Traditional measurements

Mean sphincter pressures are shown in **Figure 5.3**. Mean anal resting pressure was 73 mmHg (SD: 19.2 mmHg; range: 42-122 mmHg) in nulliparous HV, 66 mmHg (SD: 17.2 mmHg; range: 34-102 mmHg) in parous HV and 59 mmHg (SD: 26.1 mmHg; range: 13-115 mmHg) in FI. Anal resting pressure was significantly lower in FI women compared with nulliparous (p=0.010), but not parous HV (p=0.269). No significant difference was observed between HV groups (p=0.216). Mean squeeze increment was 172 mmHg (SD: 81.0; range: 45-387 mmHg) in nulliparous HV, 139 mmHg (SD: 66.3 mmHg; range: 45-309 mmHg) in parous HV and 80 mmHg (SD: 66.1 mmHg; range: 5-339 mmHg) in FI. Anal squeeze increment was significantly lower in FI women compared with nulliparous (p<0.001) and parous HV (p=0.001). No significant difference was observed between HV groups (p=0.158). By conventional HR-ARM measures, 20% of patients had hypotonia,

38% had hypocontractility, 6% had hypotonia with hypocontractility, and 36% had normal findings.

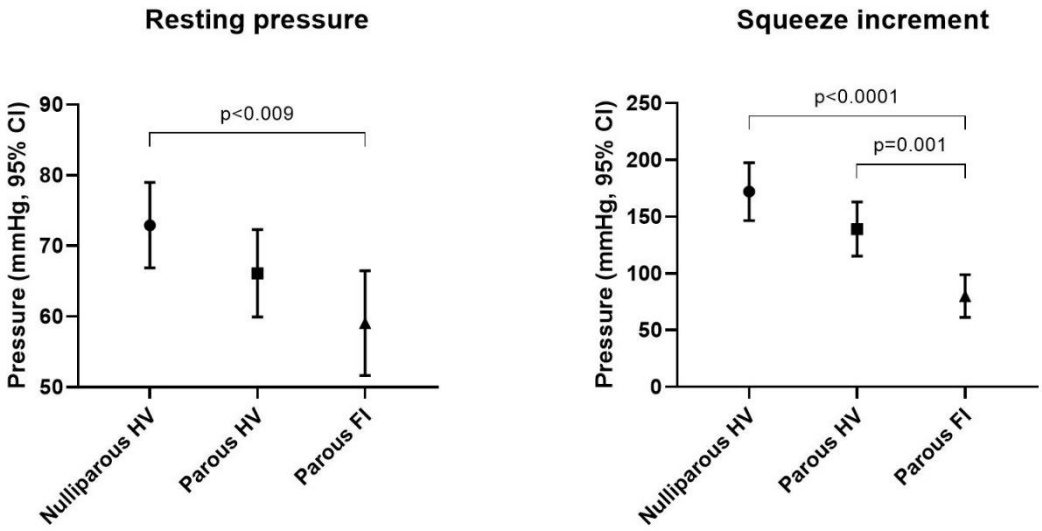


Figure 5.3 Mean resting pressure and squeeze increment

Frequency and amplitude of slow-wave activity

Two dominant SW frequencies emerged in each group, the first at 1.5 cpm and the second at 16 cpm, represented by peaks in the (grouped average) amplitude (Table 5.1), or "power" of the signal (**Figure 5.4**). Grouped comparisons showed that the power of the signal was highest in nulliparous subjects and lowest in FI patients at all frequencies (**Figure 5.5**). Significant differences in power were observed between nulliparous HV and parous HV, and nulliparous HV and FI patients, at all frequencies. Between parous HV and FI patients the difference in power was not significant at frequencies greater than 6. Including age as a covariate in the regression model did not impact these findings.

Anal SW frequency (cpm)	**Nulliparous HV**	**Parous HV**	**FI**
0.5	1.43 (2.5)	1.05 (2.39)	0.84 (2.43)
0.71	1.57 (2.58)	1.13 (2.41)	0.86 (2.29)
1	1.89 (2.65)	1.34 (2.53)	0.98 (2.44)
1.4	2.18 (2.36)	1.55 (2.33)	1.12 (2.39)
2	2.13 (1.99)	1.52 (1.98)	1.11 (2.16)
2.8	2.02 (1.84)	1.46 (1.81)	1.09 (1.95)
4	1.87 (1.75)	1.35 (1.68)	1.05 (1.79)
5.7	1.55 (1.73)	1.13 (1.64)	0.91 (1.76)
8	1.39 (2.04)	1.02 (1.86)	0.84 (1.93)
11	2.33 (2.55)	1.72 (2.3)	1.43 (2.33)
16	3.43 (2.44)	2.54 (2.24)	2.13 (2.5)
23	1.49 (2.03)	1.11 (1.89)	0.94 (2.15)
32	0.33 (1.81)	0.25 (1.7)	0.21 (1.77)
Values represent mean amplitude (SD)			

Table 5.1 Amplitude of SW frequencies between groups.

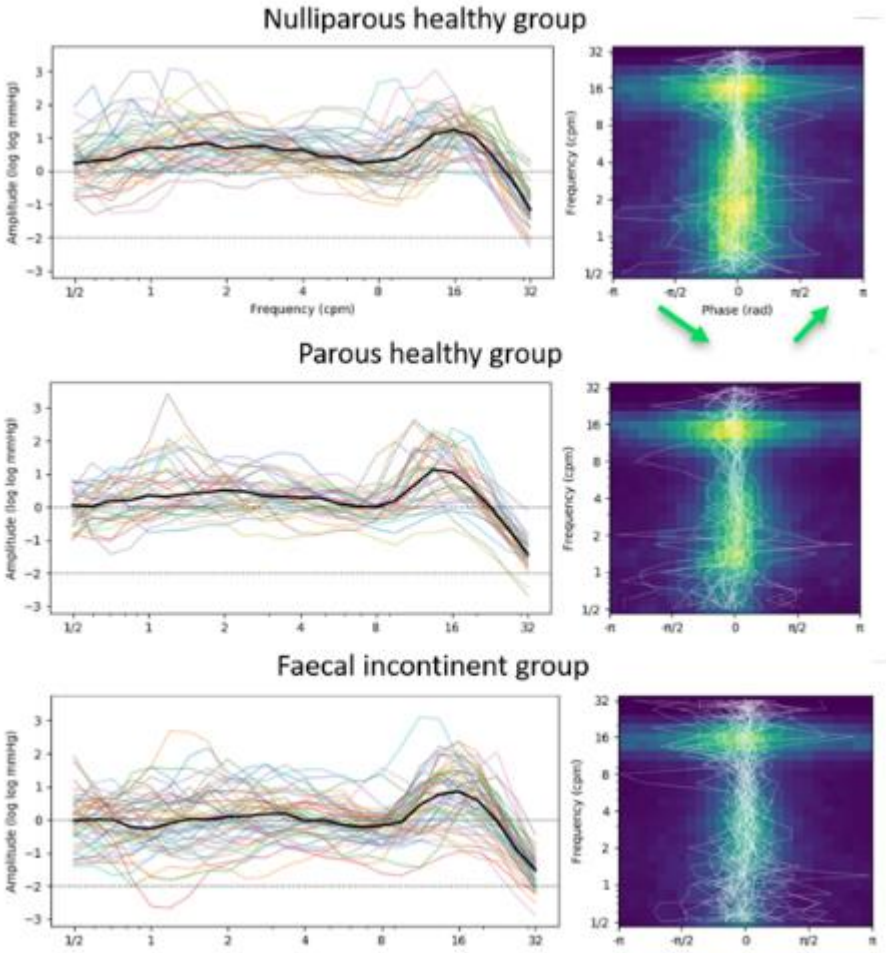


Figure 5.4 Peak "power" at each tested amplitude. The panels on the left represent the amplitude (power) results for everyone, while panels on the right represent the average amplitude per frequency (the brighter the higher the power) while spread represents the average propagation direction.

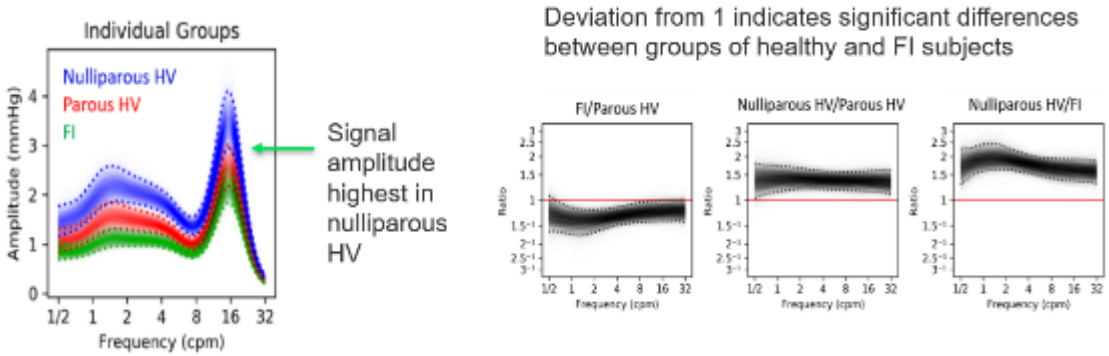


Figure 5.5 Comparison of average slow-wave amplitude between groups

Propagation of pressure

Figure 5.6 shows the propagation of pressures between adjacent channels where positive phase difference corresponds to antegrade propagation (forward in time from the upper anal canal toward the lower anal canal) and negative phase difference corresponds to movement from low anal canal (nearest anal verge) propagating to upper anal canal (towards anorectal junction). The same biphasic direction of propagation of SW was observed in all three groups: retrograde at 16 cpm and antegrade at ~4 cpm. No direction could be identified at other frequencies.

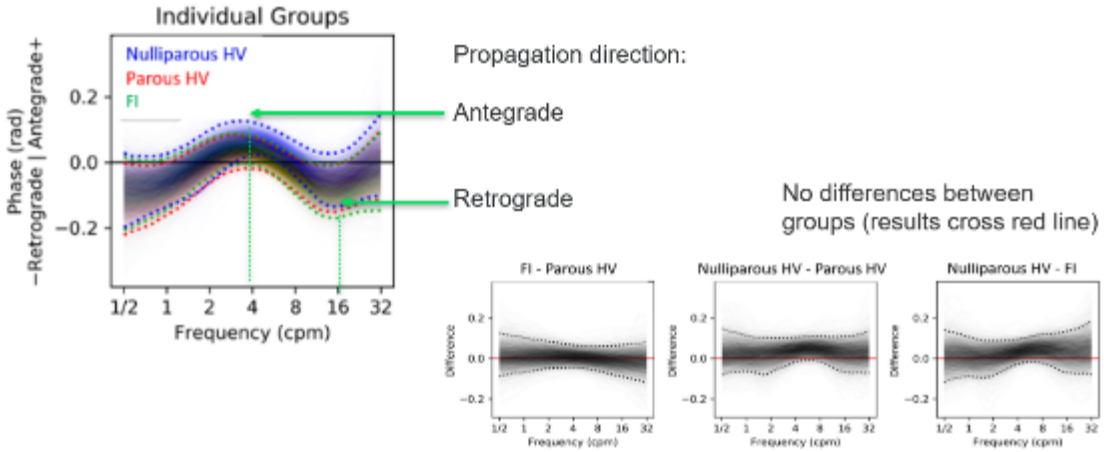


Figure 5.6 Comparison of average slow-wave propagation direction between groups

Discussion

Rhythmic pressure oscillations, or SW, observed during manometry may be important indicators of tone generation and the ability to maintain high anal resting pressure [36]. Wavelet transform has been used previously to quantify the amplitude of SW at multiple frequencies; the dominant frequency was shown to distinguish between healthy men and men with LARS [191]. This exploratory chapter aimed to identify whether subtle differences in tone (revealed by the frequency domain) would reveal differences in SW activity between healthy nulliparous and parous women and women with FI.

Using traditional measures (resting pressure and squeeze increment), no significant differences in tone or contractility between the nulliparous and parous HV were

observed. In contrast, wavelet analysis demonstrated significantly higher amplitudes across the frequency spectrum of nulliparous women. To avoid confusion with amplitude described as the ”height” of pressure waves observed on HR-ARM (indicating greater sphincter strength), the amplitude in the frequency spectrum will be referred to as the “power” of the signal henceforth. Lower power was also observed in parous FI patients at all frequencies compared with nulliparous women. These results suggest that parity, with or without FI symptoms, may be associated with worse IAS function or that there could be differences in “fine-tuning” of resting pressure [402] when compared with nulliparous women.

The dominant frequency was 16 cpm in all groups, consistent with findings in previous studies [5,189,191,394]. However, peaks on the wavelet transformed signal are unlikely to directly correspond to those peaks visible during qualitative assessment of the manometry trace [5,191]. As pointed out previously, most frequencies present in the data are ‘lost’ within a sprectrum of coexisting frequencies on the manometry trace and hence cannot be identified by simply looking at the trace [191]. Rather, we used a novel computational method that allows greater data yield by converting pressure-time information into frequency data. The method provides fast analysis of manometric signals at levels of detail and orders of magnitude beyond what was previously available [400]. However, the concept of multiple overlapping frequencies of SW is not altogether new: in 1968, Wankling *et al* observed cyclical electrical and force wave activities originating from the IAS, which were not always in phase concluding that different ”types” of slows waves acting independently may exist [398].

At the dominant frequency, no significant differences were observed between parous healthy and FI women, however there was some evidence for progressive decline in sphincter function associated with birth and FI (compared with nulliparous women) which was not affected by age. Women who present for investigation of FI often only do so after a number of decades since the initial event, usually involving vaginal delivery [6,80,90]. In the years following an intial injury (whether of muscular or neurological origin), further vaginal deliveries, menopause, and general ageing, all of which increase the risk of FI [6,403,404] may take place over time. Thus, the progressive decline in anal sphincter function leading to FI is likely due to ‘multiple-hits’ over time [405]. Unfortunately, we did

not have detailed obstetric history or endoanal ultrasound results for healthy volunteers or information on menopausal status of subjects to test this hypothesis.

By comparing the amplitude at set frequencies between adjacent sensors with respect to time, the propagation direction at each frequency was determined. In all three groups, there was a biphasic direction of propagation of slow waves; retrograde at 16 cpm; antegrade at ~4 cpm. This did not differ between the groups. Cyclical increases in rectal pressure, which are associated with respiratory artefact, are also often visible on a manometry trace. The fact that the propagation direction was toward the rectum (retrograde) at the dominant frequency means that the peaks were unlikely to be associated with respiration. Rather, this type of sweeping action, may serve to help clean the anal canal as has been suggested previously [192]. Our results support the notion that the mechanism by which SW occur may in itself be important to continence (in addition to contributing to anal tone).

In absolute pressure terms, following baseline removal[401], only very small (2-3 mmHg) pressure differences were observed between groups per frequency (Table 5.1). The amplitude of wavelets was averaged across all the channels which were identified within the anal region. As evident on HR-ARM, the upper and lower anal canal tend to exhibit lower pressure compared to the mid-anal canal ("high pressure zone"), thought to represent the overlap between the IAS and EAS. If SW activity is the result of smooth muscle activity, whether myogenic or related to ICC, we might expect the amplitude at each frequency to be greater in the upper channels compared to the lower channels (assuming these correspond to the IAS and EAS respectively). The location where SW occur, relative to the pressure trace, could be something to investigate in future studies. However this is only feasible in subjects with a sufficiently long anal canal (or using a catheter with reduced sensor spacing) so that the number of channels in the distal anal canal can be distinguished from those in the high-pressure zone.

Study limitations

In addition to those limitations already mentioned, the study cohort was chosen based on meeting criteria designed to study the cough response (Chapter 4). However, as only a limited number of HR-ARM traces in the FI group were excluded from that study based on the fact that they didn't have any 'analysable coughs' (i.e. a single cough), we felt

that the number of patients excluded from the current study on this basis, but may have had a suitable resting pressure trace, was small. Nevertheless a degree of selection bias was inherently introduced.

This study was retrospective in nature and thus sampling was somewhat opportunistic based on the pool of HV data available at the time. For example, the duration of the resting period was considerably longer in more recent HV studies and studies in FI patients. Hancock et al[395] noted that it was impossible to evaluate SW amplitude by conventional manometry, due to the regular waxing and weaning behaviour and variability from one minute to the next. Although the process of extracting data for wavelet analysis was the same for participants, individual differences in where the chosen block of time was located within the stabilisation/resting period (e.g. at the start, in the middle, or immediately before squeeze) due to avoidance of artefacts may have influenced these results.

There is currently no data available on how amplitudes at a given frequency change over time. However, the very purpose of the first 3 minutes of the HR-ARM protocol (so-called stabilisation period, which was included in analysed data) is to allow resting pressure to stabilise following irritation of anal canal mucosa during probe insertion [146,393]. Future studies to validate the use of this method in the evaluation of anal sphincter function might: a) assess how wavelet amplitude changes over time; and b) evaluate the effect of artefacts or voluntary manouvres such as squeezing on results. Finally, future studies should explore the impact of obstetric factors and sphincter morphology (integrity and thickness) with regard to wavelet amplitude.

Conclusion

Wavelet analysis represents a novel, computational method for analysing anal SW captured by HR-ARM. The dominant frequency demonstrated herein agrees with findings from conventional manometry [192] and animal studies[406]. This analysis indicates that slow wave amplitude is reduced in female FI compared to both healthy nulliparous and parous women. Childbirth also appears to reduce SW amplitude in comparison to nulliparous women. Retrograde propagation observed at ~16 cpm may represent an important physiological mechanism to 'clean' the anal canal and help maintain continence.

Chapter 6 Prospective, multimodal assessment of anorectal function in healthy volunteers: method and results of established tests of anorectal function

Introduction

This chapter describes the methods used to prospectively evaluate anorectal function in healthy volunteers using established contemporary investigation methods and novel instruments. The first part of the chapter will provide the background and rationale for why studies of health are important for the interpretation of clinical and research data. The second part of the chapter will outline the methods and protocol used for data collection, followed by a selection of performed data analyses and early observations. Detailed analyses of novel methods (rapid barostat and EndoFLIP) will be covered in greater detail in subsequent chapters (7-8).

Background and rationale

Faecal incontinence (FI) represents a growing socio-economical and clinical problem[407]. Targetted treatment from the onset is made difficult by distant temporal relationships between events resulting in pathophysiology and symptom development[85]. Often, bothersome symptoms develop only when diverse structural, functional and neurological components of continence have become sufficiently compromised as a whole and it is generally this group of patients in whom clinical investigations are performed[371].

Interpretation of specialist diagnostic investigations used to assess several inter-related functions of the anorectum relies upon a firm understanding or the variability observed in health. The concepts and definition of normality with regards to colonic, rectal, and anal structure and function have been previously investigated in our unit [23,99,166,190,211,335,408-411]. Understanding the normal range, as well as any factors which might influence normal ranges such as gender, parity or age, is a prerequisite for introduction of any novel diagnostic modality into clinical practice so that individual patients can be reliably identified as having normal or abnormal function.

As detailed in Chapter 1, aspects anorectal structure and function require the use of multiple technologies each developed to assess a particular structural or functional component of the continence mechanism. With regards to anorectal function in particular, high resolution anorectal manometry (HR-ARM) assesses anal canal pressure and sphincter contractility in response to voluntary and involuntary manouvres and is also used to assess rectal sensitivity. Anal mucosal electrosensitivity assesses afferent pathway function in the anal canal using an electrosensitivity probe. Endoanal ultrasound assesses the integrity of the internal and external anal sphincters. Rectal sensation, capacity and compliance are measured using an electromechanical barostat. Finally, balloon expulsion can be used as a screening tool to assess bowel evacuatory dysfunction. In addition, several novel technologies have emerged in the last few years to comlement current routine testing. The Rapid Barostat Pump (Mui Scientific, Canada) has been proposed as a bedside alterantive to rectal sensory, compliance and capacity testing. Anal distensibility (or compliance) measured using EndoFLIP® (Crospon/Medtronic) evaluates anal competence to distension.

This single centre, prospective, observational study in health aims to define normal ranges for emerging techniques (EndoFLIP, Rapid Barostat) based on asymptomatic volunteers and to expand existing normal datasets for manometry (HR-ARM), and tests of anal and rectal sensation. Secondary aims were to determine the effects of gender, parity and age on anorectal function studies, to compare Rapid barostat with gold standard barostat, and to qualitatively/quantitatively decribe previously undocumented physiological phenomena/novel functional parameters in health.

Methods

Recruitment

Healthy volunteers were recruited via advertisement and mailing lists of public involvement groups including the National Bowel and Cancer Research and Queen Mary University of London. To make members of the local community aware of the study, presentations about the study were delivered locally and leaflets were handed out in several community locations. Information about the study was also posted on social media groups for Tower Hamlets residents. Targetted recruitment of parous women was done through parent support groups acting in the local community. The National

Bowel and Cancer Research website also hosted information about the study online, through which a wider population could be reached.

Pre-screening

Participants who expressed an interest in taking part by contacting the research team via phone or email were asked to provide an email address and sent a participant information sheet (PIS; **Appendix 4**). Individuals interested in participating after reading the PIS underwent a pre-screening by telephone (**Appendix 5**). Gender, parity and age of subjects was enquired to maintain a focussed and stratified approach to recruitment to ensure recruitment spanned a wide age range.

For participants meeting study criteria on pre-screening, a date and time for the study visit was scheduled. Participants received a reminder by email or text the day before their appointment. Although no bowel preparation was required for the study, participants were asked to fast for three hours before the study and to open their bowels as normal on the day of the test.

The original aim was to recruit 150 healthy volunteers consisting of 50 men and 100 women (50 nulliparous and 50 parous). Problems encountered in recruitment meant that final numbers were smaller than intended. Because the primary aim of the study was to expand upon existing normal datasets, no power calculation was performed to indicate the number of participants required for meaningful comparisons between groups. Furthermore, such a power calculation could prove meaningless, because of the multitude of different tests and variables measured during even a single investigation.

Screening and study visit

All study visits were held in the Wingate Institute for Neurogastroenterology (London). Participants were greeted and taken to the investigation lab where they received information about the study, investigations, and the researcher performing the investigations. Ample time was given to discuss any questions the participant may have regarding the research process (**Figure 6.1**). The participant was asked to provide written informed consent (**Appendix 6**). Due to a change in GDPR, the PIS and informed consent forms were modified part-way through the study in collaboration with Research Governance at Queen Mary, University of London.

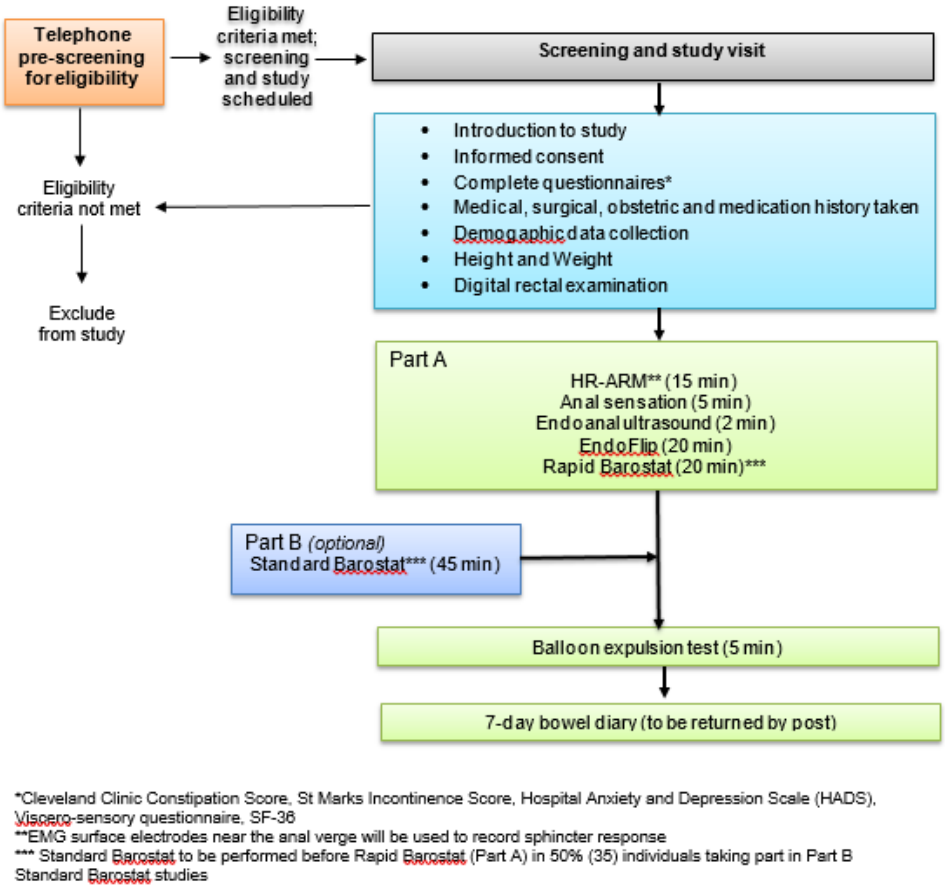


Figure 6.1 Study schematic

A detailed medical, surgical, obstetric, and medication history was taken including completion of validated diagnostic questionnaires to assess FI (St Marks Incontinence Score [SMIS] [332]) and constipation (Cleveland Clinic Constipation Score [CCCS] [333], Patient Assessment of Constipation Symptoms [412] [PAC-SYM]) symptoms. Beighton score [413] was also determined. Participants meeting full eligibility criteria (**Appendix 5**) were given the opportunity to continue to be enrolled into the study. Only enrolled participants were asked to complete further bowel and health related questionnaires (**Appendix 7**) including the Hospital Anxiety and Depression Score (HADS), a viscero-sensory questionnaire, and SF-36.

Eligibility criteria

Inclusion criteria

Inclusion criteria were healthy males or females aged 18-75 years without coexisting acute or chronic disease, average Bristol stool type[414] between 2-5 and bowel frequency between 3-21 spontaneous bowel movements per week[367,368]. Participants had to have a Cleveland Clinic Constipation Score (CCCS)[333] <8 and a St Marks Incontinence Score[332] ≤5. The ability to understand the PIS and instructions in English and ability to provide informed consent were also required.

Exclusion criteria

Exclusion criteria were not meeting the inclusion criteria above, latex allergy, ongoing diagnosed lower gastrointestinal disease or complication (such as IBS, Crohn's disease, coeliac disease, chronic diarrhoea etc.), any red flag symptoms (sudden weight loss, rectal bleeding, change in bowel habit, abdominal pain or stool positive for occult bleeding in the last 3 months), prior GI surgery (except cholecystectomy and appendectomy), history of neurologic disease known to affect GI function (such as multiple sclerosis, stroke, spinal cord injury, Hisrchsprungs disease), ongoing therapy with medications known to affect lower GI function including antidepressants, any current or recent illness (of acute or chronic nature) that may confound the study outcomes including cardiovascular, endocrine, renal, or other disease likely to affect gut motility or limit normal functions (eg reduced mobility), self-reported symptoms of pelvic organ prolapse, history of severe haemmorrhoids, chronic anal fissures or other anal pathology; for women, pregnancy or breast-feeding, and previous hysterectomy.

Study protocol

Participants were given a hospital gown and asked to undress from the waist down. All investigations were performed by a single investigator with the participant lying on their left hand side with the knees and hips flexed at 90-degrees. The main part of the study (Part A) consisted of HR-ARM, anal sensation, endo-anal ultrasound, EndoFLIP, Rapid barostat, and balloon expulsion. Participants wishing to volunteer for Part B of the study, which involved standard barostat assessment of rectal sensation, compliance and capacity, were asked for separate consent for this investigation. Standard barostat was performed prior to RBB in 50% of consenting individuals. All standard barostat

investigation were performed by a co-investigator. At the end of the study visit, all participants were given a 7-day bowel diary (**Appendix 8**) to complete at home. Upon return of the bowel diary, participants received financial compensation (£75-100) for taking part in the study. A digital rectal examination was performed prior to investigations to check for stool in the rectal ampulla and ensure safe intubation.

Part A

High-resolution anorectal manometry (HR-ARM)

High resolution anorectal manometry (HR-ARM) was performed using solid state catheter (UniTip: UniSensor AG, Switzerland) with an external diameter of 12-16 Fr. The catheter incorporates 10 micro-transducers placed 0.8 cm apart, with a total measurement distance of 8 cm and a distal, 'external' pressure sensor spaced 1.5 cm below. Pressure is measured circumferentially by means of a unidirectional pressure sensor embedded within silicone gel. An additional microtransducer was located within a 600 ml non-latex HRAM balloon (Mui Scientific, Ontario, Canada) tied to a groove cut into a metal ring 3 cm from the catheter tip using thread. Pressure data acquisition (at 10 Hz), online visualisation, and signal processing were performed using a commercially available manometric system (Solar GI HRM v9.1, Medical Measurement Systems [MMS]/Ardmore, Enschede, The Netherlands). Prior to each study, the catheter sensors were soaked in tepid water for at least 3 minutes prior to zeroing to atmosphere under 1 cm of water. The catheter was then gently inserted into the anorectum such that the distal two microtransducers were visible (with the more proximal one situated just outside the anal verge) and connected to an automated air pump (Ardmore Enschede, The Netherlands) by silicone tubing.

The standardised protocol [146,190] used during HR-ARM is summarised in **Figure 6.2.** A prolonged stabilisation period of 6 minutes was chosen to enable analysis of anal slow waves, this was followed by 1-minute of rest. Squeeze increment and maximum pressures were recorded during three consecutive voluntary contractions at maximal effort lasting 5 seconds each. A 30-second resting period followed each squeeze. A prolonged squeeze, during which participants were instructed to maintain squeeze for 30 seconds followed by 60 seconds of rest was performed. Two consecutive, single coughs were performed 30 seconds apart. Once pressures had normalised, 60 ml of air

was injected into the balloon by an automated pump for assessment of RAIR. Participants were asked to verbally report whether they felt something or not. The balloon was deflated and the return of pressures (to resting level) observed. Balloon distension was performed, again by automatic insufflation, at a rate of 2ml/sec. Participants were asked to report first sensation, urge, and maximum tolerable volume to distension.

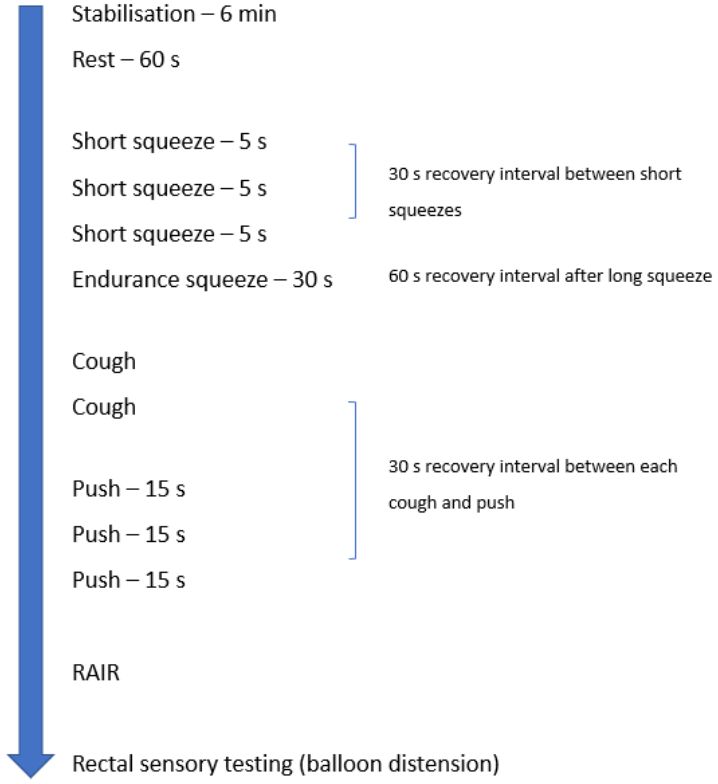


Figure 6.2 HR-ARM protocol (adapted from Carrington *et al* [146])

High-resolution anorectal manometry (HR-ARM) with EMG evaluation of cough response

Pre-gelled, self-adhesive, 15 mm x 20 mm surface electrodes (Friendship Medical, Shaanxi, China) connected to an EMG unit (The Solar Neuro Module, Ardmore, Ensched, The Netherlands) were attached to measure EAS muscle contractility. Prior to their placement, the skin surface was wiped of any excess gel and moisture using paper towel to promote adhesion. Electrodes were placed at 9 and 3 o'clock as close as possible to the anal orifice[415]. A third electrode was placed on the skin of the right outer thigh to limit artefact in the recorded signal. Manometry incorporating EMG assessment was

performed at 50 Hz and the position of the manometry catheter adjusted to avoid artefact due to contact pressure from adjacent surface electrodes as much as possible, while maintaining the quality of the pressure recording (**Figure 6.3**).

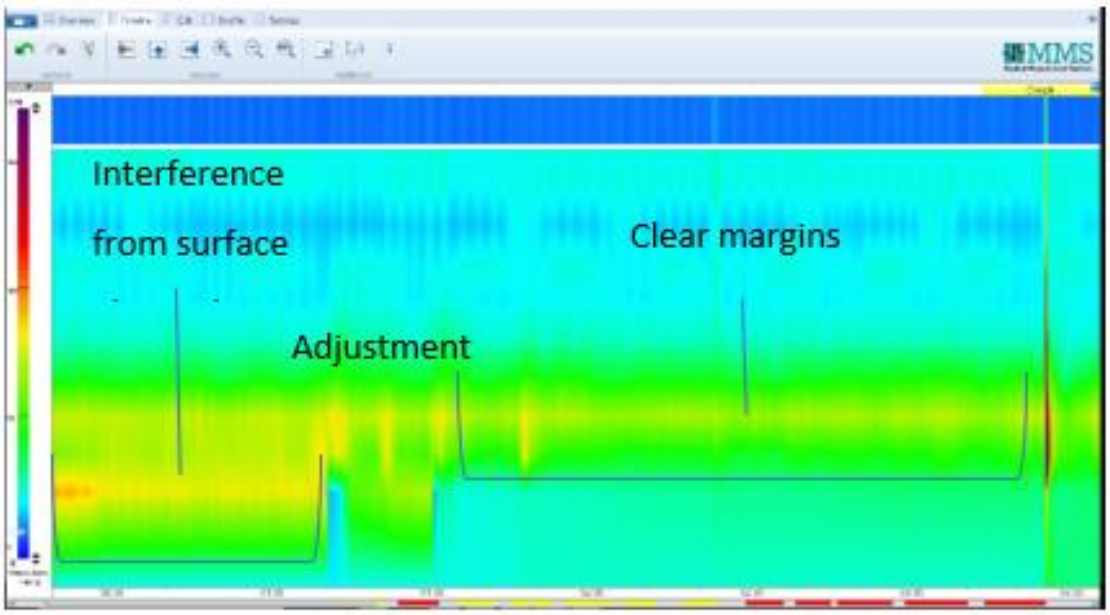


Figure 6.3 Adjusted positioning of the EMG surface electrodes during manometry to avoid pressure artefact at the distal anal verge.

Following a 3-minute stabilisation period, an enhanced cough protocol was performed (**Figure 6.4**). Participants were asked to perform two maximal single coughs 30 seconds apart. After this, 5 single coughs were performed into a peak flow meter (Respiratory monitor, asmaPLAN standard, Model 4300, Vitalograph) via a single use cardboard mouthpiece. The peak flow rate achieved for each cough was noted by the investigator. Participants were able to practice coughing into the mouthpiece prior to the commencement of the investigations. For the last component of the cough assessment, participants were asked to perform 3 coughs (without the use of the peak flow meter) of increasing intensity (small, medium and large cough) separated by 30 seconds rest. Finally participants were asked to perform two 5 sec pushdowns. Prior to the second pushdown, the rectal balloon was inflated with 50ml air and deflated after the manouvre.

Once anal canal pressures returned to resting tone, the electrodes, manometry catheter and balloon were removed concluding the investigation. Data were saved for further processing *post-hoc*.

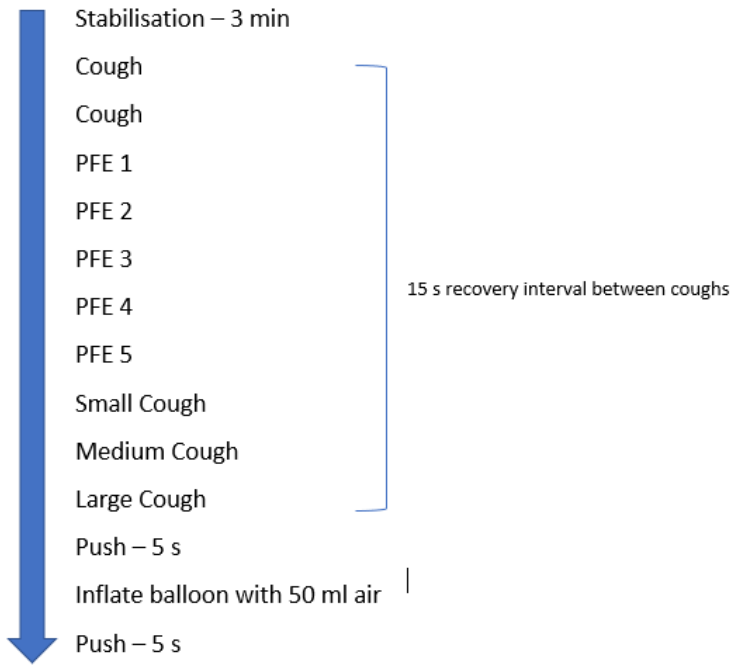


Figure 6.4 EMG-cough study protocol (HR-ARM)

Anal sensation

Anal mucosal electrosensitivity was measured using a bipolar ring electrode mounted onto a catheter (Gaeltech, Isle of Skye, UK), which was marked at 1 cm intervals starting at the midpoint between the two electrodes (see **Chapter 1, Figure 1.7**). The electrodes had an inter-electrode distance of 1 cm. The catheter was connected to a constant current stimulator (Neuropack, Nihon Kohden, UK) connected to a Shuttle computer. A ground electrode was placed around the ankle.

The probe was lubricated with conductive gel and introduced into the anal canal such that the first sensory measurement was taken 0.5 cm inside the anal verge. A constant electrical current was delivered to the mucosa at a stimulus of 5 Hz frequency and 100 ms pulse duration, starting at 0 mA and gradually increasing the stimulus intensity until the subject reports a sensation (typically either burning or tingling)[212]. Three recordings were performed to obtain minimum and mean sensory threshold. Measurements were repeated at 1.5 cm and 2.5 cm from the anal verge approximating to the mid and proximal anal canal.

Endo-anal ultrasound

Two dimensional, cross-sectional axial images of the anal canal were acquired from the level of puborectalis through to the anal verge at either 11MHz (Echoson ALBIT R-510, Pulawy, Poland) or 13 MHz (B-K Medical Pro Focus, 2101, Berkshire, UK) using 360-degree endoanal ultrasound probes. IAS and EAS integrity were categorised as intact or abnormal (disrupted, degenerate/atrophic or focally abnormal). Sphincter disruption was defined as described in previous chapters[68].

EndoFLIP

Anal distensibility was measured using the EndoFLIP® system (Model 1.0, Medtronic, Minneapolis, USA). A re-usable EndoFLIP® probe was attached to the EndoFLIP® system and calibration checked. The catheter was zeroed as part of the initialisation process. The tip of the probe and balloon were lubricated and inserted into the rectum, such that 4 sensors were visible outside the anal verge. If the pressure had failed to zero or appeared unstable or negative after intubation, the catheter was re-zeroed inside the rectum. The bag was distended to 10 ml and then deflated to ensure proper opening.

During distensions, the probe was held in place manually. A total of 5 distensions were performed at 10, 20, 30, 40 and 50 ml (**Figure 6.5**). At the end of each round of filling, resting pressure was measured for up to 30 seconds (until stable measurements were observed). The participant was asked to squeeze maximally for 5 seconds. Once the intra-bag pressure had stabilised and returned to resting level, the participant was asked to perform a single, maximal cough. Images were captured using the EndoFLIP system at rest and separate clips were captured during squeeze and cough. This was done to facilitate data analysis post-hoc. The balloon was deflated fully and the position of the catheter checked. Following the procedure, the EndoFLIP probe was removed for cleaning and disinfection.

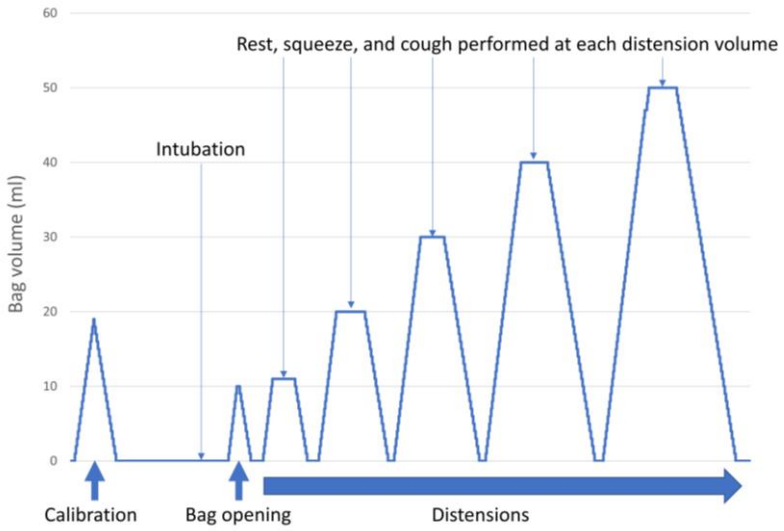


Figure 6.5 EndoFLIP distension protocol.

Rapid barostat

Rectal sensation, capacity, and compliance were assessed using The Rapid Barostat Bag (RBB) Pump (Mui scientific, Ontario, Canada). A 700 ml, non-compliant, barostat bag (S7-BR-1018, Mui scientific, Ontario, Canada) was connected to the device and deflated. The tip of the probe and the bag were lubricated and inserted into the rectum approximately 5 cm above the anal verge so that the 10 cm marker on the probe was visible just outside the anal canal. The position of the bag was verified digitally to ensure that the edges of the bag were well beyond the anal canal. Two filling cycles were performed at a rate of 120 ml/min. During the first 'compliance' cycle, the bag was filled continously until a bag pressure of 40mmHg was reached or the participant indicated discomfort/pain, at which point the bag was automatically deflated. During the second 'sensation' cycle, the bag was filled continously and the participant was asked to inform the researcher when they first became aware of the balloon (first sensation), when they felt a continuous desire to defaecate (urge) and when they felt discomfort/pain (maximum tolerable volume). Compliance and sensory data in the auto-generated report were reviewed online, prior to exporting the report and raw data to a personal computer for further processing. Detailed description for calculating compliance from raw data files will be described in Chapter 8 Evaluation of Rapid vs Standard Barostat.

Balloon expulsion

A latex balloon was attached to a 16 Fr Foley catheter with thread (**Figure 6.6**). The lubriated balloon was carefully insterted into the rectum using digitation to guide the balloon beyond the anal canal margins. A bladder syringe was used to fill the balloon with 50 ml of room temperature water and the tail of the catheter clamped shut using a removable plastic clip. The participant was asked to carefully walk to the adjoining private bathroom and to sit on the toilet. A kidney bowl was placed into the toilet a priori to catch the balloon. The participant was asked to expel the balloon and the time taken to expel the balloon was recorded.

Figure 6.6 Latex party balloon (filled with 50 ml air) used for balloon expulsion.

Bowel diary

A bowel diary (**Appendix 8**) was completed over 7 days following the study visit. Participants were asked to note down defaecation frequency, stool consistency, perception of desire to defaecate, and whether they experienced straining or urgency related to bowel opening. A pre-paid, addressed envelope was provided for the return of the questionnaire.

Part B:

Standard barostat

A 600 ml, infinitely compliant barostat bag (S7-CB-R002, Mui scientific, Ontario, Canada) was inserted into the rectum 5cm beyond the internal anal verge. The bag was inflated with 75 ml of air using a pre-calibrated barostat pump (Distender Series, Model II device, G&J Electronics, Toronto, ON, Canada), to ensure proper unfolding of the bag.

Basal operating pressure and minimum distension pressure (MDP) (Bell et al 1991) were established. An initial conditioning distension (Hammer et al 1998) was performed by increasing bag pressure in 4 mmHg increments at 60 second intervals until 20 mmHg. After a 10 minute resting period, following an ascending method of limits protocol, the bag pressure was increased in 4 mmHg intervals and held for 60 seconds. Thirty seconds into each distension, participants were asked to report sensory response using a visual analog scale: 1- No sensation, 2- first sensation, 3- desire to defaecate, 4- urgency, 5- discomfort. When discomfort/maximum tolerated volume was reached, the bag was deflated and the balloon removed.

Data processing and Statistical analysis

Manometry traces were analysed in accordance with guidelines [146]. Analysis of electromucosal sensitivity thresholds was based on the lowest threshold of three repetitions for each level studied. Ultrasound images were assessed for integrity and impression of sphincter quality as described in previous chapters.

All statistical analyses were performed in SPSS, GraphPad, or R. Parametric and non-parametric tests were used as appropriate given the distribution of variables to compare results between men vs women, and nulliparous vs parous analyses. Linear regression models in R were used to assess differences between multiple groups (ANOVA). A p-value < 0.05 was considered signficant.

Results

Participant demographics

In total 51 participants (aged 19-67 years) were recruited for the study (**Table 6.1**). There were no significant differences ($t(49)=0.1005$, $p=0.923$, **Figure 6.7**) in mean age between males (n=14, 38.8 yrs, sd= 17.2) and females (n=37, 38.4 yrs, sd=11.0), or between nulliparous (n=19, 35.2 yrs, sd=11.8) and parous (n=18, 41.8 yrs, sd=9.1) females ($t(35)=1.905$, $p=0.0651$). Mean BMI similarly did not differ between groups ($p>0.05$) and was not associated with age ($p=0.1241$, $R^2=5.1\%$, 95% CI of the slope -0.02 to 0.18). The majority of participants were white (70.6%). All participants had a CCCS ≤2 and SMIS ≤4. Of constipation type symptoms, incomplete emptying 'rarely' or 'sometimes' was reported by 15.7% and abdominal pain pain during evacuation 'rarely'

or ‘sometimes; by 25.5%. Incontinence to gas ranging from ‘rarely to daily’ was reported by 19.6%.

Of women included in the study, 48.6% (18/37) were parous. The majority, 72.2% had had at least one vaginal delivery. In four subjects, at least one delivery was described as either instrumental or traumatic. Five women (27.8%) had only ever given birth by Caesarean-section. In all cases, a C-section was performed before reaching the second stage of labour (pushing phase). Overalll, the median number of births was 2 (range 1-3).

Variable	Total (N=51)	Females (N=37)	Males (N=14)
Age [median (IQR)]	36 (31-45)	34.5 (25-53)	37 (32-42)
BMI [median (IQR)]	22.7 (21.7-25.4)	22.5 (21.5-25.1)	23.8 (22.2-25.7)
SMIS [median(range)]	0 (0-4)	0 (0-4)	0 (0-2)
CCCS [median(range)]	1 (0-2)	0 (0-2)	0 (0-2)
Beighton score [median(range)]	0 (0-6)	1 (0-6)	0 (0-2)

Table 6.1 Demographics

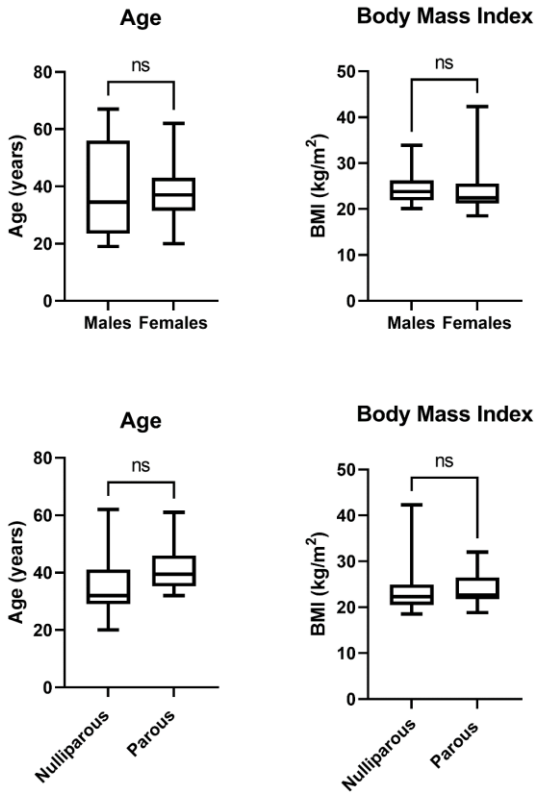


Figure 6.7 Boxplots of age and BMI in males and females, and in nulliparous and parous women. The midline represents the median; whiskers represent minimum and maximum values in each group.

Investigation performance and acceptability

The majority of subjects completed all six investigations in the study protocol, as described in Chapter 7 (Methods). All investigations were generally well tolerated. The average acceptability score for each investigation are shown in **Figure 6.8**, along with the proportion of participants in whom studies were performed. The acceptability score for rapid barostat (RBB) was signicantly lower than for anal electromucosal sensitivity (Bonferroni-adjusted p-value=0.0217, 95% CI for mean difference= 0.08 to 2.14) and balloon expulsion (Bonferroni-adjusted p-value=0.0199, 95% CI for mean difference= -2.1 to -0.09). There were no other significant differences between scores at a 95% significance level.

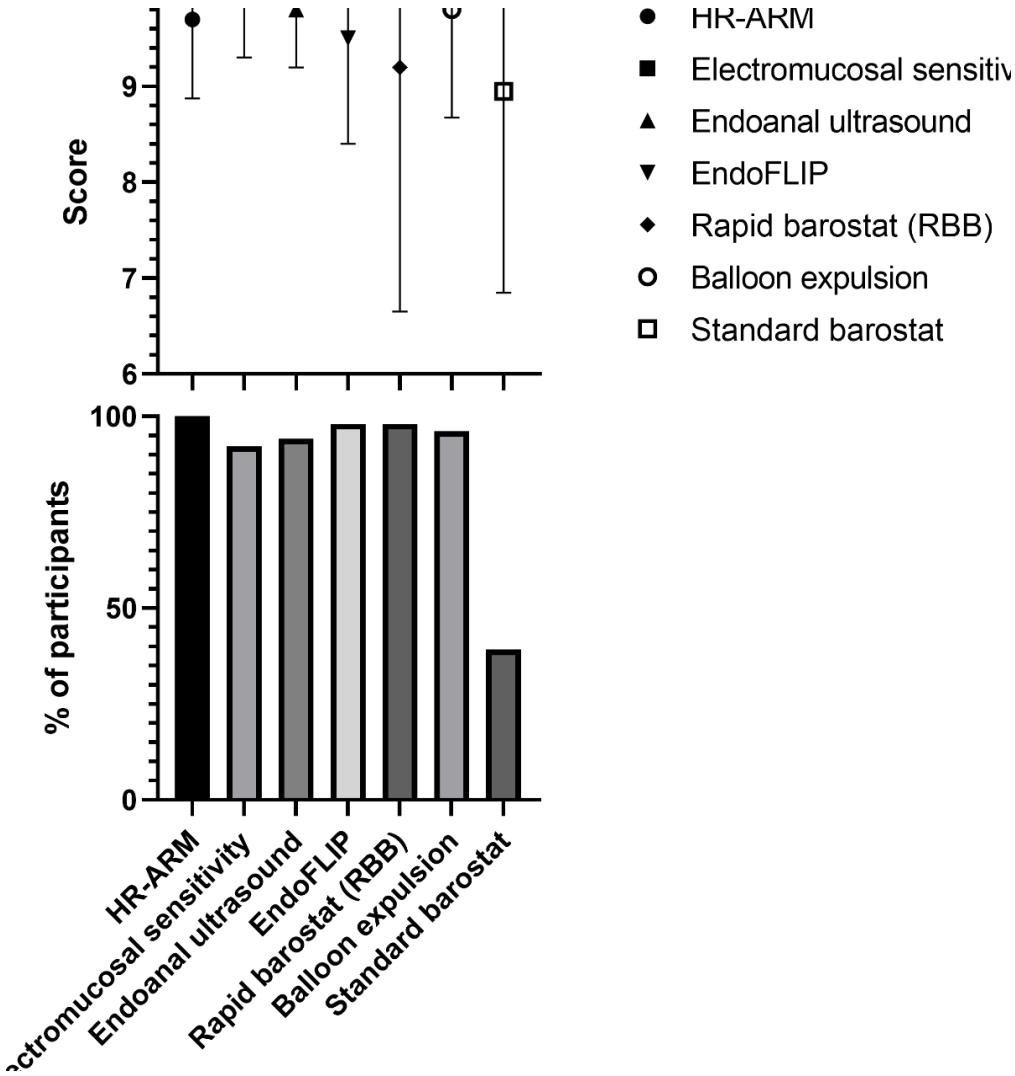


Figure 6.8 Frequency and acceptability of completed investigations

Due to the COVID-19 pandemic, restrictions on studies prevented coughing during high-resolution anorectal manometry (HR-ARM) and EndoFLIP. For this reason, no coughs or EMG were performed in a proportion of participants. Of the 36 subjects in whom an EMG study was performed prior to the pandemic, a reliable EMG and pressure recording was obtained in 26 subjects.

High-resolution anorectal manometry

Resting pressure

High-resolution anorectal manometry (HR-ARM) was performed in all subjects (**Table 6.2**). Mean resting pressure was 13.1 mmHg (95% CI 1.4-24.9 mmHg) higher in men than in women (p=0.03). This result has 51.3% power to detect a true difference. There was no significant difference in resting pressure between nulliparous and parous women

(p=0.113). Given the observed differences, a sample size calculation based on these data would indicate that a total sample size of 128 participants would be needed to observe a difference between groups at the 95% significance level with 80% power in future studies. Age was not significantly associated with anal resting pressure (p=0.2996). BMI was significantly associated with resting pressure only in men (p=0.0417, R^2= 30.22%, 95% CI of slope 0.15 to 6.55) (**Figure 6.9**).

	All	Males	Females	Nulliparous	Parous
n	51	14	37	19	18
Mean (sd)	69.3 (19.4)	78.8 (22.2)	65.7 (17.2)	70.0 (18.2)	61.0 (15.3)
Median	66.3	77.9	66.3	76.1	64.4
IQR	57.8 - 81.2	61.9 - 97.1	55.7 - 78.3	56.2 - 82.7	49.3 - 69.8
95% CI	63.8 - 74.7	66.0 - 91.6	59.9 - 71.4	61.3 - 78.8	53.4 - 68.7
5th	34.3	40.4	33.1	31.5	33.3
95th	101.2	121.6	95.6	95.3	98.5

Table 6.2 Resting pressure within groups

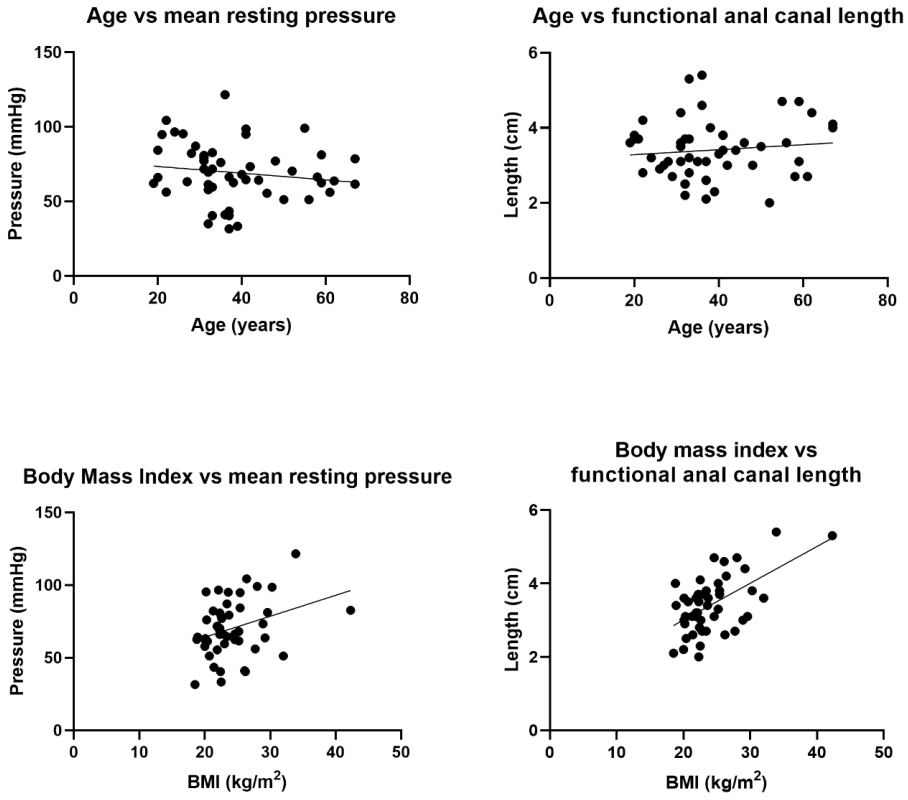


Figure 6.9 Association between age/BMI and functional anal canal length (FACL) and mean resting pressure

Slow-wave activity

In addition to evaluating resting pressure, anal canal slow wave activity was evaluated during the resting and stabilisation periods. Wavelet analysis of the resting and stabilisation periods showed that the highest amplitude of oall tested frequencies occurred at 16 cpm. Across all frequencies, females had significantly lower amplitudes ("power"; see Chapter 5 on anal slow waves) than males (**Figure 6.11**). There were no differences in slow wave amplitude between nulliparous and parous women.

Both antegrade and retrograde movement were observed at all tested frequencies with no preference for direction (on average). At 4 cpm, there was about 80% chance of preferentially antegrade movement (on average). No differences in phases (direction of propagation) were observed between groups.

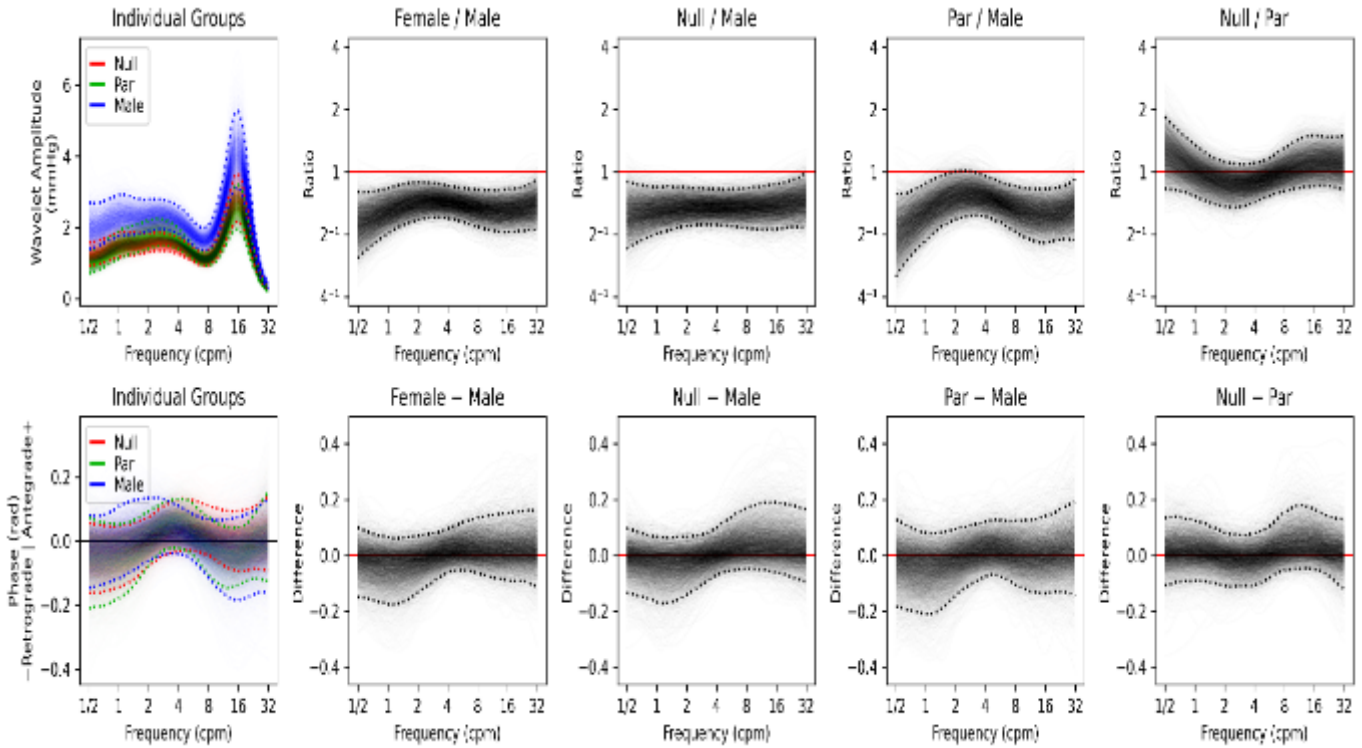


Figure 6.10 Wavelet analysis. The top panel indicates amplitude ("power") at each tested frequency and differences between groups. The lower panel indicates the average propagation direction (with no significant differences between groups.

Functional anal canal length

Mean FACL was 3.4 cm (95% CI 3.2-3.6 cm) (Table 6.3). There was no significant effect of sex (p=0.31) or parity (p=0.382) on FACL. **Figure 6.10 (above)** summarises the effect of age and BMI on FACL. BMI was associated with FACL in all groups except parous women (p=0.3233). In men, for every unit increase in BMI, FACL increased between 0.05 to 0.25 * BMI (p=0.0053, R^2= 48.97%) and in women, FACL increased between 0.04 to 0.13*BMI (p=0.0005, R^2= 31.79%). In nulliparous women, for every unit increase in BMI, FACL increased between 0.06 to 0.15 * BMI (p=0.0002, R^2= 64.38%). FACL had a significant positive linear association with resting pressure for the group as a whole (p= 0.0007, R^2= 20.96%, 95% CI of slope= 5.08 to 17.9) and in males (p=0.0345, R^2= 32.16%, 95% CI of slope= 1.380 to 30.55) only (**Figure 6.12**)

	All	Males	Females	Nulliparous	Parous
n	51	14	37	19	18
Mean (sd)	3.4 (0.8)	3.8 (0.8)	3.3 (0.7)	3.4 (0.8)	3.2 (0.7)
Median	3.4	3.7	3.2	3.4	3.2
IQR	2.8 - 3.8	3.1 - 4.3	2.7 -3.7	2.8 - 3.7	2.7 - 3.7
95% CI	3.2 - 3.6	3.3 - 4.2	3.0 - 3.5	3.0 - 3.7	2.8 - 3.5
5th	2.2	2.6	2.1	2.1	2
95th	4.9	5.4	4.7	5.3	4.6

Table 6.3 FACL descriptive statistics

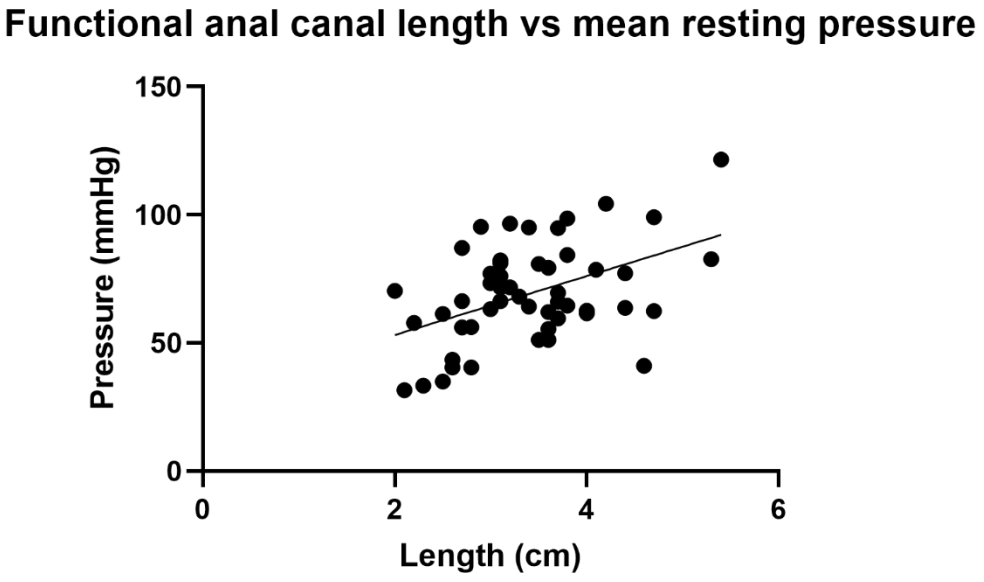


Figure 6.11 Association between functional anal canal length and mean resting pressure

Squeeze increment

Assuming unequal variances, maximum squeeze increment was, on average, 132 mmHg greater in males than in females (p=0.001, 95% CI= 59.2 – 204.8 mmHg, power= 96.8%). Amongst women, there were no significant differences between nulliparous and parous subjects (p=0.858 with equal variances) although this result lacks power (3.8%). However, a sample size calculation based on our findings would suggests that 9100 women would be needed to detect a significant difference at alpha level of 0.05 and 80% power. Maximum squeeze increment was not associated with age (p=0.271) or with BMI (p=0.196) for the group as a whole. In men, BMI was significantly associated with maximum squeeze increment (p=0.006, R^2= 48.33%, 95% CI of slope= 8.1 – 38.15).

	All	**Males**	**Females**	**Nulliparous**	**Parous**
n	51	14	37	19	18
Mean (sd)	201 (104.9)	296.8 (121.2)	164.8 (71.1)	166.9 (66.4)	162.6 (77.7)
95% CI	171.6 - 230.6	226.8 - 366.9	141.1 - 188.5	134.9 - 198.9	124.0 - 201.2
Median	171.4	266.9	159.3	159.3	156.9
IQR	133.5 - 258.0	209.6 - 391.0	113.5 - 216.2	112.4 - 244.2	109.6 - 214.4
5th	96.1	148.6	51.8	60.5	21.6
95th	520		276		

Repeatability of squeeze

According to a mixed model, there were no significant differences in maximum squeeze increment between the first, second, and third squeeze attempts (**Figure 6.13**). The squeeze attempt did have a statistically significant impact (p=0.0063) on mean squeeze increment, which was significantly greater during the 1st attempt than the 2nd (adjusted p-value=0.0493, mean diff 8.294, 95% CI 0.02 to 16.6) or 3rd (adjusted p-value =0.0039, mean diff 12.01, 95% CI 3.4 to 20.6) attempts. There was no statistically significant difference between attempts 2 and 3 (adjusted p-value = 0.2925).

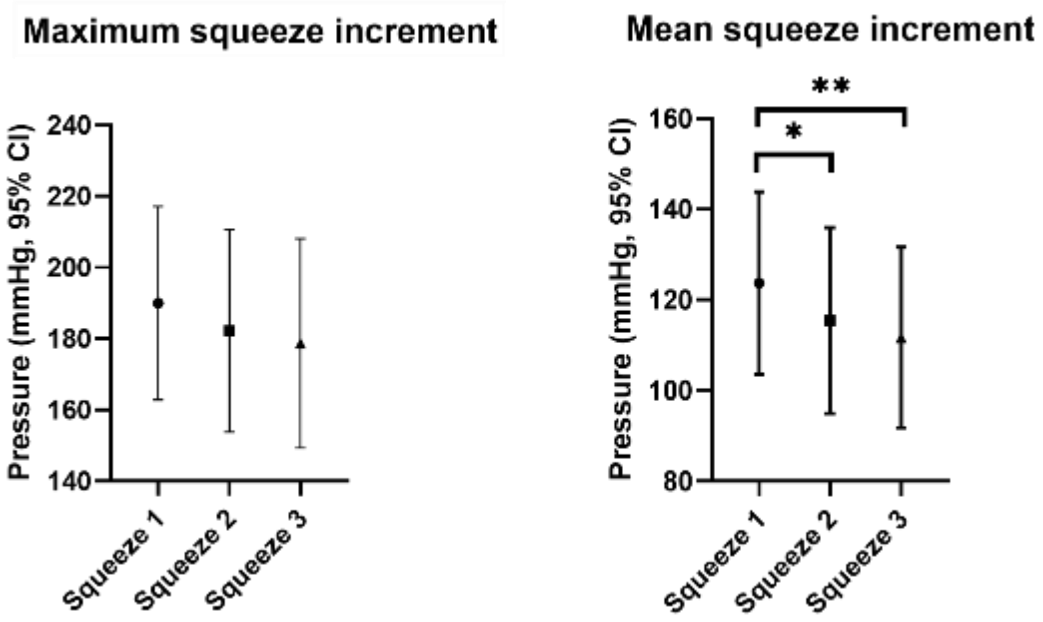


Figure 6.12 Repeatability of squeeze

Endurance squeeze

Endurance squeeze time (Table 6.4) was not significantly different between males and females (p=0.992, 95% CI -7.0 to 7.0 sec) or between nulliparous and parous women (p=0.692, 95% CI -9.2 to 6.2 sec). There was no significant correlation between age (p=0.241) or BMI (p=0.126) and endurance squeeze time.

	All	Males	Females	Nulliparous	Parous
n	51	14	37	19	18
Mean (sd)	12.5 (11)	12.5 (10.4)	12.5 (11.4)	11.7 (5.5)	13.2 (12.2)
95% CI	9.4 - 15.6	6.5 - 18.5	8.7 - 16.2	6.5 - 16.9	7.1 - 19.3
Median	6.5	8.8	5.5	5.5	5.6
IQR	3.7 - 27.5	4.1 - 19.4	3.7 - 28.3	4.4 - 21.1	5.6 - 29.5
5th	1.9	1.5	2.2	1.4	2.1
95th	30	30	30	30	30

Table 6.4 Duration during which endurance squeeze pressure was maintained at >50% of maximum increment.

Overall, endurance squeeze time was significantly negatively correlated with maximum pressure (r= -0.339, p=0.015) and maximum pressure increment (r= -0.373, p=0.007). However, the effect was only statistically significant in parous women (p=0.016, R^2=31.19%, 95% CI of slope -5.265 to -0.62) (**Figure 6.13**). In nulliparous women the 95% CI for the slope was -5.6 to 0.42 tending towards a statistically significant associtation.

Of 37 women, 12 were able to maintain squeeze pressure >50% of maximum (endurance squeeze) increment for >15 seconds. These women (50:50, nulliparous:parous) had a significantly lower maximum (endurance) squeeze pressure (157 mmHg, sd=73 vs 222mmHg, sd=69; p=0.14, 95% CI of mean difference 14 to 116 mmHg) and increment (91 mmHg, sd=64 vs 153 mmHg, sd=50; p= 0.006, 95% CI of mean difference= 19.6 to 105 mmHg). The difference in men was not statistically significant (p=0.716 for maximum pressure and p=0.673 for maximum increment), however the number of men who were able to maintain squeeze pressure >15 seconds was small (n=4 vs n=10).

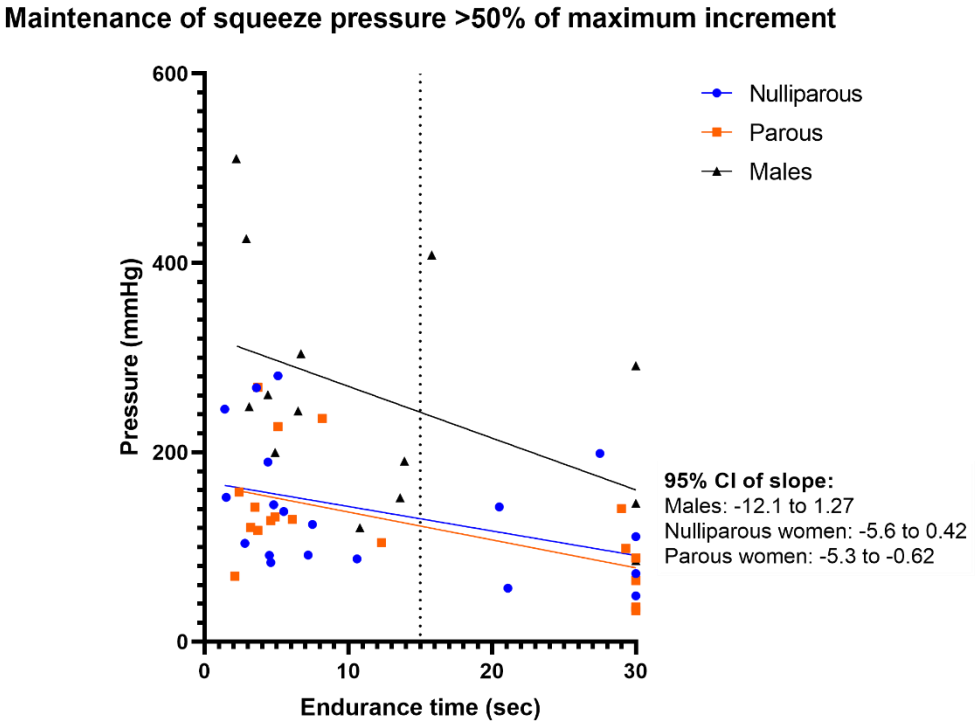


Figure 6.13 Duration during which endurance squeeze pressure was maintained above 50% of maximum increment by group. Dotted line shown at 15 seconds.

Assuming unequal variances, the area under the curve (AUC) (Table 6.5) during endurance squeeze was significantly higher (p=0.001) in men than in women by 2166 units on average (95% CI 1062 – 3270). There was no significant difference in AUC between nulliparous and parous women (p=0.632, 95% CI of mean difference -470 to 764 units). AUC was significantly associated with maximum pressure (Pearson's correlation 0.836, p<0.001) and maximum pressure increment (Pearson's correlation 0.853, p<0.001) during endurance squeeze, but not endurance squeeze time (p=0.068).

	All	Males	Females	Nulliparous	Parous
n	51	14	37	19	18
Mean (sd)	1996 (1567)	3568 (1860)	1402 (914)	1473 (970)	1326 (873)
95% CI	1556 - 2437	2493 - 4642	1097 - 1707	1006 - 1941	892 - 1760
Median	1443	2977	1189	1208	1171
IQR	941 - 2629	1918 - 5020	695 - 1885	679 - 2094	766 - 1532
5th	381	1125	342	349	283
95th	5972		3520	3279	

Table 6.5 Area under the time-pressure curve (AUC) during endurance (sustained) squeeze.

Push

Push effort (rectal pressure) was evaluated in 50 participants. There was no significant difference in mean (p=0.124, 95% CI -28.4 to 3.5) (**Table 6.6**) or maximum (p=0.130, 95% CI -33.7 to 4.5) (**Table 6.7**) rectal pressure during push between men and women based on the average of three pushes. Similarly, there were no significant differences in mean (p=0.449, 95% CI -23.4 to 10.6) or maximum (p=0.600, 95% CI= -25.5 to 15.0) between nulliparous and parous females. At the 95% confidence level no significant association between age or BMI and push pressures was observed (p>0.05).

	All	Males	Females	Nulliparous	Parous
n	50	14	36	18	18
Mean (sd)	51.6 (25.6)	55.0 (25.8)	48.1 (25.0)	44.9 (25.9)	51.4 (24.3)
95% CI	44.4 - 58.9	45.7 - 75.4	39.7 - 56.6	32.1 - 57.8	39.2 - 63.5
Median	47.9	55	44.2	38.1	49.2
IQR	31.1 - 69.2	43.2 - 77.5	27.6 - 60.6	27.3 - 59.4	28.1 - 70.3
5th	17.5	13.2	18	16.7	18.2
95th	106		105.1		

Table 6.6 Mean rectal pressure during push

	All	Males	Females	Nulliparous	Parous
n	50	14	36	18	18
Mean (sd)	65.9 (30.6)	76.4 (31.7)	61.8 (29.5)	59.2 (31.8)	64.4 (27.8)
95% CI	57.2 - 74.6	58.1 - 94.7	51.8 - 71.8	43.4 - 75.0	50.6 - 78.2
Median	62.1	75.9	61.2	53.5	63.1
IQR	41.1 - 88.4	53.7 - 95.8	37.2 - 71.1	36.6 - 69.4	39.8 - 89.3
5th	25.9	17.7	26.6	27	24.5
95th	128.2		120.8		

Table 6.7 Maximum rectal pressure during push

Repeatability of push

There were no significant differences between push attempts (propulsive effort) for either mean (p=0.6549) or maximum (p=0.5582) push pressure (i.e. rectal pressure during push) (**Figure 6.14**).

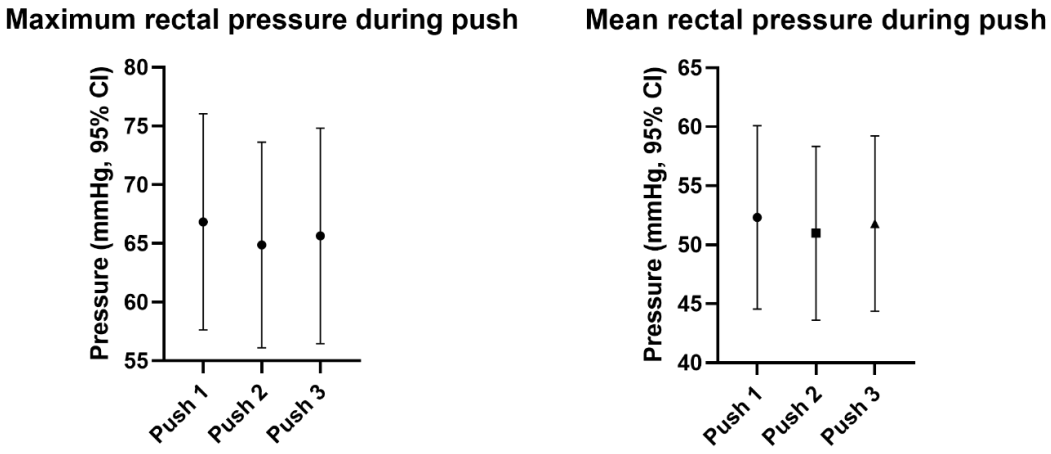


Figure 6.14 Repeatability of push effort

Cough

Cough parameters are summarised in **Table 6.8**. Between males and females, rectal pressures (maximum pressure p=0.006, 95% CI 7 – 48 mmHg; maximum increment p=0.011, 95% CI 6 – 42 mmHg) and anal pressures (Kruskall-wallis, maximum pressure p-value= 0.024; maximum increment p= 0.024) were significantly different. There were no significant differences in rectoanal gradient (p=0.089) or anal-rectal incremental difference (p=0.196) between groups. The anal-rectal duration difference was also not significantly different (p=0.059). Between nulliparous and parous women, no significant differences were observed for any cough measures (p>0.1 for all comparisons).

	All (n=44)				Males (n=14)				Female (n=30)			
	Mean (sd)	95% CI	Median	5^{th} -95^{th}	Mean (sd)	95% CI	Median	5^{th} – 95^{th}	Mean (sd)	95% CI	Median	5^{th} -95^{th}
Maximum rectal pressure during cough	84 (34)	74 - 95	80	34 - 149	103 (40)	81 - 126	99	37 -	76 (26)	66 - 85	74	33 - 128
Maximum rectal increment during cough	77 (30)	67 - 86	71	36 - 141	93 (35)	73 - 113	84	41 -	69 (24)	60 - 78	68	33 - 120
Maximum anal pressure during cough	188 (78)	164 - 212	177	99 - 387	230 (100)	173 - 288	201	81 -	168 (57)	147 - 189	157	99 -287
Maximum anal increment during cough	118 (71)	96 - 139	90	47 - 302	152 (91)	100 - 205	127	46 -	101 (54)	81 - 122	83	41 - 233
Absolute anal-rectal pressure difference	104 (63)	84 - 123	94	22 - 243	127 (73)	85 - 169	110	43 -	93 (50)	72 - 113	90	21 - 211
Incremental anal-rectal pressure difference	43 (58)	25 - 60	24	-22.1 to 171	59 (72)	18-101	30	-11	35 (50)	16 - 54	22	-25 to 161
Anal-rectal duration difference (sec)	1.3 (0.8)	1.0 - 1.5	1.2	0.2 - 2.7	0.9 (0.6)	0.6 - 1.3	0.9	0.1	1.4 (0.8)	1.1 - 1.7	1.4	0.4 - 3.4

Table 6.8 Cough parameters

For the group as a whole, maximum rectal pressure was significantly positively correlated with age (r=0.407, p=0.006) and BMI (r=0.364, p=0.019). Maximum rectal increment also correlated with BMI (r=0.347, p= 0.26) but not age. None of the other variables were significantly associated with age or BMI. In grouped analyses, the correlation between BMI and rectal pressure remained present in men only (maximum pressure r=0.586, p=0.028; maximum increment r= 0.568, p=0.034). Conversely, older age (maximum pressure r=0.482, 0.007; pressure increment r=0.375, 0.041) was associated with rectal pressures in females only, and more specifically in nulliparous females (maximum rectal pressure r=0.557, p=0.02). These result indicate differences in cough effort between groups of participants.

The maximum rectal pressure was correlated with maximum anal increment for the group as a whole (r=0.926, p<0.001), in men (r=0.960, p<0.001), and in women (r=0.867, p<0.001). When the correlation between rectal pressure and anal increment in women was split by parity, neither group showed a significant effect (nulliparous r=0.398, p=0.114; parous women (r=0.093, p=0.762).

Impact of cough effort on anal pressure

Cough effort was evaluated in terms of rectal pressure increment during sequential coughs of increasing intensity (small, moderate, and maximal cough effort) and in terms of peak flow rate.

During sequential coughs, rectal pressure increment increased significantly with cough effort. Mean rectal pressure increment during small, moderate and maximum coughing was 37 mmHg (95% CI 31 – 42 mmHg, 5^{th}-95^{th} percentile 9-79 mmHg), 65 mmHg (95% CI 56 – 72 mmHg, 5^{th} - 95^{th} percentile 29 – 99 mmHg), and 112 mmHg (95% CI 98 – 125 mmHg, 5^{th}- 95^{th} percentile 61 - 182) respectively.

During sequential coughs, anal pressure and pressure increment increased significantly (p>0.05). The anal EMG increase was similar between small and moderate cough effort (p> 0.05), but there was a significant difference against the maximum cough effort (p<0.0001 against small cough and p=0.0012 against moderate). These results suggest that greater cough effort resulted in higher rectal pressure increase and greater

pressure response in the anal canal. Coughing with maximal effort also resulted in a significantly greater EMG response, suggestive of increased muscle fibre recruitment.

Higher peak flow rate showed a significant, positive linear association with rectal pressure increment ($p<0.0001$, $R^2= 7.3\%$, 95% CI of the slope= 0.05 to 0.15). Using the cut-offs for small, moderate, and maximum cough effort identified previously, the majority of coughs produced during the peak flow measurement were classed as "small" (n=135) effort (45 were moderate and 65 maximal). Small coughs had a significantly lower mean PFE than moderate and maximum effort coughs; there was no significant difference in mean PFE during moderate and maximum effort coughs.

Furthermore, peak flow rate was not associated with anal pressure (p=0.436), anal pressure increment (p=0.663) or muscular recruitment measured by EMG (p=0.691). These results demonstrate that peak flow rate was significantly positively associated with rectal pressure increment during coughing, but was not associated with anal pressures or EMG response.

RAIR

Qualitative assessment identified RAIR to be present in all participants. When FACL using a 20 mmHg pressure contour was used to measure the extent of anal canal relaxation (n=41), on average, FACL decreased by 44% (95% CI 39 - 49%). Of the 10 individuals in whom FACL could not be measured during RAIR, 6 individuals had complete relaxation of the anal canal (pressure <20 mmHg throughout the length of the anal canal). In the remaining 4, although anal pressure decreased, anal canal pressure remained > 20 mmHg.

Balloon distension

There were no significant differences in sensory threshold volume (**Table 6.9**) between men and women for first sensation (p=0.650, 95% CI -16 to 25 ml), desire to defaecate (p=0.296, 95% CI -18 to 57 ml), or MTV (p=0.321, 95% CI -28 to 84 ml). In nulliparous women, the volume required to elicit a desire to defaecate was, on average, 45 ml higher (95% CI= 4 to 86 ml) than in parous women (p=0.032). The threshold volumes at first (p=0.491, 95% CI= -15 to 31 ml) and MTV (p=0.331, 95% CI -29 to 85 ml) were similar between groups (**Figure 6.15**).

	All	Males	Females	Nulliparous	Parous
n	51	14	37	19	18
First sensation					
Mean (sd)	53 (32)	56 (26)	51 (35)	55 (42)	47 (26)
95% CI	43 - 62	41 - 71	40 - 63	35 - 75	34 - 60
Median	41	45	38	38	39
IQR	29-65	39 - 74	28 - 63	28 - 63	29 - 66
5th	20	26	19	19	18
95th	119	111	135	178	105
Urge to defaecate					
Mean (sd)	124 (60)	139 (44)	119 (65)	141 (72)	96 (48)
95% CI	108 - 141	114 - 164	97 - 141	106 - 175	72 - 120
Median	124	137	104	138	79
IQR	75 - 153	114 - 160	68 - 146	77 - 164	66 - 120
5th	55	62	62	60	42
95th	235	222	249	352	233
Maximum tolerable volume					
Mean (sd)	215 (89)	235 (97)	208 (86)	221 (95)	193 (74)
95% CI	190 - 240	180 - 291	179 - 236	175 - 267	156 - 230
Median	190	222	183	194	174
IQR	147 - 267	158 -297	146 - 263	151 - 276	132 - 253
5th	86	123	85	85	84
95th	405	475	401	412	365

Table 6.9 Descriptive statistics for sensory thresholds to balloon distension at first, desire to defaecate and maximum tolerable volumes.

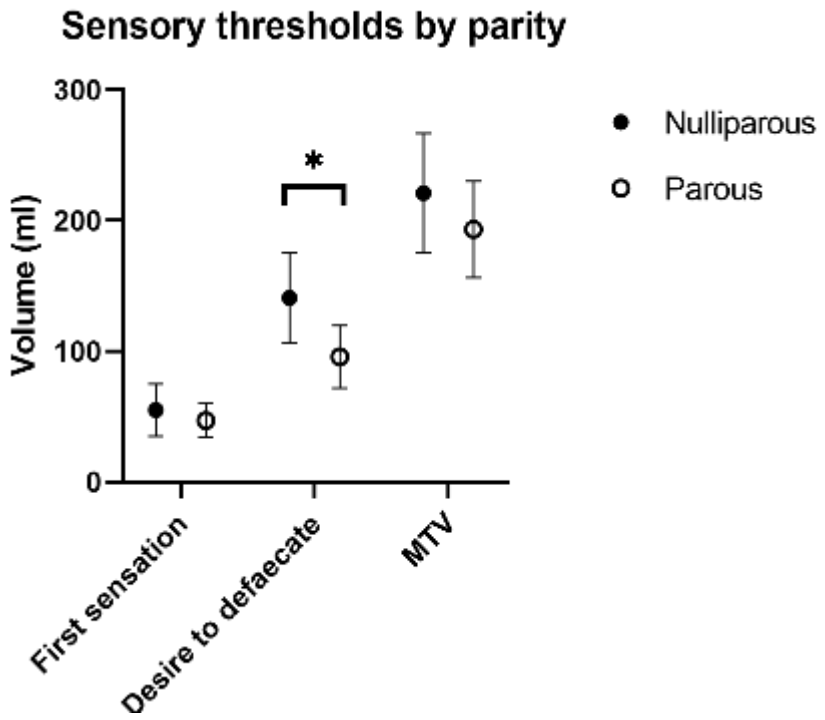


Figure 6.15 Boxplot of sensory thresholds to balloon distension in women.

Endoanal ultrasound

Endoanal ultrasound was performed in 48/51 (94.1%) participants; imaging was not undertaken in 3 subjects (2 parous women and 1 nulliparous woman) due to technical issues with the ultrasound machine. All 48 (100%) participants had an intact IAS on endoanal ultrasound. Similarly, an intact EAS was identified in 81.3% (39/48) of participants. Of subjects with EAS abnormalities, 5 were parous women (3 had occult sphincter defects <45 degrees following a history of vaginal delivery, 1 had a history of forceps/traumatic delivery, and 1 had only ever given birth by C-section) and one woman was nulliparous

Electromucosal sensitivity

Anal electromucosal sensitivity was measured in 47 participants (**Table 6.10**). In five participants, no sensation was perceived at 10 mv at 3 cm. There were no significant differences between sensory thresholds at 1 cm, 2 cm, and 3 cm ($F(2, 122)= 1.090$; $p=0.3391$). Sensory thresholds at each level showed a significant ($p<0.05$) positive correlation with other levels. On average, males (mean 5.0 uV, sd=2.0) had a significantly higher sensory threshold at 1 cm (mean difference -1.3, 95% CI= -2.4 to -0.1, $p=0.031$) than females (3.7uV, sd=1.7). This results was likely affected by extreme

values (10mv at 1cm) in one man. There were no significant differences at 2 cm (t(45)=-1.012, p=0.371) or 3 cm (t(40)=-1.235, p=0.224) between the two groups. No significant differences in sensory threshold at any level were observed between nulliparous and parous women (p>0.4) (**Figure 6.16**). There was no significant effect of age on sensory thresholds at any level for the group as a whole. In parous women, there was a significant effect of age (p=0.0212) on anal electromucosal sensitivity at 3 cm (R^2= 36.9%, 95% CI 0.02 to 0.2) (**Figure 6.17**). BMI was not significantly associated with sensitivity thresholds (p>0.05).

	n	Mean (sd)	95% CI	Median	IQR	5th	95th
1 cm	47	4.1 (1.8)	3.5 - 4.6	3.8	2.6 - 5.4	1.2	7.4
2 cm	47	3.9 (1.6)	3.5 - 4.4	3.8	2.6 - 5.2	1.2	6.5
3 cm	42	4.5 (2.3)	3.8 - 5.2	3.9	2.8 - 5.9	1.2	9.2

Table 6.10 Anal electromucosal sensitivity thresholds for the group as a whole

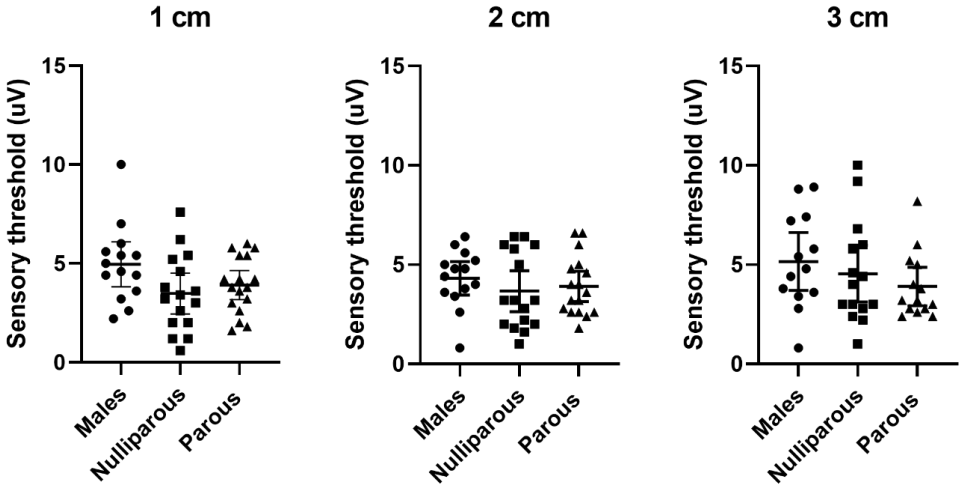


Figure 6.16 Anal electromucosal sensitivity at 1 cm, 2 cm, and 3 cm in males, nulliparous- and parous females. Lines indicate mean ± 95% CI.

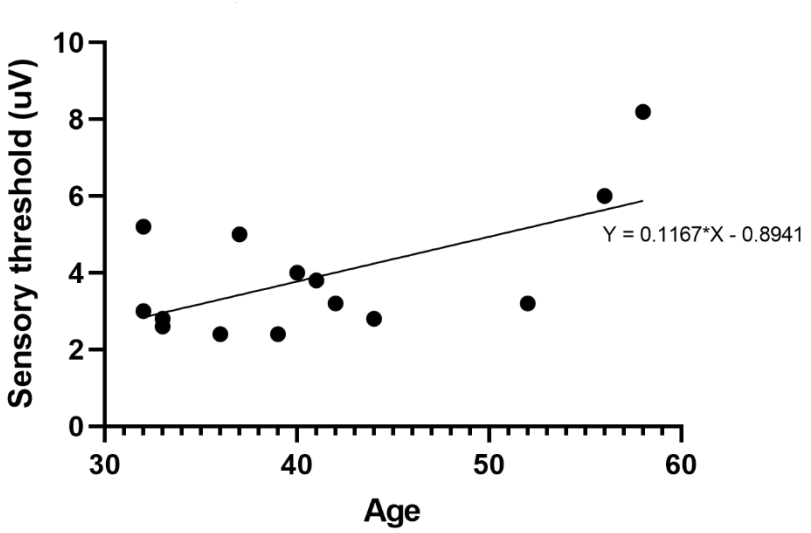


Figure 6.17 Effect of age on anal electromucosal sensitivity threshold at 3 cm in parous women

Balloon expulsion

Balloon expulsion time was evaluated in 48 participants (**Table 6.11**). There were no significant differences in BET between males and females (t(46)=0.3370, p=0.7377) or between nulliparous and parous women (t(32)=1.033, 0.3094). Of 48 participants, 85.4% (41/48) had a BET <60 seconds, 93.8% (45.48) had BET <120 seconds, 97.9% (47/48) had a BET <180 seconds, and 100% of participants had a BET <300 seconds. There was no significant effect of age (p=0.0914) or BMI (p=0.5274) on BET (Figure 6.18).

	All	Males	Females	Nulliparous	Parous
n	48	14	34	17	17
Mean (sd)	36 (45.0)	40 (39.4)	35 (47.6)	43 (61.5)	26 (27.2)
95% CI	23 - 49	17 - 62	18 - 51	11 - 75	12 - 40
Median	22	26	19	23	14
IQR	13 - 43	16 - 43	19 - 45	18 - 47	7 - 40
5th	4	8	4	2	4
95th	128	130	143	270	101

Table 6.11 Balloon expulsion time (BET) according to group.

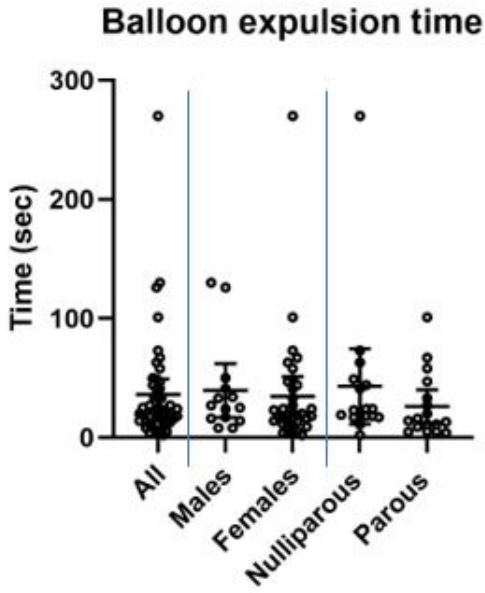


Figure 6.18 Balloon expulsion time. Blue lines indicate division into whole group, males vs females and nulliparous vs parous women. There were no significant differences between groups.

Discussion

The concepts and definition of normality with regards to colonic, rectal, and anal structure and function have been previously investigated in our unit [23,99,166,190,211,335,408-411]. The single centre, prospective, observational study in health, the study design, methods, and early results of which have been described, serve to continue that history. The aims of the study are to define normal ranges for emerging techniques (EndoFLIP, Rapid Barostat) and to expand existing normal datasets for manometry (HR-ARM), and tests of anal and rectal sensation (electromucosal sensitivity, standard barostat, balloon distension) and BET. Preliminary analysis has been performed to investigate the effects of gender, parity and age on findings although any conclusions drawn should be made with caution due to the small number of recruited individuals to date.

Unique to this study, in addition to incorating novel techniques, is the multimodal approach, which will provide the opportunity to assess rectal and anal continence mechanisms as an integrated, functional unit and provide understanding of how the results of individual tests interact in the continent individual. In addition to FI symptoms, we screened participants using stringent criteria and validated questionnaires for functional bowel disorders.

We planned to recruit 150 HV with equal numbers of males, and nulliparous and parous females. However, the recruitment effort fell far short of the target. Challenges included recruitment, researcher availability, and the COVID-19 pandemic, which halted the research in March 2020. Although studies were briefly able to resume, without any evaluation of anorectal response to coughing, further lockdowns impacted study continuation. In recruiting in the local community, social stigma associated with the anorectum and the large number of tests involved were some of the reasons why people who had initially expressed their interest in taking part declined. Other common reasons why interested subjects were exlcuded were included hysterectomy, thyroid medication, and mental health problems. In addition, many parous women declined to participate due to lack of childcare.

To date, 51 HV have taken part in the study. Most completed the full study and in general acceptability scores were high (9 or 10/10). Only one subject withdrew consent half-way through the study. In other cases of missing tests, these were due to technical problems or access to equipment on a particular study day. We received no complaints about the study from volunteers. As the aim was to recruit a heterogenous study sample that represented the diverse cultural community in East London, UK, numerous community groups, social platforms, mailing lists etc. were utilised to draw in volunteers. Unfortunately, we were not able to approach healthcare staff working at the Royal London Hospital directly, due to restrictions by ethical approval received. Hospital staff may have been more approachable or willing to participate than lay people.

Although summary statistics have been presents, limited conclusion can be drawn as we know from previous studies that variability in health is large. It is unlikely that these analyses will have reached power to assess true differences associated with gender, parity and age. All testing procedures followed either local or internationally recognised protocols and thus these results will be used to expand currently available datasets and hopefully gain meaningful results in the future. For this reason, the current discussion is not intended to compare these findings with others. Neverthless certain findings and novel concepts are discussed in brief below.

The effect of parity:

- No effect on resting pressure or amplitude of slow waves
- BMI was associated with functional anal canal length in males and nulliparous females but not parous women.
- No effect on squeeze increment
- No effect on endurance squeeze time, however in parous women there was a significant negative correlation between endurance squeeze time and endurance squeeze pressure/pressure increment. This suggests that women who achieved lower pressure to start were more likely to be able to maintain that pressure longer (this points towards a greater proportion of slow-twitch fibers and has been observed previously; may be related to the proportion of slow vs fast twitch fibres and preferential loss of fast-fibres over time. However, this result was also tending toward significance in nulliparous women.
- No effect on pushdown
- No effect of cough measures
- The volume required to elicit urge (balloon distension) was significantly lower in parous women than nulliparous women
- Anal mucosal sensitivity a 3 cm was significantly associated with age in parous women (although no significant differences in sensitivity were seen at different anal canal levels)

Squeeze:

There was no difference in maximum squeeze pressure between squeezes attempts however, mean squeeze increment was significantly lower during the 2nd and 3rd attempts compared to the first. This suggests an element of fatigue may be present. Alternatively subjects simply used less less effort (having first demonstrated to themselves that they are able to perform the manouevre).

Endurance squeeze was evaluated in three ways: the duration of time 50% of maximum pressure is maintained, maximum and maximum increment, and AUC. The best method for assessing endurance squeeze has not been established.

Push:

There was no significant difference maximum or mean rectal pressure during consecutive attempts at push down, suggesting that fewer repetitions (currently 3) may be appropriate.

Cough:

Rectal and anal cough pressures were greater in men than women, consistent with the higher resting and squeeze pressures seen in males. The anal-rectal incremental difference was not significant between men and women, suggesting that 'reserve function' available to respond to the intra-abdominal pressure rise is similar between the sexes. However men, on average, coughed with greater effort; increasing cough effort (on small, medium and large coughs) was associated with progressively higher rectal and anal pressures. Coughing with greater effort also yielded a higher anal pressure and EMG response.

We tested if a peak flow meter could be used to measure cough effort (with a view of standardising coughs by either asking patients to produce a certain flow during coughing or correcting results for effort). However, the majority of PFE metered coughs performed resulted in rectal and anal pressure responses equivalent to a "small" cough. From these assessments we concluded that although peak flow rate was significantly positively associated with rectal pressure increment during coughing, 'effort' was not associated with anal pressures or EMG response.

Balloon Expulsion

97.9% of subjects passed the balloon within 180 seconds. Many subjects reported the need to pass urine before attempting balloon expulsion.

These findings summarise the preliminary observations made in a cohort of 51 HV and will be used to expand normative datasets. When (if) the study can resume in the future, greater use of social platforms be help drive numbers and generally help to promote healthcare research. Based on acceptability scoring performed by participants to date, there are no issues with the study design/protocol which need addressing. In general, once through the door, partcipants were not bothered by the procedures.

Chapter 7a Validation of the Rapid Barostat Bag (RBB) pump and comparison with conventional electromechanical barostat

Introduction

Awareness of rectal filling is critical to normal bowel function; abnormal visceral sensitivity and/or rectal compliance are common in faecal incontinence and other evacuation disorders [9,23,92]. Indeed, abnormal sensory thresholds to balloon distension indicative of rectal sensory dysfunction were found in a tenth of women with FI and 1 in 5 men with FI (Chapter 3- systematic review). Demonstration of altered sensation can guide toward therapeutic measures aimed at normalising sensory thresholds and relieving bowel symptoms[416-419]. Similarly, rectal compliance is a mechanical property of the rectal wall which can be altered by pathologic processes which adversely affect faecal continence[207,420].

While balloon distention is cost-effective and easy to perform in a clinical setting, determination of rectal sensory dysfunction by balloon distension alone cannot determine the *cause* of abnormal thresholds [421]. Pathoaetiology leading to abnormal balloon distension may relate to rectal capacity and/or compliance (the ability of the rectum to stretch to accommodate stool), or afferent pathway function[99]. For example, hypersensitivity (heightened sensation) leading to incontinence may result from a small capacity or 'stiff' hypocompliant rectum that lacks 'reservoir' function, and hence the sphincter barrier may be overwhelmed on arrival of stool. Alternatively, sensitisation of extrinsic peripheral pathways and/ or central afferent mechanisms or abnormalities in perceptual and behavioural processes causing hypervigilance could lead to urgency and incontinence[92]. Conversely, in a hyposensate (blunted sensation) individual, reflex inhibition of the internal anal sphincter (IAS) may occur before the presence of stool in a large capacity rectum is perceived, leading to incontinence[199].

The electromechanical barostat is the gold standard method for assessing rectal sensorimotor function[422]. Studies performed with a computerised barostat enable distension at a specified and precise rate providing a more accurate measurement of

rectal function[9]. Unlike the use of a latex or rubber balloon which has its own inherent elasticity known to affect compliance, the standard barostat uses an infinitely compliant bag that is larger than the viscus it is distending (in this case the rectum)[2]. However, lengthy study protocols (~1h minimum), cumbersome equipment (**Figure 7a.1**), and poor standardisation with regard to the optimum inflation paradigm to use, means this method is rarely used outside of research[62] or is limited to those patients in whom abnormal rectal compliance or capacity are highly suspected[2,9,201].

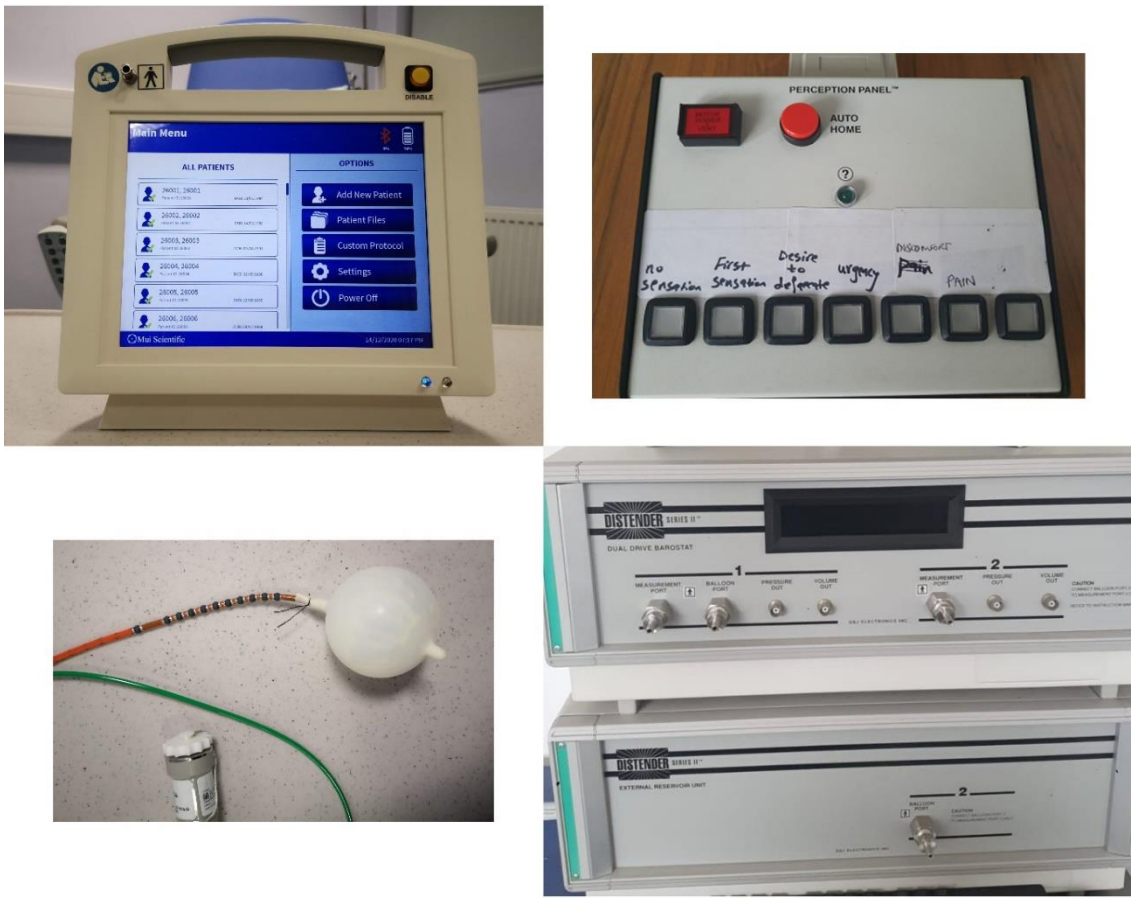


Figure 7a.1 Equipment for assessing rectal sensorimotor function: RBB pump (top left), non-latex, rubber balloon assembled onto HR-ARM catheter (bottom left), standard barostat machine and control panel (right).

In an attempt to to overcome limitations associated with the conventional barostat, including user-dependent variation in distension rate and pattern (Sun 1990 Gastroenterology), the hand-held Rapid Barostat Bag (RBB) technique has been developed[150]. This is a portable bedside medical device designed to inflate a barostat bag at a constant pre-defined rate[423] to reduce operator bias since the rate of inflation is known to influence rectal motor and sensory responses[199,232,235]. The device seeks to

enable accurate and consistent measurement of rectal capacity, compliance, and sensory thresholds by performing the two-step RBB Test[150,232]. Although a previous handheld RBB device showed good agreement with standard barostat measures[150], the RBB pump is yet to be validated against the standard barostat. Furthermore, the RBB protocol is yet to be tested in parous women, since the only existing study using the RBB excluded parous women due to the prevalence of occult sphincter lesions and anorectal dysfunction[150]. Finally, normative data using the technique are currently lacking.

Aims

The aim of this chapter is to compare measures of rectal sensorimotor function in health using the RBB pump (Mui Scientific) against the standard electromechanical barostat. Specific aims relating to each of the measurements are detailed below.

Part 1: Sensation

a) To summarise data on sensory thresholds using descriptive statistics for standard barostat and rapid barostat.
b) To compare mean sensory thresholds between the two methods (using t-tests for matched data).
c) To assess agreement between RBB sensation measurements against standard barostat sensation measurements using Bland and Altman[424] plots.

Part 2: Compliance

d) To calculate compliance by manual slope and delta change methods [201] for (i) standard barostat, and (ii) RBB
e) To assess agreement between RBB compliance measurements by each method against standard barostat measurements (gold standard) using Bland & Altman[424] plots
f) To assess RBB inter- and intra-rater variability for compliance measurement for the best method identified in steps a-b.

Part 3: Capacity

g) To summarise capacity using descriptive statistics for RBB
h) To compare mean capacity between the two methods

i) To To assess agreement between RBB capacity against standard barostat using Bland & Altman plots

Method

Healthy volunteers were recruited prospectively to undergo standard barostat and RBB assessment on the same day in randomised order (QMREC2017/33). The study method and details of each investigation have been described in Chapter 6 (Prospective study-methods). The steps for each study are summarised in **Figure 7a.2**.

Standard barostat Distender series II, G&J Electronics Inc., Toronto, Ont., Canada	**Rapid Barostat Bag pump** Mui Scientific, Canada
1. Connect 600ml rectal barostat bag to outlet. 2. Perform calibration 3. Intubate the rectum. 4. Unfold the bag in the rectum by inflating with 75ml of air. 5. Identify Basal operating pressure (BOP). 6. Rest 60 sec 7. Initial conditioning – inflate the bag to 20 mmHg in increments of 4 mmHg followed by complete deflation to 0 mmHg. 8. Rest 10 min 9. Ascending method of limits protocol – increase bag pressure in 4 mmHg intervals. Hold each volume constant for 60 seconds. During each interval, prompt the subject to report on sensation after 30 seconds using a keypad or visual aid. Continue filling until discomfort (MTV) is reported (the subject should report discomfort unprompted). 10. Deflate the bag and extubate	1. Connect 700ml barostat bag to outlet. 2. Deflate bag 3. Intubate the rectum 4. Begin capacity cycle. The pump will automatically deflate when 40 mmHg is reached. 5. Begin sensation cycle. Ask the subject to report when they first feel the bag, when they feel urge, and when they reach MTV (discomfort). The pump will automatically deflate when discomfort is reached or when bag pressure reaches 50 mmHg. 6. Extubate.

Figure 7a.2 Summary of standard barostat and rapid barostat protocols

One subject did not tolerate standard barostat and standard barostat data were lost due to a technical failure in 2 individuals. All participants underwent RBB, however 6 individuals surpassed the 50 mmHg safety limit during sensory testing resulting in

automatic termination of the test. As a result no RBB report was generated in these individuals, although raw data were available for manual processing.

Data processing

Standard barostat

The raw data file for each individual was downloaded to a memory stick and saved onto a PC. The .dat file was opened as an Excel file and saved as a .xlsx file. Average pressure (P1) and corrected volume (V1 corrected) were calculated for each distension, until maximum tolerable pressure/volume was reached. The threshold volume and pressure for initial sensation, urge, and maximum tolerable volume were noted. Capacity was taken as the corrected volume when pressure first reached 40 mmHg. If 40 mmHg was not attained, the corrected volume at maximum tolerated pressure was used.

A pressure-volume curve was plotted in GraphPad Prism (Version 9.0.1, GraphPad Software, LLC) and the steepest part of the curve identified visually (**Figure 7a.3 panel A**). The (x,y) co-ordinates at the start (V1, P1) and end (V2, P2) were noted in a separate data sheet in Excel and the change in volume was divided by the change in pressure (V2-V1/P2-P1); this was defined the 'delta change' method. Data for the curve were delimited between these points and a new pressure volume curve was plotted. The slope of the line of best fit was identified in Prism (defined as the 'manual slope' method).

Rapid barostat

For the RBB, raw data in Excel format were downloaded off the RBB pump using a memory stick. Continuous pressure and volume data from the 'capacity' cycle were copied into GraphPad Prism (Version 9.0.1, GraphPad Software, LLC) and a pressure-volume curve was generated. The same steps for 'delta change' and 'manual slope' methods were followed as for standard barostat (**Figure 7a.3 panel B**).

Using the RBB report (or information in the raw data file if no report was generated), the volume and pressure at first sensation, urge and MTV/discomfort were identified. Capacity was taken as the reported volume when pressure first reached 40 mmHg. If 40 mmHg was not attained, the maximum volume tolerated during the 'capacity' cycle was used instead.

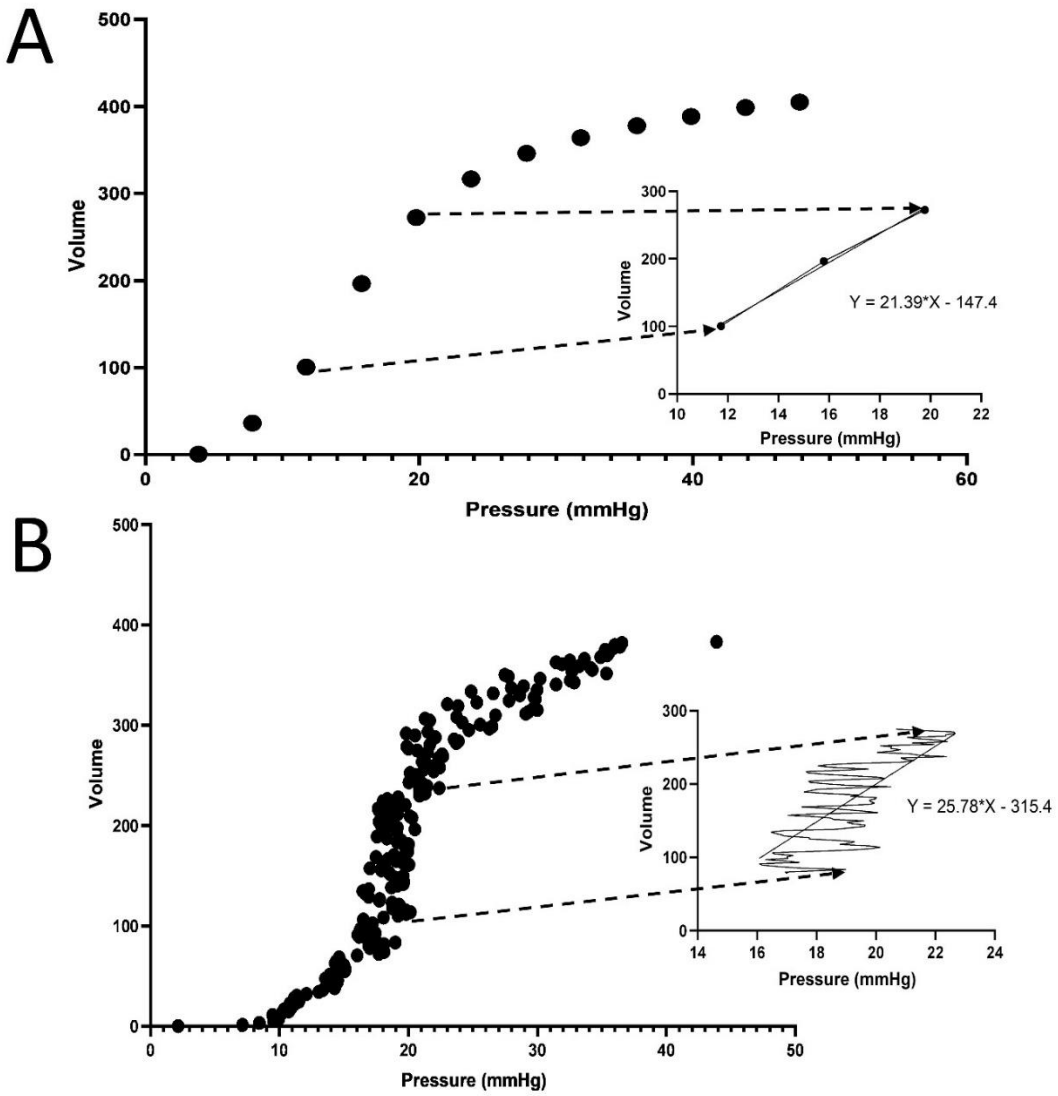


Figure 7a.3 Summary of data processing for (A) standard barostat and (B) rapid barostat in a single participant. The straight part of the sigmoid curve was identified. For the 'manual slope' method, data points were re-plotted including a line of best fit to identify the slope of the line. For the 'delta change' method, individual data points were selected at each extreme (of the straight line) and compliance calculated as (V2-V1)/(P2-P1).

Statistical analysis

Summary statistics were performed to describe sensory thresholds, compliance values and capacity by different methods. Differences in means between standard and rapid barostat were compared by t-test; multiple method comparisons for compliance were analysed using a repeated measures ANOVA. A p-value <0.05 was considered significant. Bland & Altman plots[424] were generated to assess agreement between standard and rapid barostat for sensory thresholds (volume and pressure), compliance,

and capacity (volume). Inter- and intra-observer variability in for compliance assessment was assesed in a similar way to above; the average compliance between two observers was used for the x-axis. Exact parametric confidence intervals for the Bland & Altman limits of agreement (LoA) were calculated using 2-sided tolerance factors[425]; a published spreadsheet[426] was used to assist calculating these limits in Excel (Microsoft 360).

Results

In total, 19 HV has standard barostat and RBB studies (Table 7a.1).

	Mean
n	19
Female	13 (68.4%)
Nulliparous	7 (53.8%)
Age (yrs, SD)	36.8 (12.9)
BMI (SD)	25.0 (5.8)
CCCS (median, range)	1 (0-5)
SMIS (median, range)	0 (0-4)
PACSYM (median, range)	2 (0-6)
Beighton (median, range)	0 (0-3)

Table 7a.1. Demographics

Part 1: Sensory thresholds

Summary statistics

Data on sensory thresholds for standard and rapid barostat are summarised in **Table 7a.2**. First sensation volume was significantly lower using the standard barostat (mean 74 ml vs 112 ml, $p<0.007$) (**Figure 7a.4 panel A**). No significant differences were observed between the two modalities for urge ($p=0.2548$) or MTV ($p=0.4115$). There were no significant differences in pressure at any sensory threshold (**Figure 7a.4 panel B**).

	Standard barostat		**RBB**	
n	15		15	
	Volume	Pressure	Volume	Pressure
First				
Mean (SD)	74 (51)	12.9 (2.5)	112 (59)	11.7 (3.3)
5th percentile	4.0	8.2	17.0	6.0
95th percentile	183.0	16.3	227.0	16.0
Urge				
Mean (SD)	202 (97)	21.9 (4.7)	221 (66)	18.8 (6.1)
5th percentile	69	15.7	117	10.0
95th percentile	366	31.7	368	31.0
Max tolerable volume				
Mean (SD)	335 (107)	45.3 (11.4)	345 (112)	44.2
5th percentile	113.0	27.7	160.0	22.0
95th percentile	500.0	60.2	531.0	50.9

Table 7a.2. Summary data for sensation

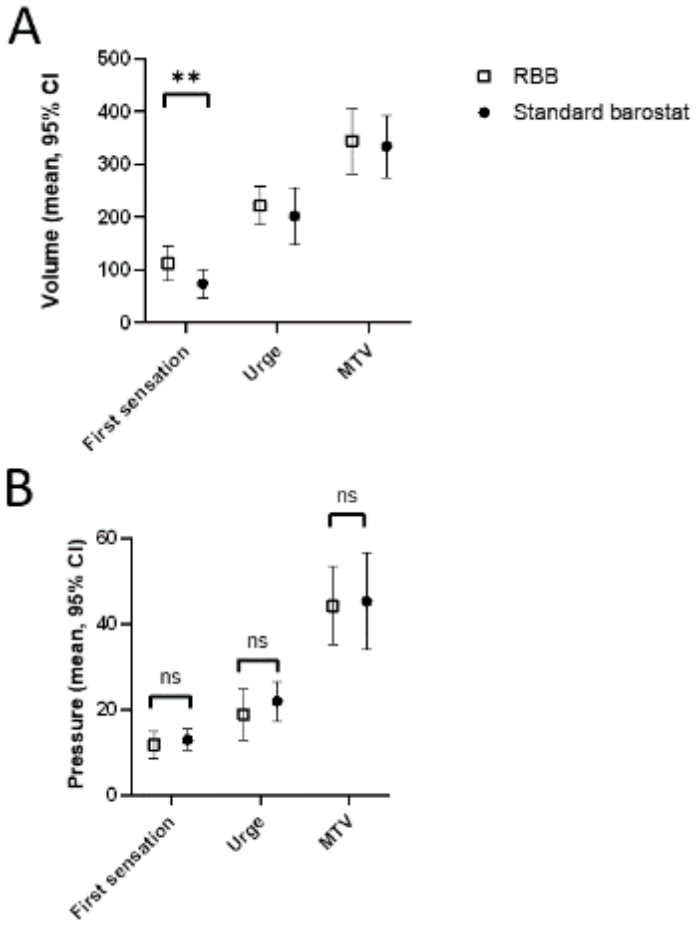


Figure 7a.4 Comparison of sensory threshold volumes (Panel A) and pressures (Panel B) using standard and rapic barostat.

Agreement

Sensory threshold volumes measured by RBB were generally higher than for standard barostat (**Figure 7a.5 panel A).** Assesment revealed clinically significant mean difference (38 ml) for first sensation, and clinically insignificant mean difference for urge (20 ml) and MTV (10 ml). For urge sensation a proportional disagreement was observed. At lower thresholds (up to ~200 ml), urge threshold with standard barostat was below that measured by RBB (resulting in a negative difference); at thresholds >200 ml, urge thresholds with standard barostat were higher than that measured by RBB.

Considering pressure at each of the sensory thresholds, the mean difference between the methods was small, between 1 and 3 mmHg. Bland & Altman plots showed no bias for first sensation and urge (**Figure 7a.5 panel B**). Meanwhile, pressure at MTV showed some proportional bias. **Table 7a.3** provides a summary of all Bland & Altman analyses

performed. Mean differences, 95% CI (limits of agreement, LoA), and the 95% confidence intervals (CI) for the LoA for all analyses are given. The wider the inner and outer limits of LoA, the more inaccurate either (or both) methods are.

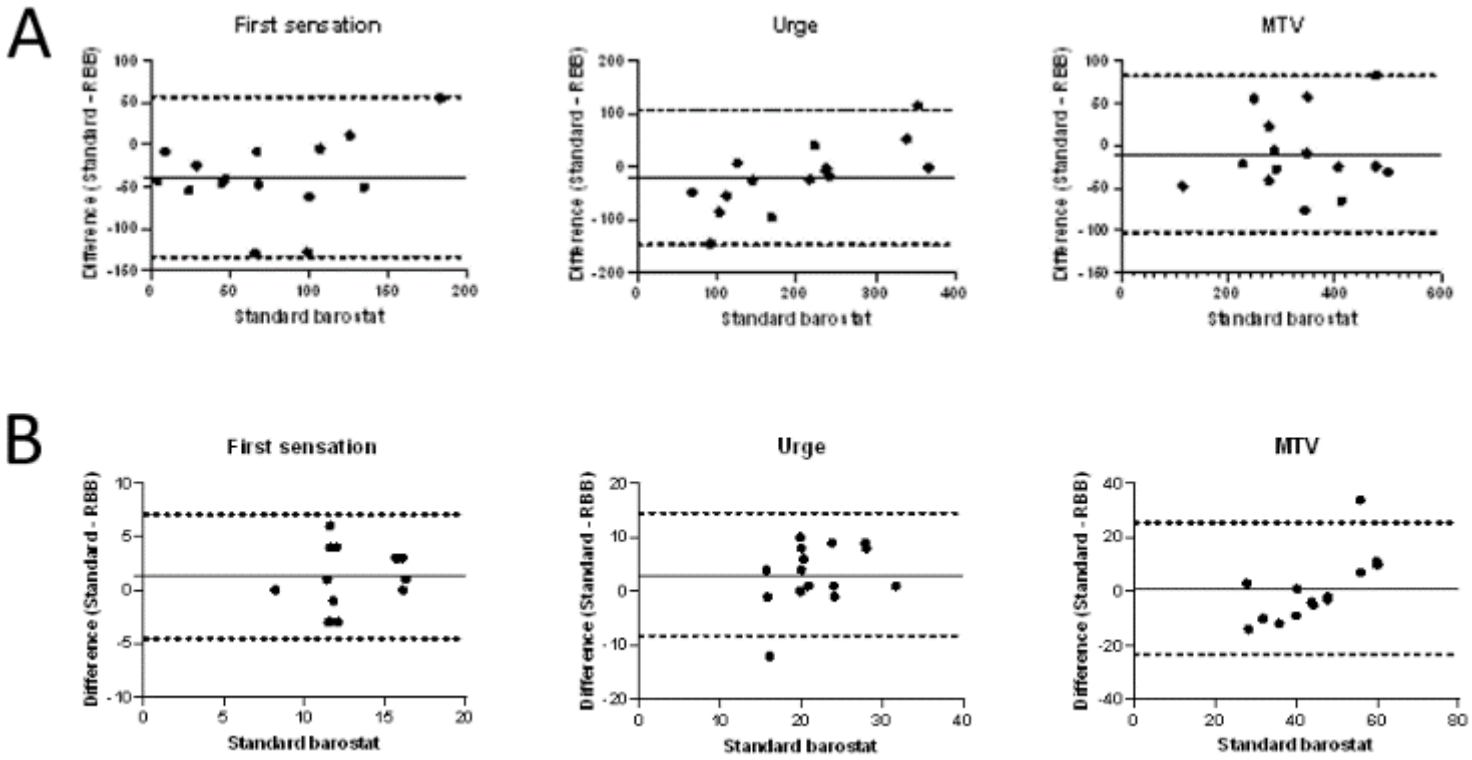


Figure 7a.5 Bland & Altman plots to compare sensory threshold volumes (panel A) and pressures (panel B) using standard and rapid barostat.

			Mean difference	95% CI	Limits of agreement		Outer Confidence Limits for LoA	Inner Confidence Limits for LoA
					Lower limit	Upper limit		
Standard VS RBB	Compliance	Manual slope	-2.4 (5.1)	-5.3 – 0.4	-12.7	7.8	-18.8 – 14.0	-9.9 – 5.1
		Delta change	-16.4 (20.3)	-27.7 – (-)5.2	-57.0	24.1	-81.4 – 48.5	-46.1 – 13.3
	Sensation	First sensation (volume)	-38.7 (47.6)	-65.1 – (-)12.4	-133.9	56.3	-191.5 – 113.8	-108.7 - 31.1
		Urge (volume)	-19.5 (63.5)	-54.6 – 15.7	-146.5	107.5	-223.1 – 184.1	-112.72 – 73.7
		MTV (volume)	-10.2 (46.2	-35.8 – 15.4	-102.5	82.3	-158.3 – 138.1	-77.9 – 57.7
		First sensation (pressure)	1.26 (2.9)	-0.3 – 2.9	-4.5	7.1	-8.0 – 10.6	-3.0 – 5.5
		Urge (pressure)	3.0 (6.0)	-0.1 – 6.3	-8.3	-23.3	-16.2 – 22.2	-5.8 – 11.8
		MTV (pressure)	1.0 (12.0)	-5.6 – 7.9	14.5	25.8	-37.5 – 39.5	-16.6 – 18.6
	Capacity	Capacity	-10.9 (40.5)	-33.4 – 11.5	-91.8	70.2	-140.7 – 119.1	-70.3 – 48.7
RBB VS RBB	Compliance	Intra-observer	0.1 (2.7)	-1.2 – 1.4	-5.3	5.5	-8.0 – 8.1	-4.0 – 4.1
		Inter-observer	2.6 (4.6)	0.4 – 4.8	-6.5	11.7	-11.0 – 16.2	-4.3 – 9.5

Table 7a.3 Summary table of Bland & Altman assessments

Part 2: Compliance

Summary statistics

Compliance using the manual slope and delta change methods for standard barostat and RBB data are summarised in Table 7a.4. Mean compliance using the manual slope method was 16.8 (95% CI: 14.65-18.95) for standard barostat and 17.9 (95% CI: 14.67-21.11) for RBB. Using the delta change method the equivalent ranges were 16.9 (95% CI: 14.74-19.06) for standard barostat and 29.1 (95% CI: 19.08-39.12) for RBB. Mean compliance was significantly higher when using the delta change method to analyse RBB results compared with all other methods (**Figure 7a.6**).

	Standard barostat		Rapid barostat	
	Manual slope	Delta change	Manual Slope	Delta change
n	15	15	19	19
Minimum	8.6	8.6	6.7	7.2
Maximum	24.50	23.90	31.92	73.8
5% Percentile	8.6	8.6	6.7	7.2
95% Percentile	24.5	23.9	31.9	73.8
Mean	16.8	16.9	17.9	29.1
Std. Deviation	3.9	3.9	6.7	20.8
Lower 95% CI of mean	14.6	14.7	14.7	19.1
Upper 95% CI of mean	19.0	19.1	21.1	39.1

Table 7a.4. Summary statistics for compliance using two different manual processing techniques.

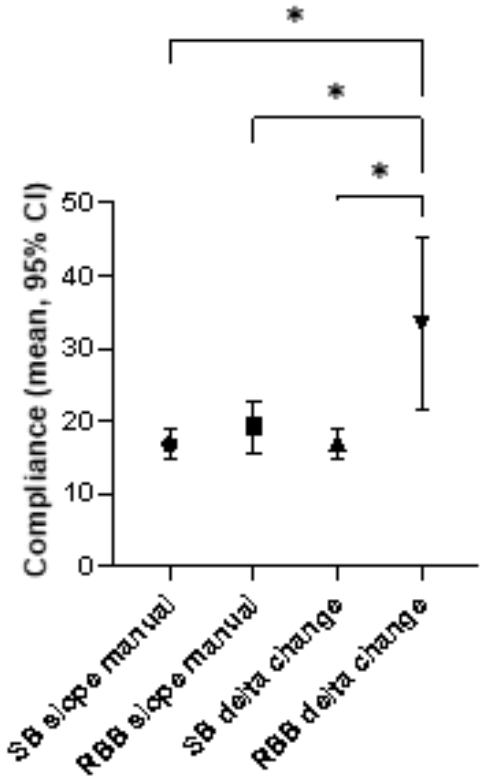


Figure 7a.6 Mean compliance compared using ANOVA using manual slope and delta change methods using standard and rapid barostat data.

Agreement

No proportional bias between standard and rapid barostat were noted by either method of compliance calculation (**Figure 7a.7**). However, compliance measurement by the delta change method resulted in clinically significant mean difference (mean -16.4 ml/mmHg; 95% CI: -57.0 to 24.1). These results indicate that the manual slope method is a superior method for calculating compliance from RBB data.

RBB intra- and inter-observer variability of manual slope compliance

An asssessment of intra- and inter-observer variability for the calculation of RBB manual slope was performed in all individuals (**Figure 7a.8**). Intra-observer differences were relatively small and not clinically significant (mean 0.06; 95% CI: -5.33 to 5.45). There were no systematic or proportional bias. Likewise, inter-observer assement (mean 2.6; 95% CI: -6.5 to 11.7) did not reveal any systematic or proportional bias, but limits of agreement around the mean were slightly wider.

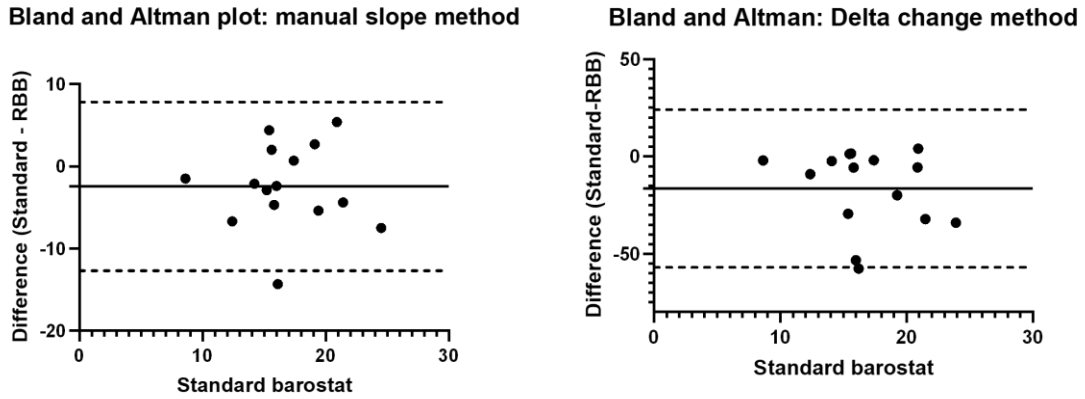


Figure 7a.7 Compliance agreement (Bland and Altman plot) between standard barostat vs RBB.

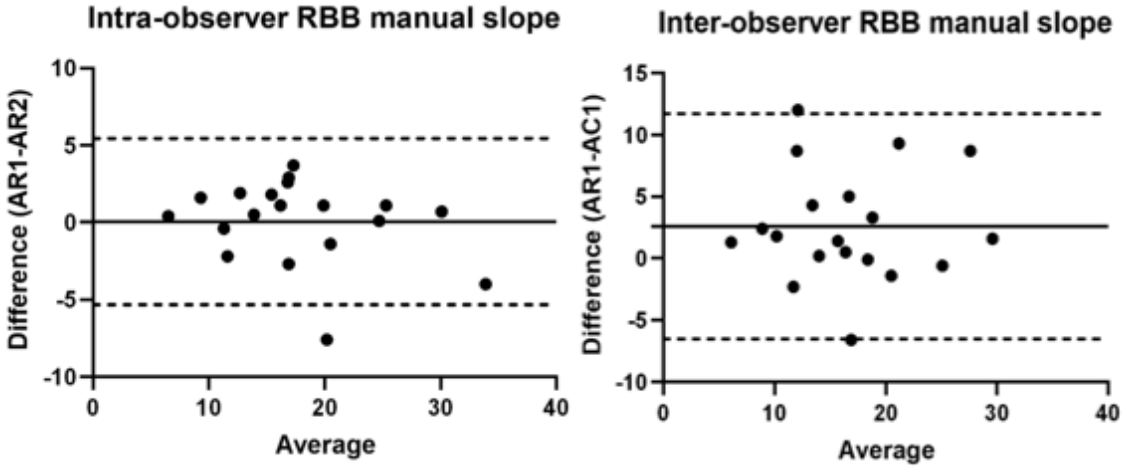


Figure 7a.8 Intra- and inter-assessor agreement in RBB compliance using the manual slope method of calculating compliance.

Part 3: Capacity

Summary statistics

Data on capacity are summarised in Table 7a.5. There was no significant difference in capacity measured by the two modalities (**Figure 7a.9**).

	Standard barostat	**RBB**
n	15	15
	Volume	Volume
Mean (SD)	301.0 (84.85)	311.7
5th percentile	113.0	145.0
95th percentile	455.0	487.0

Table 7a.5 Summary data for capacity

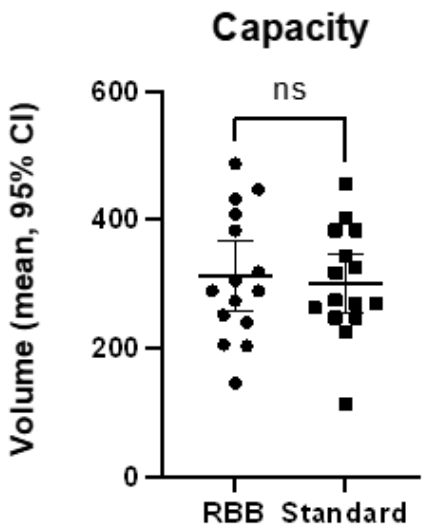


Figure 7a.9 Mean capacity

Agreement

The measurement of rectal capacity using standard and rapid barostat had a mean difference of -10.8 ml (95% CI -33.4 – 11.5). There was no evidence of proportional bias (**Figure 7a.10**).

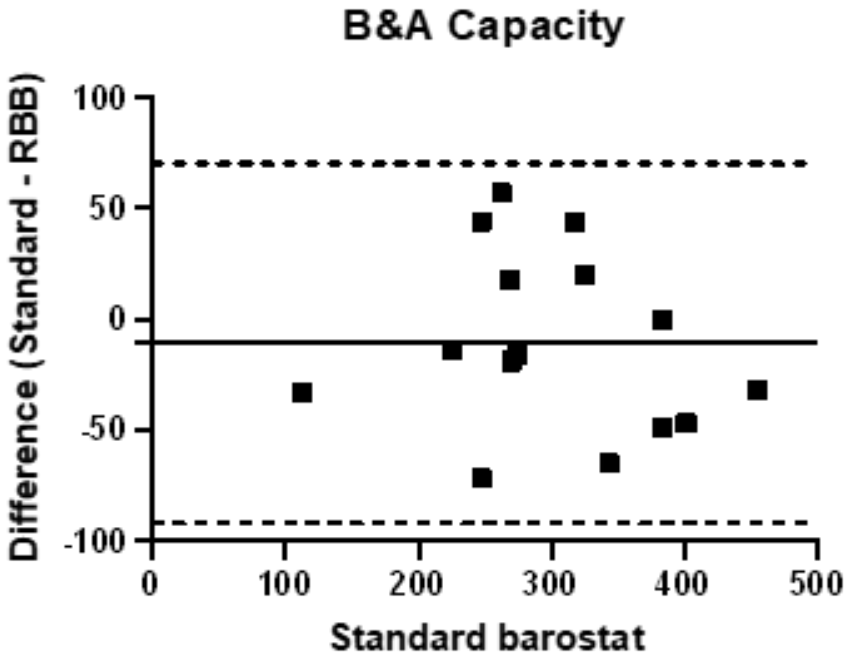


Figure 7a.10 Bland and Altman plot to assess agreement in capacity by standard and rapid barostat.

Discussion

Determining visceral sensitivity is a crucial part of assessing physiology in FI[234]. Although practical and cost-effective, simple balloon distension using a handheld syringe does not comply with the principles of objective physiological measurements[427] nor does it accurately measure physical alterations in the rectal wall[207]. Balloon shape, distension rate, distension protocol, and the inherent characteristics of the balloon material are known to impact sensory values and compliance[420]. Predefined and standardised distensions of the bowel wall using a barostat device are the current standard for the assessment of sensorimotor function in experimental trials in health and disease[234], however the sparcity of equipment and time-consuming protocol mean that routine clinical assessment is often impractical.

The RBB pump (Mui Scientific) has been developed to overcome some of these issues, but it is currently unknown how it compares with standard barostat. We aimed to measure the agreement of sensory threshold measurements, rectal compliance, and capacity in healthy volunteers between the RBB pump and standard barostat.

Sensory threshold measurements

The threshold volume for first sensation was significantly higher using RBB than with standard barostat. This difference may be related to the way sensation was assessed. Using standard barostat, the operator provides a prompt at each distension asking the patient to report a sensation. Conversely with RBB, the operator provides no prompt and waits for the individual to report sensory responses. The provision of a prompt may make the participant 'more aware' of filling triggering a response. Previous standard barostat studies have also shown greater variability and less reliability of standard barostat at low rectal volumes[232]; this could be because the participants feels 'expected' to report a sensation in response to a prompt rather than waiting for a 'true' sensation. There were no significant differences for measurements of urge or MTV. Like previous studies, threshold sensations for first sensation, urge and MTV increased progressively from 74ml to 202 ml to 335 ml for standard barostat and 112 ml to 221 ml to 344 ml for RBB. However, absolute volumes were lower than previously reported (with the exception of first sensation using RBB)[150]. However, both studies demonstrated wide confidence intervals for the mean and broad LoA on Bland & Altman analysis.

In general, rapid barostat resulted in higher sensory thresholds than standard barostat with the biggest difference observed at first sensation (-38 ml), consistent with studies that show that the greatest variability in rectal sensation occurs at low volumes[232]. The threshold volume at urge was the only sensory threshold to exhibit proportional bias, consistent with previous studies, which also showed proportional bias with early sensory thresholds (initial perception and urge)[150]. The mean difference in MTV (10 ml) observed in this study was considered clinically insignificant and MTV did not show proportional bias, however, as with all measures, the limits of agreement were generally wide. Previously Sauter et al[150] reported <50 ml differences in MTV in most subjects. Likewise, we showed differences <50 ml in 73% of subjects (individual data

points not shown). Sensory pressure thresholds were comparable with previous literature as summarised by Cremonini et al[237].

Rectal compliance

In surgical literature, compliance refers to the distensibility of the rectum and is commonly defined as the change in rectal volume per unit change in rectal pressure[207]. Our results showed compliance measured by standard barostat (mean 16.8 ml/mmHg) and the RBB pump (17.9 ml/mmHg) yielded comparable results on average with good agreement between the two methods though compliance measured by rapid barostat was, on average, 2.4 ml/mmHg (95% CI around the mean: -5.3 – 0.4 ml/mmHg) higher than standard barostat.

Compliance in our study was higher than previously reported using a similar method for calculating compliance, but a different barostat machine (Synetics Medical Ltd. Visceral Stimulator). Gladman et al[201] reported a mean compliance of 12.9 ml/mmHg in health with an upper limit of normal (mean +- 2SD) of 17.9 ml/mmHg. However, intra-individual and inter-individual variations in compliance are known to be large with normal values varying up to 300 percent between centres owing to differences in study protocol, equipment used and method of calculating compliance[207,237,428]. Indeed, compliance values with standard barostat showed wide variability in the current study (range: 15.9 ml/mmHg), with even greater range observed using RBB (25.2 ml/mmHg). Due to the limited number of subjects included, no comment has been made on the 'normal range'. Furthermore, the wide LoA and the error of these limits (Table 7a.3) suggest that the RBB method provides an innaccurate measure of compliance compared with standard barostat (assuming the standard barostat is the gold standard). Nevertheless, minimal intra- and inter-rater variability were found using the manual slope method for calculating compliance using the RBB pump suggesting that it is reliable and clinically feasible. This is in contrast to the delta change method, which did not provide reliable measures of compliance for the RBB pump (**Appendix 9**). The main reason leading to greater differences was the large number of data points available; although two observers may have visually chosen the same part of the curve, it was unlikely that the data points used for making the delta change calculation would be the same. Small variations in pressure/volume as a result of breathing or movement

could have a large impact on individual data points. This was in contrast to the standard barostat method where volume data were averaged for a given pressure/distension resulting in fewer data points on which the pressure/volume curve was based.

The only other study to assess differences in compliance measurement between standard and rapid barostat (using a hand-held pump), reported similar differences to our study[150]. Specifically, Sauter and colleagues reported greater distensibility using standard barostat (9.4 +- 2.8 mmHg) compared to rapid barostat (13.4 -+ 2.7 mmHg). However, because they estimated compliance based on the rectal distension pressure at 50% of rectal capacity, the values themselves are not comparable with our study.

Rectal capacity

Maximum tolerable volume (MTV) can be used as a surrogate for rectal capacity in healthy volunteers[90]. Others have suggested that rectal capacity defined by intra-bag volume at 40 mmHg is a more important determinant of rectal sensation and continence during rectal filling[429]. Furthermore, in clinical practice, an objective measurement of rectal capacity that is independent of subjective reports of sensation is desirable because rectal sensitivity may be increased in some conditions[150]. Such measurements of rectal capacity are highly reproducible, but vary greatly between individuals (250–600 mL). In the current study, mean rectal capacity at 40 mmHg was 301 ml (95% CI: 245- 348ml) for standard barostat and 312 ml (95% CI 256-367 ml) for rapid barostat with no significant difference between the two methods and good agreement between the tests.

In another study[150], hand-held rapid barostat also showed comparable capacity with standard barostat (388 +- 108ml vs 410 +- 93ml), although mean values were higher than observed in this study. The authors noted <50ml difference in volume measurements in the majority of 26 healthy volunteers. In our study, mean difference in capacity between standard and rapid barostat (pump) was only 11 ml, though LoA were between -92ml and 70 ml, with differences up to 72 ml recorded. However, not all patients (5/15) reached a pressure of 40 mmHg using standard barostat; in these cases, pressure at MTV was used instead yielding greater differences (data not shown). Because rectal capacity is associated with rectal compliance, such that a large rectum appears to be more compliant than a small rectum independent of wall properties[232],

accurate measurement of both capacity and compliance are desired for distinguishing rectal pathophysiology related to biomechanical and afferent nerve dysfunction.

Limitations

This study was performed as part of a larger study, the general limitations of which will be discussed in Chapter 9. Specific limitations to this analysis include, firstly, that the number of subjects was small. Previous studies have suggested that an n-number of 21-25 would be sufficient to detect meaningful differences in rectal sensation[150,237]. We calculated differences in compliance, capacity, and sensory thresholds in 15 subjects (men, nulliparous and parous women). Intra- and inter-observer variability was available for 19 individuals, but in four subjects, standard barostat data were not available.

Second, we assessed the RBB pump against standard barostat, which is the recognised gold standard method for assessing rectal sensorimotor function[9]. In Bland & Altman analysis, the x-axis can represent either one method (the "reference method" or "gold standard") or the average of two methods[430]. We plotted the difference between methods against standard barostat values, which we recognised as the "gold standard" in accordance with findings by Krouwer[431] despite the original Lancet paper by Bland and Altman[424] recommending that the average of the two methods be used. This approach is common when one is looking to replace and existing method with an alternative.

Some of the pressures measured by standard barostat were very small. For example, the 5th percentile for first sensation was 4 ml. It is highly unlikely that such a small amount of air in at 600 ml bag would elicit a response especially in a healthy volunteer. However we checked extreme data points with raw data files in case of any transcription errors; as far as we know there were no known technical difficulties during any of the tests, except for those already excluded as per the description given in Methods. We assessed standard barostat using the ascending method of limits protocol because it is the best accepted method for assessing rectal sensorimotor function[234]; the RBB protocol follows a similar filling pattern but which is based on consistently increasing volume rather than pressure. Participants were prompted to report sensation 30 seconds into each distension during standard barostat assessment. Only

maximum tolerable volume (discomfort or pain) was recorded unprompted. Indeed, MTV showed the highest agreement with rapid barostat. Of note is that all subjects reported sensations "in order" on standard barostat possibly due to the expectation that sensations should increase sequentially during the test. The use of repeated or alternative protocols (such as random phasic distensions) may have yielded different results although research shows that the ascending method of limits technique is as useful as more complex paradigms designed to avoid subject bias (Whitehead 2000 Gastroenterology).

With rapid barostat, it is possible to repeat sensory testing to obtain an average threshold. Although repeated distensions evoke increasingly reproducible measurements of sensation and compliance[235], we based our results on a single sensation cycle. One of the main advantages of the RBB pump is to provide the means for speedy (see chapter 7b for evaluation of protocol duration), routine assessment of rectal sensorimotor function. To more appropriately simulate the clinical use of the RBB pump, RBB testing was limited to two cycles: (1) conditioning cycle for compliance and capacity assessment and (2) sensory threshold assessement. Limiting the protocol may have increased variability especially at low volumes/first sensation[232]. This is of clinical importance especially in FI women, who are more likely to show hypersensitivity (Chapter 3-systematic review). It is possible that repeating distensions may be desired until consistency is achieved if optimal measures of rectal sensations at low volumes are needed[150].

The standard barostat bag used in the study was slightly smaller (600 ml) than the bag used for RBB (700 ml max). This, together with differences in distension rate, protocol (pressure vs volume driven increase), and technology used to monitor intrabag pressure will have contributed somewhat to the differences between methods. While introducing the RBB pump into routine physiology assessment is desirable, it is not our purpose to recommend the replacement of standard barostat entirely. In fact the current assessment confirms that while the RBB pump may be both reliable and practical as a stand-alone machine, the results obtained are not interchangeable with the standard barostat. For this reason, we recommend that separate normal ranges are established using the RBB pump and the influence of sex, age, parity, and body mass

index [150,237,432] are investigated since these have been previously shown to influence anorectal physiology parameters.

Many different analysis techiques have been used to calculate compliance[7,201,207,237,433]. Rectal compliance computed from pressure-volume curves during rectal distention is commonly used to describe rectal wall properties[428] and these approximate closely to a power exponential function where the estimated pressure at half-maximum value (Pr1/2) summarises the entire curve [434]. The use of Pr1/2 has been decribed in several other studies [150,237,240,435], but requires specialist statistical software (NLIN procedure in the SAS software package [436] to calculate K- and β- constants). Instead, we used the method used by Gladman et al [201] (described in Diamant *et al* 1999 [158]) to calculate compliance based on the slope of the line of best fit fitted to the straight part of the pressure volume curve (calculated as the change in volume divided by the change in pressure). This is in part because this is the method used to automatically generate compliance for the report generated by the RBB pump. Unfortunately due to the small number of subjects (n=13) in whom we had all three measures of compliance (standard barostat, RBB pump raw data, and RBB pump report) comparison with the auto-generated report was not feasible. From a clinical perspective, it will be useful to know how auto-generated compliance and manually processed RBB compliance (using the manual slope method) compare.

Conclusions

Barostat assessment complements the clinical investigation of FI. The RBB pump has been developed to facilitate routine measurement of compliance, capacity, and rectal sensation. We have demonstrated that despite clinically insignificant differences between mean measurements by standard barostat and RBB pump, wide LoA suggest that the two methods do not provide entirely comparable measures of compliance (i.e. elasticity). However, the term “compliance” is sometimes used more simply to imply a functional measurement made on the rectum [207]. In this sense, the RBB showed excellent intra- and inter-rater variability supporting its routine clinical use to measure rectal function so long as the manual slope method was used. Measurement of capacity and sensory thresholds using the RBB pump showed similar results when compared with standard barostat, but wide CI of the LoA indicate that, once again, the methods

(and any existing normal ranges based on standard barostat) are not interchangeable. While the RBB may be clinically useful as an alternative for balloon distension, sensory thresholds are clearly markedly different to previously reported thresholds. This supports the need for the development for a large normative RBB dataset. Further studies are needed to define normal cut-offs for clinical purposes and further assessment of how sex, age, parity, and body mass index may increase inter-individual variability.

Chapter 7b Rapid barostat assessment of rectal compliance, capacity and sensory thresholds in healthy volunteers

Introduction

The previous chapter confirmed the validity of rectal compliance and sensory measurements obtained using the recently developed Rapid Barostat Bag (RBB) pump (Mui Scientific, Ontario, Canada) compared with the gold-standard, electromechanical barostat. While the RBB pump was found to provide an appropriate means by which to assess sensory thresholds and the pressure-volume relationship in the rectum, RBB results were not interchangeable with values obtained by standard barostat. This is not surprising given the methodological differences. Hence, normative ranges for the RBB pump are required.

Altered rectal compliance and/or sensation may contribute to disturbed rectal function. For example, increased compliance may result in constipation due to reduced rectal tone and contractility while decreased compliance could lead to frequent defaecation and faecal incontinence (FI)[437,438,439]. In both instances, visceral perception can also be affected. Hyposensitivity of the rectum has been proposed as a relevant factor in the aetiology of chronic constipation [201,330,421,440,441] and FI [238,442] while, rectal hypersensitivity is especially relevant in irritable bowel syndrome [443-445] and in the development of urgency. In patients with abnormal sensory thresholds, barostat is required to differentiate between those with normal, a hyper- or hypo compliant rectum and sensory abnormalities.

Normal values for the RBB protocol have been previously reported in 26 healthy volunteers [150]. However these ranges were actually derived from a different, handheld device rather than using the automated RBB pump. Furthermore, given the wide inter-individual variability observed, a larger normative dataset for the RBB pump is desired to define normal cut-offs for clinical diagnostic purposes. Furthermore, there is a general paucity of information on the role of sex, age, parity, and body mass index on sensory thresholds and compliance using the RBB pump.

The aims of this chapter are therefore: (a) to generate normal ranges for rectal compliance, capacity and sensory thresholds in healthy volunteers (HV) using the RBB

pump; and (b) to explore the influence of gender, parity, age, and BMI on values. While raw data can be easily downloaded from the RBB pump and manually processed to calculate the slope of the pressure-volume curve, such a curve is also displayed on the RBB pump display and can be used to generate a compliance value using automated features for more practical, clinical purposes. It is not known how well the automated analysis (which calculates compliance using the formula $\Delta V/\Delta P$, where the changes in volume (V) and pressure (P) are determined at two points identified by the pump operator) performs compared with manual data processing. In the previous chapter, we demonstrated that compliance based on the slope of the line of best fit ('manual slope method') resulted in smaller intra-observer differences than compliance using the 'delta change' method based on raw data analysis. However, because the necessity for offline, manual processing of raw data could cause significant delays in reporting as well as increasing the risk of data mis-reporting (e.g mistakes made during data transfer) which could deter users from adopting the RBB into clinical practice, the accuracy of the automated analysis requires establishing against the manual slope method. Therefore, this chapter will also aim to: (c) compare rectal compliance measured by manual and automated processes. Finally, the RBB protocol is based around two independent filling cycles (the conditioning and index cycles), but in theory, it is possible to perform all analyses on data gathered during a single (index) inflation. Therefore this chapter will (d) compare compliance and capacity during the two cycles (to determine repeatability of these measures).

Methods

Participants

RBB was performed as part of a multimodal assessment of anorectal sensorimotor function in healthy individuals as described in Chapter 8. Of the 51 individuals recruited for the study, RBB was performed in 50 due to a failure of the pump to inflate the rectal balloon in one individual. This was subsequently recognised to have resulted from user error rather than any technical failure of the pump itself. The RBB pump has a 'disable' button in the top right hand corner of the machine (**Figure 7b.1**). When raised, the pneumatic components of the pump are disabled and air in the bag is released (for example in case of an emergency). Prior to the start of the test in one subject, it was

not noticed that the button, which is usually maintained in a depressed state, had been inadvertently pressure disabling the machine. The RBB pump user manual was revised in April 2021 to include information regarding the disable button (Mui Scientific, Ontario Canada, 2021).

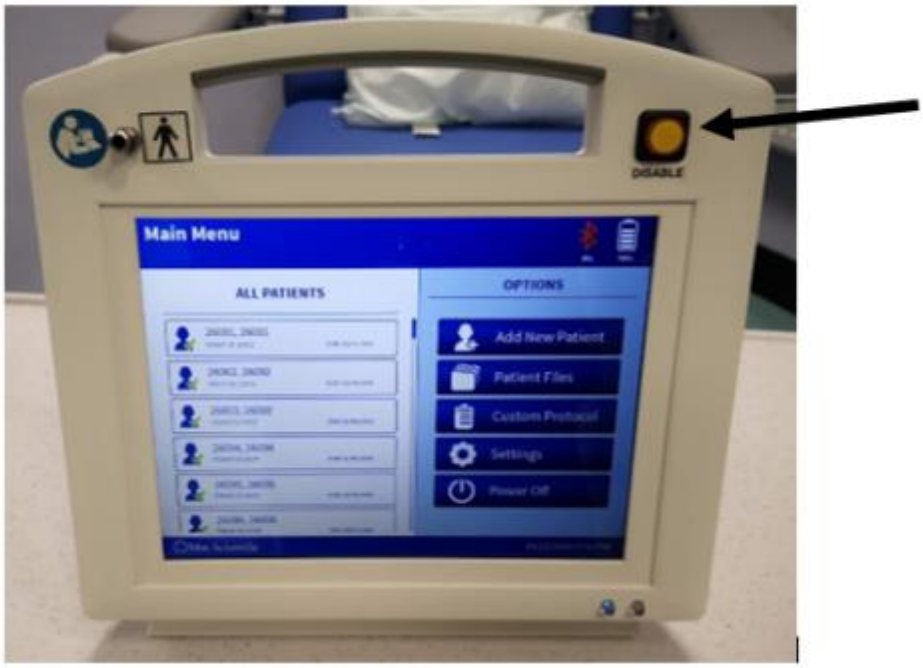


Figure 7b.1 RBB pump showing the DISABLE button in the top right-hand corner. The machine's pneumatic components are only operational if the button is depressed (as per photo).

Data collection and generation of the RBB report

Following rectal intubation with the bag, participants underwent two cycles of inflation. During the first 'conditioning cycle', the bag was inflated (with air) at a rate of 120 ml/min up to a pressure of 40 mmHg or up to the volume/pressure at which the participant reported discomfort and the bag deflated. A second 'index' inflation (also at 120 ml/min) was performed during which the participants were asked to report first sensation, urge, and maximum tolerable volume (MTV). When MTV was reached, the bag automatically deflated and was subsequently removed by the operator.

After saving the test, the RBB pump user interface was used to generate a report. This included measures of rectal capacity (ml) at 40 mmHg (or the pressure at the maximum

achieved volume), balloon pressure at 50% capacity, and compliance (derived from a pressure-volume curve) based on the 'conditioning cycle'. The pressure-volume curve generated by the pump was first reviewed in 'editing' mode and the location of the black triangles on the graph manually adjusted using the touch-screen interface to define the start and end points on the steep part of the pressure-volume curve (**Figure 7b.2**). Compliance was calculated by the RBB software using the formula ΔV/ΔP based on the selected data. For evaluation of rectal sensation, the RBB report expressed the volume at each sensory threshold in absolute terms and as a percentage of capacity at 40 mmHg (obtained during the conditioning cycle). An example of an RBB report is shown in **Appendix 10**. The report for each individual along with the raw data files was downloaded on to a USB stick and transferred to a personal computer. However, in some instances, a 50 mmHg intrabag pressure safety limit was reached before the participant reported MTV. In such cases, the RBB pump considers the test as a failure and no report is generated for that individual (unless the sensation cycle is repeated and terminated prior to reaching 50 mmHg or alternatively the safety limit is adjusted). The raw data file can still be downloaded as normal for post-hoc (manual) analysis.

Post-hoc analysis

For all individuals, compliance was assessed using the 'manual slope' method as described in Chapter 8c (Rapid vs Standard barostat). To summarise, a line of best fit was added to the linear part of the pressure-volume curve generated from raw data captured during the 'conditioning cycle'. Compliance was determined by the slope of the line of best fit. The intrabag pressure at each sensory threshold was noted in a separate spreadsheet. The total time taken for each cycle was determined from the raw data.

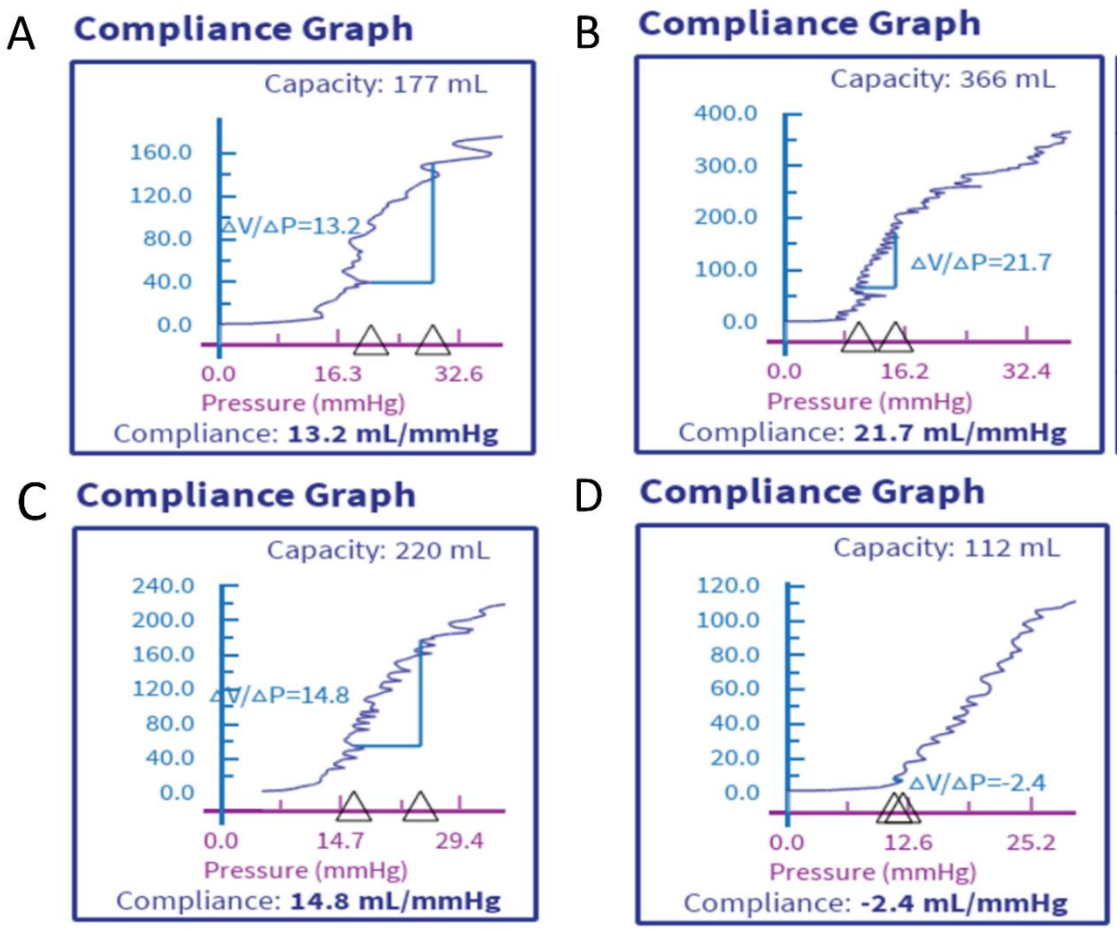


Figure 7b.2 Representative plots of intra-bag pressure and bag volume during the 'conditioning cycle' in 4 healthy volunteers. Plots A-C demonstrate how the black triangles have been adjusted to reflect the beginning and end of the steep part of the pressure-volume curve. Plot D shows the inaccurate automated placement of the triangles and resulting incorrect compliance calculation (-2.4ml/mmHg) prior to review and manual adjustment by the operator

Statistical analysis

Descriptive statistics for compliance, capacity at 40 mmHg, and sensory threshold measurements were performed for total and grouped data. Means with 95% confidence intervals (CI) or medians with interquartile range (IQR) are reported for parametric and non-parametric variables, respectively. The 5th and 95th percentiles were calculated to express the normal range. Pearson correlation coeffcient was performed to investigate the relationship between parameters and Bland and Altaman plots with limits of agreement generated to observe method-related bias. Paired-samples T-tests, ANOVA, and non-parametric equivalents (Wilcoxon matched pairs

signed rank test, Friedman test, and Kruskal-Wallis test) were used to assess for any differences between groups. P-values <0.05 were consired significant.

Results

Participants

Of the 50 participants, 72% were female; overall median age was 36 (range 19-67) (Table 7b.1). On average, the time taken to complete the conditioning cycle was 240 sec (95% CI 220-259 sec), the index cycle took 233 sec (95% CI 210-257 sec) and the total test took 487 sec (95% CI 445-529 sec) (not including set-up, intubation/extubation).

	Total	**Males**	**Females**		
			All	**Nulliparous**	**Parous**
N	50	14	36	20	16
Age (median, IQR)	36 (19-67)	35 (24-56)	37 (31-44)	32 (30-40)	41 (36-50)
BMI (median, IQR)	22.6 (21.6-25.8)	23.8 (21.9-26.3)	22.4 (20.7-25.4)	22.0 (20.4-23.7)	23.1 (22.2-27.3)

Table 7b.1 Descriptive statistics

Missing data

During sensory testing, 8 subjects (1 nulliparous and 3 parous women and 4 males) failed to reach MTV before the intrabag pressure reached 50 mmHg. In addition, data from one individual was accidentally deleted from the system before the compliance graph generated by the machine had been edited. Therefore, no "automated" compliance value was available for a total of 9 individuals.

Compliance

Compliance data using the two analytical methods (manual slope and automated) are summarised in **Table 7b.2**. The two methods were significantly correlated (Pearson correlation: 0.792, p<0.001) and there were no differences in mean compliance measured by manual (15.2, SD 6.3) and automated (16.2, SD=6.8) analyses (t(40)=-1.49. p=0.143). A Bland and Altman plot showed no evidence of bias and a clinically insignificant mean difference (-0.99 ml/mmHg, p=0.143) between the two methods. BMI was significantly correlated with manual (Pearson correlation coefficient 0.328,

p=0.021) and automated (0.433, p=0.0005) compliance (**Figure 7b.3**). However, this result was no longer significant when a single outlier was removed (manual= 0.118, p=0.424 and automated 0.168, p=0.307). Age did not significantly correlate with manual (0.281, p=0.128) or automated (-0.009, p=0.957) compliance. Age and BMI were not related to each other (0.251, p=0.081).

	Manual slope	**Automated analysis**
N	50	41
Mean (SD)	16.0 (6.8)	16.2 (6.8)
95% CI	14.0-17.9	14.1-18.3
Min	4.9	6.7
Max	35.0	36.1
5th percentile	6.0	6.9
95th percentile	31.6	29.3

Table 7b.2 Summary statistics for compliance.

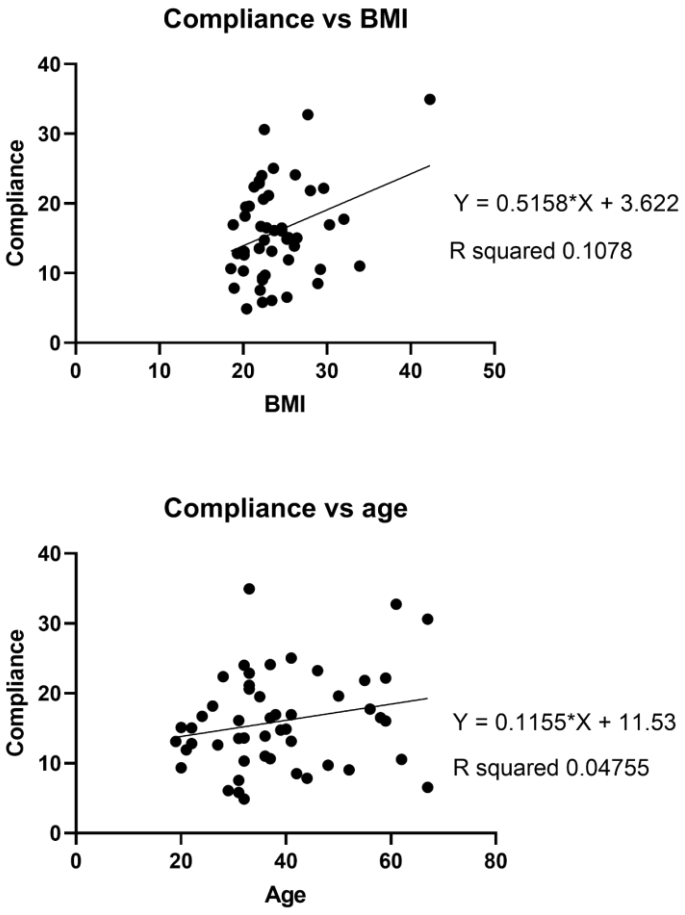


Figure 7b.3 Compliance (manual) vs BMI and age. Results of automated analysis were similar but are not shown.

There were no significant differences in compliance between nulliparous women, parous women, and males using either manual (one way ANOVA (F(2,47)= 0.094, p=0.911) or automated (one way ANOVA (F(2,38)= 0.283, p=0.755)) methods (**Figure 7b.4**).

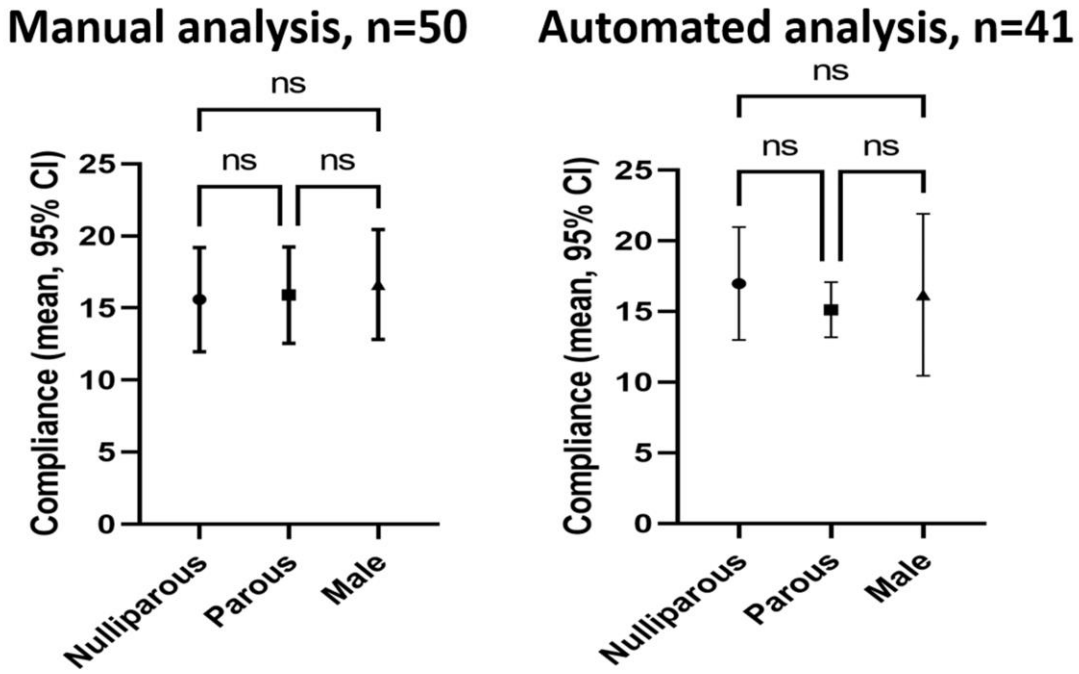


Figure 7b.4 Compliance in nulliparous and parous women and males using manual and automated analyses.

Capacity

Of 50 subjects, 32 reported discomfort before intrabag pressure reached 40 mmHg (median pressure 38.5 mmHg, IQR: 32.7-39.0). In these individuals, capacity was defined as the maximum attained volume. Mean capacity (intrabag volume at 40 mmHg or maximum volume) was 291 ml (95% CI 267-315 ml) (**Table 7b.3).** There were no significant differences between nulliparous (mean 308 ml, 95% CI: 267-350) and parous (mean 265 ml 95% CI 223-307) women and men (mean 296 ml, 95% CI 245-347, p>0.05). Capacity was significantly correlated with compliance (Pearson correlation: 0.709, p<0.001 for manual analysis and 0.666, p<0.001 for automated analysis; **Figure 7b.5**).

	Total	Nulliparous	Parous	Males
N	50	20	16	14
Mean (SD)	291 (86)	308 (88)	265 (79)	296 (89)
95% CI	267-315	267-350	223-307	245-347
Min	112	152	162	112
Max	487	487	432	419
5th percentile	149	155	162	112
95th percentile	439	485	398*	396*

Table 7b.3 Capacity (intrabag bag volume at 40 mmHg); *90th percentile

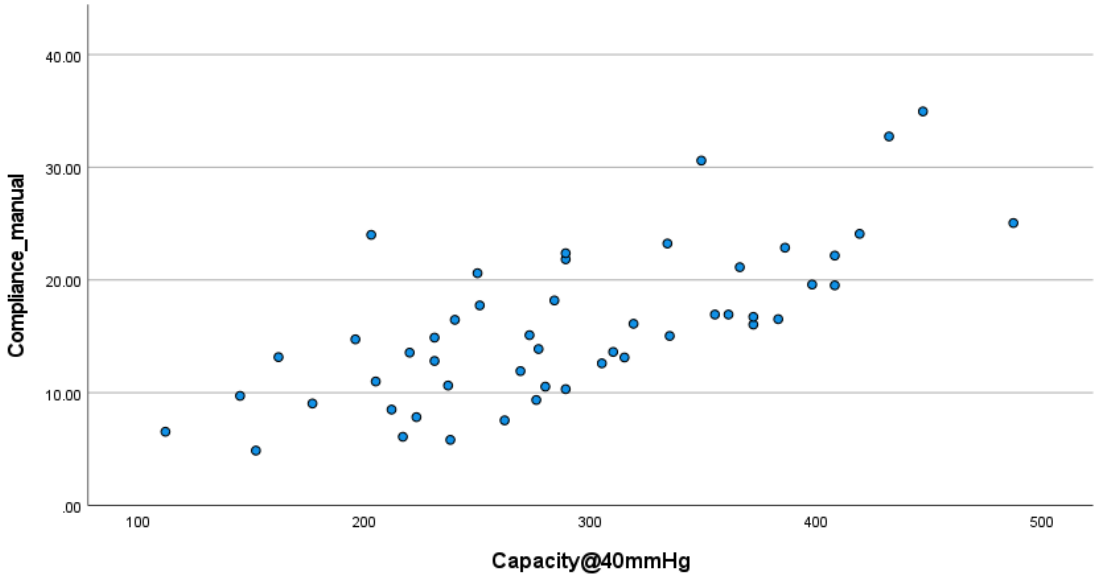


Figure 7b.5 Scatter plot of compliance vs rectal capacity measured at 40 mmHg.

Conditioning vs index cycle

Mean compliance during the index round (19.1 ml/mmHg, 95% CI 17.0-21.3) was significantly greater than mean compliance (16.0 ml/mmHg) during the conditioning round (t(49)= 3.739, p=0.0005).

During the index cycle, 23 individuals did not reach the 40 mmHg threshold for capacity (median pressure 24.7 mmHg, IQR: 21.9-30.5) vs 32 during the conditioning cycle (X^2(1, n=50) =3.76, p=0.0525). However, in 19 individuals, the outcome changed (i.e they reached the 40 mmHg threshold only during one cycle, but not the other). Considering the volume achieved either at 40 mmHg threshold or maximum attained volume, there

was no significant difference in capacity between the conditioning (mean 291 ml, 95% CI 267-315) and index (288 ml, 95% CI 263-314) cycles of filling ($t(49)=0.489$, $p=0.627$).

Rectal sensation

Rectal sensory thresholds for first, urge, and MTV are expressed in terms of volume (ml), pressure (mmHg), and a percentage of capacity (at 40 mmHg) and are shown in **Table 7b.4**. In total, 8 participants (4 men, 3 parous women and 1 nulliparous woman) failed to reach MTV. There were no significant differences in mean MTV expressed as volume (mean [sd]: 282 [94] ml vs. 298 [98] ml) or pressure (mean [sd]: 35 [11.5] mmHg vs. 36.9 [11.8]) in n=42 and n=50. Median bag volume increased significantly with each sensory threshold (Friedman test, $p<0.001$). No significant differences were observed between groups (nulliparous vs parous vs males) for any of the measurement parameters.

	Volume (ml)	Pressure (mmHg)	Percent Capacity at 40 mmHg (%)
First sensation			
N	50	50	50
Mean (SD)	104 (65)	10.7 (4.8)	36.5 (20.3)
95% CI	85-122	9-12	31-42
Median	90	11	
IQR	53-150	7-14	
Min	13	4	4
Max	276	24	87
Urge			
N	50	50	50
Mean (SD)	193 (76)	17.6 (7.7)	67
95% CI	172-215	15-20	62-73
Median	179	16	
IQR	125-242	12-21	
Min	79	6	32
Max	369	44	114
MTV			
N	42	44	42
Mean (SD)	282 (94)	35.2 (11.5)	100 (15)
95% CI	253-311	32-39	95-105
Median	259	38	
IQR	212-334	24-46	
Min	126	12	65
Max	531	50	142

Table 7b.4 Descriptive statistics for sensory thresholds according to volume, pressure, and percentage capacity in healthy volunteers.

Sensory thresholds	Volume (ml)			Pressure (mmHg)			Percent of Capacity (%)		
	First sensation	Urge	MTV	First sensation	Urge	MTV	First sensation	Urge	MTV
5th	21	93	155	4	9	17	8	32	70
95th	262	362	498	21	36	50	76	101	119

Table 7b.5 Normal reference ranges in health

Sensory threshold volumes correlated with compliance (irrespective of the method of analysis used) for urge and MTV, but not first sensation for the group as a whole, in nulliparous females and in males (**Figure 7b.6)**. None of the sensory threshold volumes correlated significantly with compliance in parous women. Sensory threshold pressures

correlated significantly with compliance at all thresholds for the group as a whole and in nulliparous women. No significant correlation was observed at MTV in parous women. In men, only the pressure at first sensation was significantly correlated with compliance in males.

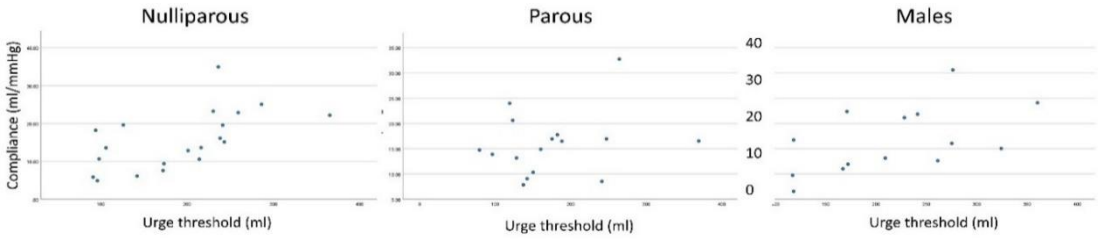


Figure 7b.6 Scatter plots showing the relationship between urge volume and compliance

Discussion

The aims of this chapter were to describe the results of RBB testing in HV and to generate normal ranges for clinical purposes. Compliance, capacity and sensory thresholds to distension were evaluated in 50 HV, representing the largest healthy cohort ever studied using this method. The influence of gender and parity on measurements was explored and the association between age and BMI with rectal compliance evaluated. Compliance values 'read' directly from the RBB pump/report were compared against analysis of raw data using the best 'manual' method as established in previous chapters (Chapter 7 RBB vs standard) to validate the use of the automated compliance graph. Finally, the repeatability of compliance and capacity measurements during consecutive filling cycles was evaluated. Based on our experience of using the RBB pump in healthy volunteers, the short duration of the RBB protocol (mean test duration was 8 minutes) makes the RBB widely practical within routine clinical assessment; a fair estimate for completing the investigation, from start to finish, is around 15 minutes.

To summarise, the main findings of this study were as follows:

- there were no significant differences in compliance, capacity or sensory thresholds based on participant demographics, except BMI;

- the significant association between rectal compliance and BMI should be interpreted with caution due to the small number of obese individuals included in the study;
- manual and automated assessment of compliance yielded similar results;
- compliance was significantly higher during the second (index) cycle of filling;
- rectal capacity during two consecutive cycles was not signficantly different. However, a considerable number of subjects did not reach the 40 mmHg threshold either during the first or second cycle. Furthermore, 38% of subjects did not consistently 'meet' or 'not meet' the set 40 mmHg pressure threshold during consecutive rounds of filling.
- parous women were the only group in whom sensory threshold volumes for urge and MTV did not correlate with compliance.
- sensory threshold pressures correlated significantly with compliance for the group as a whole and in nulliparous women. Only MTV correlated with pressure in parous women, and first sensation in men.

Results in health and the influence of gender, age, and parity

Compliance, capacity and sensory thresholds measured by RBB were not affected by gender or parity and therefore normal ranges were presented for the group as a whole. These findings are consitent with results in healthy volunteers and patients with lower GI disorders studied by balloon distension methods [420]. The effects of demographics on rapid barostat have not been explored in previous studies [150,446]. Sensory thresholds did not differ between groups of males, nulliparous and parous women, however there may be some differences in the interaction between sensation and compliance between groups. For example, nulliparous women and men with a less distensible (stiff) rectal wall also tended to have lower sensory thresholds. No such association was observed in parous women.

As with most physiological measurements, there was wide variability in recorded results. As this is the first study to be performed using the RBB pump in health to our knowledge, the ability to compare our findings with previously published data is limited. Sauter *et al* [150] who have published only other rapid barostat study in health, used a handheld device and a different method of assessing compliance [232]. Mean rectal

capacity at 40 mmHg was higher than observed in this study (mean, 5th-95th percentile: 419 ml, 344-433 ml vs. 291 ml, 149-439 ml). This is likely due to the large proportion of participants reporting discomfort before the 40 mmHg threhold was reached in our study. Of note is that the upper limit of normal (95th percentile) is remarkably consistent between the two studies. On the other hand, mean sensory threshold volumes for urge (246 ml vs. 193 ml) and MTV, or "discomfort" in the Sauter *et al* study, (399 vs. 282 ml) were higher and threshold for first sensation (71 ml vs. 104 ml) lower than reported in this study. Overall the range of normal values reported by Sauter et al (2014) at each threshold were much less variable. The faster and consistent rate of filling (2 ml/sec vs. 1 ml/sec) in our study may have contributed to these differences.

A recent study in patients, some of whom have FI has been published recently [446], but no HV data are presented. In the past, compliance in HV studied at our unit by standard barostat was ~14 ml/mmHg [23,201]. Although the methods are not directly comparable as shown in the previous chapter, mean compliance using the RBB (16 ml/mmHg, 95% CI 14-18 ml/mmHg) was similar overall.

Validation of automated compliance measurement

There was good agreement between the manual and automated processes for calculating compliance. Based on these results, manual analysis of raw data to determine compliance is only necessary for those individuals who do not reach MTV during sensory testing (and therefore do not have a report). Clinically this is good news, as it saves time. The automated analysis may be improved by adding a line of best fit in between the triangles and calculating the compliance based on the line of best fit, rather than individual points along the curve. This is due to the superiority of the 'manual slope' compared to the 'delta change' method as shown in the previous chapter.

Repeatability of measures

The RBB protocol [150] is based on two filling cycles: compliance and capacity are always evaluated during the first cycle of filling, while sensory thresholds are evaluated during the second. However, using the raw data available for each cycle, we analysed compliance manual in each cycle observing significantly lower compliance during the first 'conditioning' cycle compared with the second 'index' cycle. Standard barostat

methods advocate the used of an initial cycle of inflation (confusingly, this is also referred to as the conditioning cycle) during which no measurements are performed. This serves as a training period, to allow the subject to familiarise with the sensations and afferent signals which occur as a result of distending the rectum [447] and has been shown to reduce the variability in compliance and rectal sensory thresholds measured thereafter [235].

Meanwhile, mean capacity did not differ signficantly between groups. However a significant proportion of participants did not reach the 40 mmHg threshold either during the conditioning or index cycles. This puts into question the validity/use of standardising compliance/sensory thresholds to a certain pressure and poses the question whether sensory thresholds reported as a percentage of capacity measured during a different cycle makes sense (see below).

Limitations

The number of participants included in this study represents the largest cohort of HV to have undergone assessment by rapid barostat. While we reported no significant differences between groups, the number of subjects per group remains small. The number of included participants was further reduced for analysis of MTV, as 8/50 participants failed to reach MTV by 50 mmHg, the pressure safety limit imposed (default settings were used) and therefore no report was generated for these individuals. Simply excluding these results from the analysis introduces a selection bias which, depending on the rectal compliance in the excluded individuals, could either under or over estimate normal ranges. When results were re-analysed including sensory thresholds for entire cohort using the maximum achieved volume/pressure recorded in the raw data file, there was no significant difference in mean MTV or pressure recorded (n=42 vs n=50).

The rapid barostat measures both compliance and capacity enabling the reporting of sensory thresholds with reference to these properties. However, when sensory thresholds are reported in terms of inflation volume, as is the norm with balloon distension, measurements are as subject to the same (false) assumptions as other volumetric methods, i.e. that rectal size does not affect compliance and that the rectum

is a mechanically passive structure [207,234]. For this reason, we present data on sensory thresholds expressed as a percentage of rectal capacity (i.e. rectal volume at 40 mmHg) as established during the conditioning cycle. However, in contrast to Fox *et al* [232], 64% or participants expressed discomfort, thereby terminating the test, before reaching the 40 mmHg threshold. Therefore, in these individuals, capacity reflects "discomfort" (a sensation itself) rather than a pre-determined threshold estimating rectal size (ref). This distinction is important (clinically) especially when first sensation, urge, or MTV is reported as greater than >100% of capacity. It is important to note that the values for capacity evaluated in this study includes both. Normal ranges for sensory thresholds may be different if they were based on findings in only those 36% of subjects who achieved the 40 mmHg threshold during the first round of filling.

One of the proposed advantages of expressing results as a percentage of capacity is to normalise sensory thresholds to allow comparison between individuals [232]. For this purpose to be fulfilled, however, a consistently achieveable threshold for capacity needs to be established. However, capacity, or the volume at the maximum imposed pressure, may be of clinical value in itself; Bharucha et al [90] identified reduced values in at least a subset of women with idiopathic FI. In the same study, reduced rectal capacity on balloon distension was associated with urgency and with rectal hypersensitivity. It remains to be determined if similar outcomes can be observed results using with rapid barostat in patients.

During RBB, the operator may choose to repeat sensory testing up to three times (reporting only the average of selected cycles). In only performing the minimum number of cycles (one to evaluate compliance/capacity and one to assess sensation), we missed out on the opportunity to evaluate consistency of sensory thresholds. In addition, while we did perform additional analyses on raw data which showed significantly higher mean compliance during the second round, reprodicibility was not formally evaluted (e.g. using Bland and Altman plots, Kappa coefficient, or correlation) as part of this study. We suggest that further validation work is done to: a) ascertain the reproducibility of sensory thresholds, compliance, and capacity during multiple consecutive filling rounds; b) determine if the addition of an initial 'training' cycle of filling reduces the number of subjects who report discomfort before 40 mmHg and c)

evaluate the effect of this on compliance and capacity (including reproducibility). Depending on the outcome of these studies, consideration may need to be given on the appropriateness of the 40 mmHg threshold for normalising data.

From an investigators point of view, the RBB pump is easy to use and the investigation simple to incorporate into practice (in terms of time taken, space required, and consumables needed). However, based on personal experience of inserting hundreds of manometry catheters in the past, intubation and extubation of the RBB catheter was by far the most unpleasant due to the size and roughness of the bag material, particularly in those with longer anal canals. To ensure that the distal edge of the bag was fully beyond the internal anal verge, a finger had to be inserted alongside the catheter following intubation. This was undesirable from a patient perspective (refer to discussion in Chapter 6). Due to the size of the bag, a fair amount of tugging was also required when removing the bag. In addition, feedback from patients was that the sensory experience was intense (anecdotal). Surprisingly these anecdotal observations did not seem to impact patient acceptability score, since the average score for barostat was 9.2/10 on a visual analog scale (VAS). However, variability in scores was the greatest of all tests performed as reported in Chapter 6.

Finally, hypersensitivity is the clinical finding where at least MTV and one other sensory parameter is below the lower limit of normal seen in control subjects, while hyposensitivity is the finding of 2 or 3 sensory parameters (first sensation, desire to defecate volume, and MTV) above the upper limit of normal observed in control subjects, on balloon distension [146]. Major findings by the current consensus do not extend to alternative means of rectal sensory testing, including barostat methods. Although this study presents the findings of rectal sensory testing, with proposed cut-offs for normal function, these represent only the first step required toward recognition of the RBB pump as a clinically useful tool for determining any disorder of rectal sensation [146]. In the first instance, studies are needed to determine: a) the sensory thresholds observed in disease groups (e.g. FI, constipation) as evaluated by the RBB pump and compared with healthy individuals; b) the number of abnormal sensory thresholds required to differentiate between health and disease; and c) to determine

whether particular sensory thresholds are associated with specific symptoms (e.g. urgency, urge FI) or conditions (e.g. MTV/pain in IBS).

Conclusion

Current methods to assess rectal biomechanical properties and sensory perception are subject to either limited availability and feasibility in the clinical setting (standard barostat) or alternatively, have inherent limitations owing to the material characteristics of equipment and limited procedural control (balloon distension). The RBB pump has been introduced recently to fulfil the need for routine, bed-side assessment of compliance and capacity and represents the 'middle ground' between existing tools. From a usability point of view, the RBB pump is simple to use and provides the opportunity for rapid analysis and reporting. We have presented normal ranges for compliance, capacity, and sensory thresholds in 50 HV, the largest study of health to be performed to our knowledge. While these initial results require validation in future studies and by others, it represents the first step toward implementation of this technology within routine clinical practice.

Chapter 8a The use of EndoFLIP to assess anal sphincter distensibility: principles and review of literature

Introduction

Underlying concepts

Anal canal closure is traditionally thought to rely on tonic contractile activity of the anal sphincters which "squeezes" the anal canal shuts. Indeed, the definition of a sphincter is a 'ring-like muscle or physiological configuration in the body, the primary function of which is to prevent flow of contents by controlling the opening of a body orifice or constricting the lumen of a natural body passage'[448]. Manometry measures the force of this "squeeze" using pressure as a surrogate marker for anal closure. Thus, the competency of such a barrier to flow is most often measured in terms of the presence (or absence) of an area of high intra-luminal pressure, where increased pressure implies a competent barrier[449]. As noted in previous sections, manometric assessment of anal sphincter function has demonstrated that FI patients tend to have low resting and/or squeeze pressure compared with HV[5].

The ability of pressure to provide separation between cavities to prevent flow has limited basis in physics and the meaning of 'pressure' in the anal sphincter has been questioned in the past [450]. Harris and Pope [450] hypothesised that a sphincter does not depend upon tonic contraction or squeeze to remain competent, but simply closes, and then resists being opened. This resistance to distention has been proposed as the primary determinant of sphincteric strength [449]. The concept of resistance to distension as a more meaningful measure of anal sphincter function compared with closure pressure has gained renewed traction following the commercialisation of a high-resolution impedance planimetry measurement system known as the **Endo**luminal **F**unctional **L**umen **I**maging **P**robe (EndoFLIP®, Crospon/Medtronic).

The EndoFLIP system (**Figure 8a.1**) measures the cross-sectional area (CSA) of a cylindrical bag placed within a tubular organ during volumetric distension [451]. A 12-cm-long non-compliant bag containing electrically conductive fluid surrounds a catheter containing 16 pairs of detection electrodes each placed 5 mm apart. Two excitation electrodes located at either end of the bag deliver a constant current across the

catheter allowing CSA to be calculated based on Ohm's law. The measurement of the corresponding intra-bag pressure determined by a solid state pressure transducer inside the bag, makes it possible to assess the CSA-pressure response (known as distensibility index) of the organ [452].

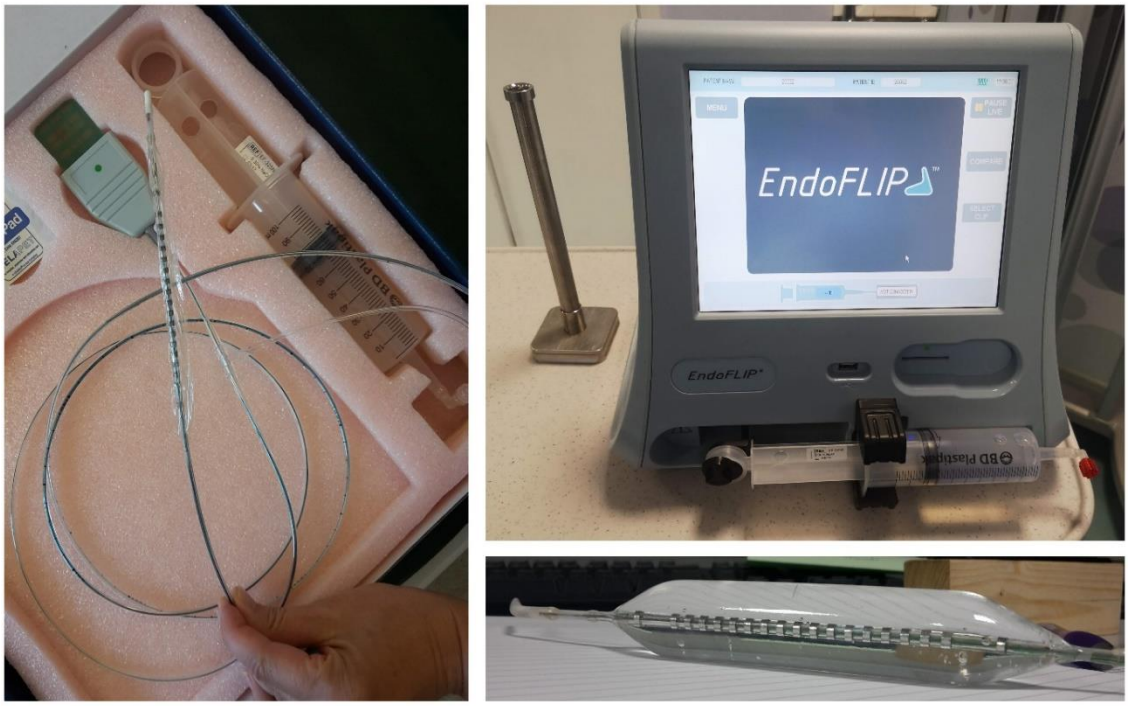


Figure 8a.1 The EndoFLIP system showing calibration tube and syringe containing infusion fluid. The catheter is also shown with the bag fully inflated revealing 8 pairs of detection electrodes. Intrabag pressure is measured by a single sensor at the tip of the probe.

The underlying concept of CSA measurement using FLIP is based on impedance planimetry [453]. According to Ohm's Law, the current (I) through a conductor between two points is directly proportional to the voltage (V) across the two points. Thus the,

Voltage (V) = current (I) x resistance (R)

where resistance (R) is:

distance / (conductivity x CSA)

By measuring the voltage between each pair of detection electrodes, the CSA of the FLIP bag can be determined mathematically at multiple, adjacent locations within the organ/anal canal. Multiple CSA measurements along the length of the anal canal are used to determine the narrowest section of the sphincter during controlled volume

distension. By assuming that the anal canal is circular, the diameter (Dest) of the anal canal can be estimated from CSA as:

$$Dest = 2\sqrt{\frac{CSA}{\pi}}$$

Gradual filling of the bag surrounding the electrodes with a fluid of known conductivity, leads to changes in CSA and intra-bag pressure. The latter, measured by a single pressure sensor at the tip of the bag, increases with progressive filling. Intra-bag pressure in FI patients was consistently lower compared to healthy volunteers (HV) both at rest and during voluntary contraction, consistent with ARM [141].

The relationship between CSA and intrabag pressure is linear [303]. However, changes in CSA occur at different rates depending on the location of the measurement, suggesting that the geometry of the lumen and the biomechanical properties along the length of the anal canal are not uniform[244,303,454] (see **Figure 8.b3**). At the start of the test (volume <10 ml) the anal canal has a cylindrical configuration with equal diameters measured along the length of the anal canal. With progressive volumetric distension, parts of the anal canal begin to distend at different times. The opening pressure (or volume) is a measure of the region's response to initial distension [303]. Typically, the proximal (upper) anal canal opens first, followed by the distal anal canal. Thus, there is a transition in the shape of the anal canal from a tubular to an hourglass shape, characterised by a narrowing in the middle part of the anal canal. With increased filling, the narrow region at the centre of the bag shortens and eventually the diameter at the narrowest region of the anal canal begins to change, known as yield pressure/volume [8,244,303,454]. This opening pattern has been consistently demonstrated in healthy individuals and in FI patients [8,141,243,303,454], suggesting that the EndoFLIP technique is a valid measurement tool in these populations [303].

Brief literature review

The majority of published studies using EndoFLIP have assessed oesophageal function, in particular lower oesophageal sphincter (LOS) distensibility in conditions such as achalasia [455] (ref) and gastro-oesophageal reflux disease (GERD) [451], which have been reviewed elsewhere[456]. To date, only a small number of studies on the use of FLIP for

the assessment of anal canal function have been published (**Table 8a.1**). Of 15 original articles, the majority have been published by researchers in Denmark (7 studies [8,243,244,303,454,457,458]), France (4 studies [141,245,246,459]), Switzerland (2 studies [460,461]) and, most recently, the USA (2 studies [246,462]).

Reference	Year	Country	Article type	Population(s) studied	n (% HV)
Alqudah	2010	Aalborg, Denmark	Abstract	HV	4 (100)
Alqudah	2012	Aalborg, Denmark	Original article	HV	21 (100)
Luft	2012	Aarhus, Denmark	Original article	HV	15 (100)
Fynne	2012	Aarhus, Denmark	Original article	HV vs FI patients with systemic sclerosis	29 (52)
Fynne	2013	Aarhus, Denmark	Abstract	HV vs FI patients with systemic sclerosis	29 (52)
Kumar	2014	UK	Abstract	HV	19 (100)
Kumar	2014	UK	Abstract	HV	19 (100)
Sorensen	2014	Aarhus, Denmark	Original article	HV vs FI	43 (62)
Gourcerol	2016	France	Original article	HV vs FI	73 (55)
Haas	2016	Aarhus, Denmark	Original article	FI patients undergoing SNS	11 (0)
Haas	2017	Aarhus, Denmark	Abstract	HV vs anal cancer patients	28 (50)
Brusa	2017	Switzerland	Original article	HV	20 (100)
Chen	2018	China	Abstract	HV vs FI patients	25 (36)
Gronlund	2018	Aalborg, Denmark	Original article	HV	24 (100)
Haas	2018	Aarhus, Denmark	Original article	HV vs anal cancer patients	27 (70)
Leroi	2018	France	Original article	FI	83 (0)
Zifan	2018	France	Original article	HV vs FI	70 (54)
Brusa	2018	Switzerland	Original article	HV	20 (100)
Tuttle	2018	California, USA	Original article	HV	14 (100)
Anschuetz	2019	California, USA	Abstract	Anal incontinent patients	4 (0)
Desprez	2019	France	Original article	STARR patients	7 (0)
Yan	2020	Georgia, USA	Abstract	HV	12 (100)
Barr	2020	California, USA	Abstract	Female patients with FI	14 (0)
Zifan	2020	California, USA	Original article	HV vs FI	28 (50)
Jalanivich	2021	California, USA	Abstract	Female patients with FI	29 (100)

Table 8.1 FLIP studies (result of literature search, excluding reviews and miscellaneous articles)

The available literature primarily focusses on the description of morphological changes during static and dynamic manouevres, application of different measurement parameters, validation of FLIP measures against other tests of anal function, and determination of clinical utility/treatment effects. Nineteen publications (including abstracts) included HV and nine publications included FI/anal incontinent patients (2 of these were in patients with systemic sclerosis). There is considerable overlap between participants included in these publications; data from a limited number of HV in particular, was used as control data (either in part or entirety) in more than one study (**Table 8a.2**). This points towards paucity of EndoFLIP studies in both HV and in patients with functional bowel problems.

Four publications used FLIP to evaluate the effects of treatment (SNS [8], radiotherapy/chemoradiotherapy [458], Naloxogol [457] or STARR [459] on anal canal function. The clinical utility of EndoFLIP, i.e its ability to differentiate between FI patients and HV compared with anorectal manometry, has been assessed in 4 studies [141,243,245,246]. Brusa et al [460,461] developed the "MR-FLIP" technique, to measure distensibility of the anal canal based on pressure and CSA measurements calculated from magnetic resonance images and compared these findings with "normal FLIP". Four other studies, published only as conference abstracts, described the use of FLIP for the assesessment of anorectal sensory or motor function in healthy volunteers (HV) (Kumar 2014, Kumar 2014b, Yan 2020) or FI patients [463]. Three other abstracts (Anshuetz 2019, Barr 2020, Jalanivich 2021) used the EndoFLIP as a resistance exercise tool during treatment of anal/faecal incontinence.

Author	Year	Type	n	Female n (% of total)	Parous (% of females)	Age	Overlap with other studies
Alqudah	2010	Abs	4	2 (50%)	nr	average 25 (23–29)	na
Alqudah	2012	OA	21	11 (52)	2 (18)	mean 36.5 ± SEM 2.5	Alqudah 2010 (Abs)*
Luft	2012	OA	15	12 (80)	nr	mean 51 (range 32-65)	na
Fynne	2012	OA	15	12 (80)	nr	median 54 (range 33-67)	Luft 2012*
Fynne	2013	Abs	15	12 (80)	nr	median 54 (range 33–67)	Fynne 2012*
Kumar	2014	Abs	19	9 (47)	nr	mean 34 (20-75)	na
Kumar	2014	Abs	19	9 (47)	nr	mean 34 (20-75)	Kumar 2014 (Abs)*
Sorensen	2014	OA	21	18 (86)	nr	median 55 (range 32-73)	Luft 2012, Fynne 2012
Gourcerol	2016	OA	40	40 (100)	40 (100)	median 51.5 (32-75)	na
Haas	2017	Abs	14	nr	nr	nr	na
Brusa	2017	OA	20	10 (50)	10 (100)	mean 70 (63-85)	na
Chen	2018	Abs	9	6 (67)	nr	Mean 57.9 SEM ± 3.69	na
Gronlund	2018	OA	24	0 (0)	na	20–60	na
Haas	2018	OA	14	9 (64)		61.4 ± 1.5	Haas 2017 (Abs)*, Sorensen 2014
Zifan	2018	OA	38	38 (100)	nr	nr	Gourcerol 2016
Brusa	2018	OA	20	10 (50)	nr	mean 70 (63-85)	Brusa 2017
Tuttle	2018	OA	14	14 (100)	14 (100)	34 (22-50)	na
Yan	2020	Abs	12	7 (58)	nr	28.2 ± 9.5	na
Zifan	2020	OA	14	14 (100)	14 (100)	34 ± 13	Tuttle 2018*

Abs: abstract OA: original article
na: not applicable
nr: not reported
* assumed overlap/not acknowledged in text

Table 8.a2 FLIP studies including HV

FLIP protocol

The methodology used to perform FLIP was similar between studies. In general, the FLIP was calibrated and then zeroed to atmospheric pressure. The lubricated tip of the probe was advanced intra-anally such that only 2 detection electrodes remain visible outside the anal verge; the probe was then held in place manually to minimise sensor migration during the test. The bag was first unfolded by infusing 10 ml of conductive fluid into the bag and then fully deflated. Further ramp inflations at 40ml/min were performed to 10, 20, 30, 40 and 50 ml with complete deflation of the bag in between distentions (refer to **Figure 6.5**). Resistance to opening was generally assessed during inflation with the anal canal in 'resting' state; only one study evaluated opening pressure during sustained squeeze [141]. At each filling volume, resting pressure measurements were followed by dynamic assessment of squeeze (typically 1-3 repetitions, each lasting 5-10 seconds). One study also performed cough as part of the protocol [303]. At the end of the study, the bag was deflated before extubation.

Data analysis

While the EndoFLIP 1.0 (Medtronic) system provides real-time data on lumenal diameter and pressure, analysis of FLIP data requires data to be transferred to a suitable PC via a portable drive (such as a USB stick) and processed offline. Extracted data includes the time stamp and number for each data sample followed by the diameter (mm) at each sensor and bag pressure, volume and temperature. Data sampling is performed at 10 Hz. Most studies used custom Python or Matlab code to create colour topography plots similar to HR-ARM (using diameter instead of pressure to define colours) and to analyse data. While the main purpose of FLIP is to describe the compliance or "distensibility" of the organ under study, there is currenlty no consensus about the best parameters to record with FLIP [456].

Anal distensibility during distension or at rest

The distensibility of the organ under study refers to the ability of the distended tissue (in this case the anal sphincter complex) to resist opening and is characterised by the CSA-pressure or pressure-volume relationship [464]. While the anal canal of patients with FI has been shown to be significantly more distensible (i.e showing less resistance to stool entry) compared to HV especially in the middle of the anal canal [141,243-245,454,458], a

variety of different measures have been used to summarise this relationship, of which the distensibility index (DI) is most commonly associated with FLIP [456].

The DI (expressed in mm^2/mmHg) is calculated as CSA (determined from the bag diameter during FLIP) divided by pressure [242]. It can be determined for any given location (along the length of the anal canal) and distension volume. The DI in the anal canal has been shown to differentiate between FI patients and HV [141]. For example, Gourcerol et al [141] demonstrated that for each distension volume (30-50 mL), anal canal opening in 34 FI women was greater at its narrowest point than that in 40 healthy female volunteers. Meanwhile, intrabag pressure was lower in FI patients than controls. The resultant DI was significantly higher in FI patients than in healthy subjects indicating a less resistant anal canal both at rest and during squeeze [141]. DI at rest and during squeeze correlated significantly with FI severity (higher incontinence score); however this finding was not replicated in a subsequent, larger study of only FI patients [245].

Currently there are no published normal ranges for DI based on the 5^{th} and 95^{th} percentiles in health, due to only small numbers of healthy controls being included in studies. However, optimum cut-off values for DI (at 40 ml distension) in women based on the ability for DI to discriminate between healthy and FI subjects were published in 2016 by Gourcerol et al [141]. No consistent correlations between DI and age, BMI, parity, or menopausal status for DI at rest or during squeeze were observed. These cutoffs (abnormal >1 mm^2/mmHg at rest and >0.5 mm^2/mmHg during squeeze) were subsequently used by Leroi et al [245] to compare FI characteristics in female patients with normal and abnormal DI. Of a group of 83 FI patients, 64% and 65% of patients exhibited DI above the cut-off value at rest and during voluntary contraction, respectively. Initial analyses suggested that patients with an abnormal DI at rest were significantly older, while patients with an abnormal DI during voluntary contraction had a longer FI duration than the others and were less likely to suffer from idiopathic FI [245]. However, when the comparisons were conducted based on a multilogistic regression, no significant differences were observed between those with and without abnormal distensibility.

Some smaller studies that included both male and female HV have suggested that there may be some impact of sex on distensibility (but not DI) which remains to be confirmed

in larger studies [303,461]. Alqudah et al [303] noted a lower opening pressure in males than in females. However, in the study by Alqudah et al [303] the location (proximal, mid, distal anal canal or narrowest part) at which opening pressure was measured is somewhat unclear and suspiciously low. For comparison, Haas et al [458] reported yield pressures of 28.0 ± 1.3 mmHg. Yield pressure, defined as continous anal canal opening at the narrowest location, may be of greater clinical relevance than the opening pressure reported in previous studies [243,303,454].

Anal distensibility during squeeze

The effect of voluntary sphincter contraction has been studied using EndoFLIP both during distension [141] and during defined volumes [244,303]. Voluntary EAS contraction resulted in significant pressure changes with peak squeeze pressure being associated with the beginning of the manouvre [141,244,303,460]. Voluntary contraction has been shown to decrease DI in healthy volunteers suggesting some contribution of striated muscle toward resisting anal canal opening [141]. Furthermore, the inverse of DI (accounting to the reduction in diameter during squeeze) showed good correlation with squeeze pressure using ARM in the same study.

During voluntary contraction, Alqudah et al [303] observed distinct proximal and distal narrow regions which were presumed to represent the puborectalis and external anal sphincter (EAS), respectively. Narrowing in the upper anal canal was maintained for the duration of the squeeze effort (10-20 sec), while narrowing at the outer anal verge was more pronounced and short-lived (3-5 sec). Meanwhile the middle of the anal canal was shown to have increased CSA during squeeze, due to trapping of fluid between the two narrow zones, leading the authors to conclude that anal closure during squeeze was not the result of tightening throughout the anal canal [303]. However some of these observations were made at low distension volumes. Conversely, a subsequent larger study showed that the diameter of the anal canal decreased throughout the anal canal during squeeze, although due to bag placement diameter changes could only be measured in the middle and distal anal canal [244]. Notably, despite poor squeeze function on anorectal manometry, FI patients were able to close a significant part of the anal canal even at the highest distension volume. In fact, the decrease in diameter during

squeeze was sometimes greater in patients compared with HV, however this finding is likely to have resulted because their anal canal was more distended to begin with [244].

Using the MR-FLIP technique to determine the location of anal canal narrow regions, Brusa et al [460] observed diameter changes related at both EAS and puborectalis levels. They evaluated the effect of squeeze based on a calculation of the mechanical work performed; a measure which aims to simultaneously take into account the amount of orifice closing and the force required for achieving this level of closing. In doing so they hoped to avoid the pitfalls of measures used to quantify muscle fitness used by others (diameter-time changes [244], DI [141], and wall tension [454]. Brusa et al [460] observed that the largest change in orifice diameter during squeeze occurred in the upper anal canal, reinforced by the observation that the maximum amount of work was performed around the proximal EAS end. These results strongly suggested that the puborectalis contributes to maintaining continence mechanically and that urge incontinence can be attributed to the mechanical weakness of the EAS and/or the puborectalis [460].

Finally, the most recently published studies on FLIP have based analysis of squeeze on the principles developed from knowledge of length-tension relationships [462,465]; this is the principle that the force generated by a given muscle is related to the length of the muscle. In general, a muscle is strongest when operating at its optimum length; under normal conditions, both the EAS and puborectalis operate at a suboptimal length [462]. Assessment of length-tension relationships using manometry have been demonstrated in the past using probes of increasing diameter [177]; squeeze pressure was shown to increase with larger diameter probes. Tension (mm/mmHg) can be calculated during FLIP using pressure and diameter readings (tension= pressure (mmHg) X radius (mm)) at different volume distensions. However, in contrast to manometry and as noted by others [244,303] because the EndoFLIP bag collapses during squeeze, there is reduction in the size of the FLIP bag with increased voluntary contractile pressure allowing concentric, rather than isometric, contractile force of the anal canal to be assessed.

To assess squeeze, Tuttle et al [462] developed tension-time plots for each bag volume. Zifan et al [465] further expanded on these ideas, using loop analysis to compare differences in area-pressure and area-tension plots to describe EAS function and compared results between FI patients and HV. Each loop represented changes in the

CSA and pressure (or tension) from resting to the peak of muscle contraction and back to resting, drawing inspiration from how shifts in cardiac loops are used to identify damaged myorcardium. According to their results, the greater shifts of loops in the right (increase in CSA) and upward (increase in pressure) directions observed in FI patients compared to HV correlated with greater severity of damage to the EAS and puborectalis muscles. Thus the authors concluded that differences in the magnitude of the shift of area-tension and area-pressure loops with increasing bag volume (muscle length) describe muscle dysfunction in FI patients. Furthermore, unlike distensibility, which implies passive function, the authors proposed that loop analysis may be a better analytical approach to assessing squeeze because it allows a more meaningful dynamic assessment which takes into account length-tension properties of muscle [465]. Inspection of the loops revealed that even though FI patients can generate comparable muscle tension to normal subjects, they achieve it at a significantly higher muscle length (CSA) referring to the phenomenon as the "muscle reserve function" [465].

Clinical utility of EndoFLIP

Several studies have tried to determine whether FLIP is comparable or complementary to ARM. In both HV and in patients, anal canal DI at rest and during squeeze showed strong inverse correlation with resting pressure and squeeze pressures measured by 3D-HRAM [141]. Similarly, the elastic-pressure strain modulus (Ep) determined by FLIP was associated with anal resting pressure determined by pull-through manometry [244]. In FI patients, there was substantial diagnostic agreement between the EndoFLIP and 3D-HRAM (pressure) values, with both techniques providing the same diagnoses regarding anal deficiency at rest or during voluntary contraction for more than 70% of the patients. These results indicated that EndoFLIP provides a reliable assessment of anorectal function and diagnosis of anal weakness [245].

The clinical utility of distensibility measured using FLIP in comparison with manometric pressure-based assessments has also been examined in terms of its accuracy in differentiating between HV and FI patients has also been examined. DI at rest [141], but not Ep [244], was superior to anal pressure at rest for discriminating between FI patients and healthy subjects. Similarly, DI during squeeze was significantly superior to anal pressure during squeeze [141]. However, the distension volume at which FLIP

measurements are made can have a significant impact on outcome; better discrimination was achieved using the 40 ml, rather than 50 ml distension in one study [141], while another study showed that a combination of measures from the 40 and 50 ml distensions provides best discrimatory ability [246]. Overall, EndoFLIP may be a more accurate tool than 3D-HRAM for estimating anal capacity to retain stools and may thus provide a more accurate portrait of positive changes in anal sphincter function that are not appreciated by 3D-HRM following FI therapy [458]. However, voluntary EAS muscle activity determined by EMG was not related to EndoFLIP parameters during squeeze, but did correlate with 3D-HRAM suggesting that the DI during squeeze may be less sensitive for detecting the isolated action of the EAS than anal pressure [141].

Similarly, in female FI patients, anal distensibility outcome (normal/abnormal) was not shown to relate to electrophysiological or ultrasound findings [245]. In particular, no significant relationship was found between the presence of anal sphincter atrophy and the DI at rest and/or during contraction [245]. This contrasts with previous findings in systemic sclerosis patients [243] in whom, contrary to expectations, the diameter at the mid anal canal was smaller in the patient group compared with HV, a finding which was related to IAS thinning on endoanal ultrasound and low anal pressures on manometry. While the authors suggested that the result may have been related to increased fibrosis common in systemic sclerosis [243], Leroi et al [245] found no such relationship in patients with history of radiotherapy who might have been assumed to have similarly fibrosed tissues. Meanwhile in HV, Brusa et al [460] demonstrated that the thicker the muscle (measured during MR-FLIP), the lower its compliance (indicating greater stiffness) and the smaller the orifice opening during traditional FLIP balloon inflation. Similarly, greater EAS muscle thickness was associated with the degree of orifice closure and mechanical work performed during squeeze. The relationships between muscle thickness and biomechanical properties may therefore be population/disease specific.

Limitations of FLIP

Although the FLIP technique looks like a promising addition to the range of tests which can be feasibly performed in a clinical context, certain limitations of the technique have been noted in the literature.

Probe placement and position of sensors within the anal canal

Current studies have all been performed using FLIP bags which have a length that is greater than the average FACL [30]. Inevitably, part of the approx. 8 cm bag therefore lies beyond the internal and external anal verges depending on the positioning of the probe during intubation. During filling, fluid will initially collect in the part of the bag showing least resistance to flow. If this is located within the rectum there is potential to induce RAIR, which could artificially lower distensibility measured in the anal canal [303,466]. To minimise these effects, bag positioning should be standardised between subjects so that the amount of rectal distension occurs uniformaly in all participants; this has been achieved to some degree by controlling the number of sensors located outside the outer anal verge (typically 2 sensors). However, this approach leaves physiological variation in anal canal length unaccounted for and assumes that the amount of distension required to induce RAIR is the same in all subjects [9].

Location of measurements

Once positioned in the anal canal, the EndoFLIP probe measures CSA at 16 points along the bag. However, opening of the anal canal is not uniform along its length indicative of differences in related to passive and active properties of the anal sphincter muscles and surrounding tissue [462]. The location where measurements are made must be defined either with reference to specific features along the length of the probe (e.g. proximal, distal, mid anal canal or narrowest region) or in relation to anatomical structures (currently only possible using MR-FLIP), in order to make meaningful comparisons between subjects and between studies.

Because identification of proximal and distal limits for determination of the middle point of the anal canal is highly subjective, the narrowest point has been proposed as a standardised clinical reference point for measurements such as DI [458,460]. At this location, the DI makes sense from a fluid mechanical point of view, as the narrowest cross-section is the main determinant of the resistance to flow. Furthermore, it is easily identifiable in all subjects and more easily “tracked” in case of bag migration. Because small amounts of movement of the endoFLIP bag occurs especially during high volume distension and high bag pressure [303], changes in the mid-point, for example, may be easily missed during analysis as its location is dependent on reference points

determined prior to bag migration (i.e. proximal and distal sensor limits identified at low volumes).

Using MR-FLIP, the narrow region during distension in healthy female volunteers was found to represent a position just a few millimetres below the proximal end of the EAS muscle, where the EAS starts to overlap the IAS and puborectalis during filling [461]. This is consistent with findings from a study incorporating transvaginal ultrasound in women [467], however it is currently not known if the anatomical reference point varies by sex or disease status. For example, one previous study using FLIP including both male and female HV and patients with systemic sclerosis and FI [243], suggested that the least distensible part of the anal canal was located in the region where the IAS and EAS overlap [243]. However, although FLIP provides the opportunity to compare distensibility in different parts of the anal canal, on its own (as was the case in the stufy by Fynne et al [243], it cannot be used to investigate the biomechanical properties of specific anal canal structures such as the IAS, EAS, or puborectalis [461].

Reliability and relevance of measurements

At low distension volumes, intrabag pressure shows high inter- and intra-individual variablity [303]. This could be due to the location of a single pressure sensor at the distal end of the probe. With little to no liquid in the bag, the pressure measurement is most likely to represent contact pressure within the rectum. The amount of volume required for reliable pressure measurement varies between 10 -30 ml [141,303]. In upper GI studies, DI should be approached with caution if bag pressure is below 30 mmHg at bag volumes >40 ml (Medtronic training video). Others have also questioned the validity of a single pressure measurement, on the basis that a narrow sphinteric region may divide the bag into two sections, especially at low volumes [458]. The benefit of this observation is that the study protocol can be significantly shortened in some cases to only include distensions at higher volumes.

With increasing distension, the narrowest part of the anal canal typically yields (opens) at bag volumes ≥40 ml [244,458]. However, in a proportion of patients and HV, the anal canal has remained closed even at the maximum distension volume (50 ml). Haas et al [458] noted a closed anal canal in 10/14 HV and 2/10 patients at maximum distension. In another study [141], approximately 10% of subjects did not show any anal canal opening.

In a third study, the diameter of the middle segment of the anal canal did not open sufficiently to evaluate distensibility based on the elastic-pressure strain modulus (Ep) in a "significant portion" of healthy male volunteers [457]. The lack of anal canal opening in some subjects should be taken into account when defining normal ranges. For example, defining an upper limit of normal yield pressure based on the 95th percentile in those who did achieve anal canal opening could be misleading if those who did not show anal canal opening were simply excluded.

To date, a wide variety of distensibility measures have been used to describe anal canal resistance to opening of which DI is perhaps the most simple to use measure. The underlying principles of impedance planimetry assume a circular cross-section, however opening cross-section of the anal canal has not been shown to be strictly circular [461](Brusa 2017). In patients with sphincter tears, circularity may be compromised even further.

Lack of normative ranges

Despite most (19/25, 76%) of the studies published to date including HV, only one study has reported normal cut-offs for distensibility [141]. This is probably because many of the published studies have included only small numbers of HV (median 15, range: 4-40). Some of these studies have indicated (or simply presumed) that baseline characteristics such as sex, age, parity and BMI may influence EndoFLIP measures (both in HV and FI patients). Larger studies are needed to either confirm or refute their impact on distensibility.

Summary

Despite its limitations, published studies have consistently shown that anal canal function, measured using EndoFLIP, differs between FI patients and HV. For example, FI patients appear to have higher distensibility [141,243,244,246,454,465], earlier anal canal opening [463], and greater degrees of muscular damage [465]. Compared to manometry, EndoFLIP has been shown to have greater sensitivity to differentiating between HV and FI patients [141] and has greater potential to identify positive changes following treatment [458]. Resting and squeeze pressures mearusred by EndoFLIP have been shown to correlate well with manometry [141,245] with EndoFLIP correctly identifying anal weakness in up to 70% of subjects leading some to suggest that manometry and EndoFLIP may be

used interchangeably [245]. Others have stressed that while 3D-HDAM and EndoFLIP perform in a similar manner for diagnosing FI, they are not complementary [246]. However, with up to 80% of female FI patients shown to have an abnormally distensible anal sphincter in one study [245], we should ask, are current normal cut-offs too forgiving?

Chapter 8b Prospective assessment of anal distensibility using EndoFLIP in healthy volunteers

Introduction

The main determinant of faecal continence is the competence of the anal sphincter complex when faced with the arrival of stool, maintained by internal anal sphincter (IAS) tone at rest. Functional incompetence can be attributed to either anatomical or physiological factors, including structural or neurological injury leading to low anal and pelvic floor tone. However, not all individuals with low anal tone (pressure) are incontinent and actually, only a minority of FI patients have abnormal tone (Chapter 2 Systematic review).

As described in the previous section, distensibility is a measure of the relationship between cross-sectional area (CSA) or volume and pressure of a spherical structure. The more distensible (lax) an organ is, the less pressure is required to change its diameter. Increased distensibility may exacerbate FI in two ways: 1) the amount of intra-abdominal pressure required to open the anal canal may be lower; and 2) the anal canal may open wider than normal under a given physiological circumstance. This could explain, for example, the reduced resistance to gas, as opposed to liquid or solid stool. The potential for an incompetent, highly compliant sphincter to exacerbate disease (e.g. gastro-oesophageal reflux disease) has been similarly described in the oesophageal literature [455].

Despite a lower distensibility index (DI) in FI patients [141,246], when FI patients were classed as having abnormal vs normal distensibility based on DI (CSA/pressure at the narrowest region) [242], no significant differences in demographics, FI characteristics or severity, probable causes, or structural and neurological deficiency were observed between groups based on multiple regression analysis [245]. One of the reasons for these findings may be that the cut-offs used to define normal DI at rest and during squeeze were based on the best sensitivity and specificity [141] rather than the true range of values observed in health [245]. Furthermore, the recruitment of the healthy subjects on whom these cut-offs are based was weighted toward middle-aged (median age 58.5 , range 26–82 years) females with a mean parity of 2 (range 1-4) [141].

While the overwhelming majority of patients presenting for investigation of FI are women with a history of (often traumatic or instrumental) vaginal delivery [6], community based studies indicate similar prevalences of FI amongst males and females [65], and between nulliparous and parous women [296]. Therefore diagnostic ranges applicable to a wider population are needed. Previous studies using EndoFLIP have suggested some impact of gender [303], age [245,246], and BMI [246] on anal canal distensibility. However the impact of parity is yet to be determined. Despite this, to avoid any *likely* impact of vaginal delivery on distensibility, previous studies have opted to include only nulliparous women [462,465] or matched FI subjects and controls for the aforementioned characteristics [244,246,458].

While the EndoFLIP system can provide measurements of luminal diameters and information on changes in tissue distensibility in real-time using representative 3D geometric plots of the organ under study [468], comprehensive data analysis and offline processing has been largely based on customised code (e.g in Matlab [The MathWorks Inc., Massachusetts, USA]) raw data analysis. Thus methods of analysis are subject to variation between centres and are often (probably) incompletely described in publications, affecting reproducibility. Recently, commercially available software (EndoVizX [Motilityviz, California, USA]) has become available. To our knowledge, this software has not been used in any published studies of anal canal biomechanics to date.

Thus, the aim of this chapter is to decribe the distensibility of the anal canal using EndoFLIP during continous inflation, at rest and during voluntary contraction in healthy subjects with a view of determining appropriate normal ranges accounting for any differences in participant demographics. Analysis of raw data files will be supported by analysis using available software.

Methods

51 normal subjects (14 males, 37 females; 19-67 yrs old) without bowel symptoms were recruited as described in chapter 7: Propective study Methods. Of female participants, 20 were nulliparous and 17 were parous. The EndoFLIP procedure was performed as previously described [303]. Briefly, a reusable (maximum 5 uses), EndoFLIP EF-325R catheter (Crospon) incorporating 17 detection electrodes (providing 16 distensibility

measurements 5 mm apart) and a solid-state pressure sensor within a 12 cm long bag was used (**Figure 8b.1**). Prior to each investigation, the diameter recording of the probe was checked within a calibration tube of known diameter. The study was performed with the particant lying on their left side with knees and hips flexed at 90 degrees. The lubricated EndoFLIP probe was inserted such that 4 detection electrodes were visible outside the anus. The length and positioning of the probe was chosen to ensure that the mid anal canal remained roughly at the centre of the bag, while limiting the amount of rectal distension and avoiding the need to reposition the bag during the test due to bag migration. The probe was held in place manually during the investigation.

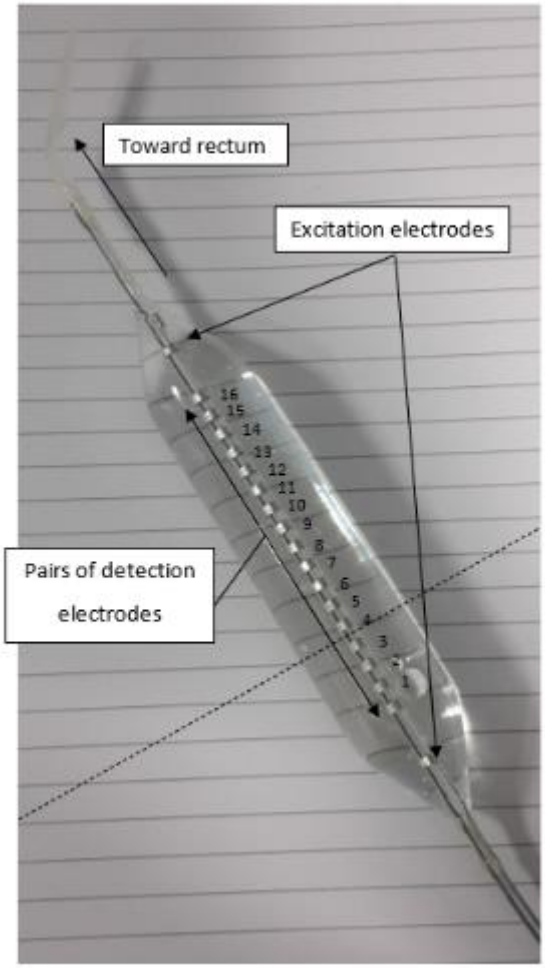


Figure 8b.1 EndoFLIP catheter (filled with 50 ml infusion liquid). Eight pairs of detection electrodes enable determination of CSA at 16 channels (locations) along the length of the probe. The dotted line represents the level of the external anal verge such that four sensors are visible outside the anus.

Distensibility

Distensibility, i.e. the relationship between the anal canal diameter and intraluminal pressure at a defined distension volume, was characterised during inflation, at rest, during squeeze, and during cough. During inflation, distensibility was defined by: a) the opening (or yield) pressure determined as the pressure at which the diameter of the most narrow region of the anal canal first began to increase continously during constant inflation of the EndoFLIP bag from 0 – 50 ml at a rate of 40 ml/minute; b) the opening volume defined as the bag volume at opening pressure; c) opening DI, calculated at the narrowest CSA at opening divided by the opening pressure; and d) compliance defined as the change in volume divided by the corresponding change in pressure from 0 ml to opening pressure. The rate of subsequent anal canal opening was described by the slope of the volume-diameter curve after initial opening. During manual data processing, only data points toward the centre of the probe (channels 5-14) were considered.

Resting pressure was defined as the average recorded pressure during the last 10-15 seconds of the resting period (immediately prior to squeeze). The DI at rest was calculated from the median diameter at the narrowest anal canal location during the resting period, converted into CSA, and divided by the median pressure over the same time period. Resting DI using the Endovizx software, was based on the minimum diameter (rather than the median) and the corresponding pressure. Distensibility measures obtained by manual and software analyses during inflation and at rest were compared. The EndovizX software was used to measure the minimum DI during squeeze and during cough.

Statistical analysis

Participant demographics between men and women and nulliparous and parous women were compared using an independent samples t-test. All other measurements were compared between three goups (males, nulliparous and parous women) using a one-way ANOVA assessed at the 95% significance level (95% confidence intervals provided).

Results

Participants

Distensibility parameters could be analysed in 46 of the 51 subjects recruited (Table 8b.1). One individual did not tolerate intubation with the EndoFLIP probe and the test was abandoned. Data from one other individual was accidentally over-written during a subsequent study which was performed prior to saving the raw data file to a USB stick. In two individuals the pressure sensor failed (values were negative) and in one individual the pressure sensor was not zeroed correctly leading to erroneous readings. These five subjects were excluded from the final dataset.

		Total	**Males**	**Females**	**Nulliparous**	**Parous**
n		46	11	35	19	16
Age	Mean (sd)	38.5 (12.4)	38.2 (16.3)	38.5 (11.2)	35.3 (11.8)	42.4 (9.3)
Body Mass Index	Mean (sd)	24.1 (4.5)	24.8 (3.8)	23.9 (4.8)	23.7 (5.5)	24.0 (3.8)

Table 8b.1 Table of participant demographics

Distensibility during inflation

Anal canal opening at the narrowest point occurred in 45/46 subjects during distension from 0-50 ml. Distensibility parameters during inflation are summarised in **Table 8b.2**. No significant differences in opening pressure (p=0.270), opening volume (p=0.232), opening DI (p=0.313), compliance (p=0.398) or rate of anal canal opening (p=0.930) were observed between men, nulliparous women, and parous women (see **Appendix 11** for summary and ANOVA tables). There was no statistically significant linear association between opening DI and age (p=0.15, R^2=4.9%, 95% CI= -0.001 to 0.009) or BMI (p=0.823, R^2=1.2%, 95% CI=-0.02 to 0.01). Thus normal ranges for the group as a whole are presented in **Table 8b.3.**

	n	Mean	sd	Median	se	95% CI
Opening pressure	45	49	15.0	47	2.3	45-54
Opening volume	45	38	4.6	38	0.7	37-40
Opening DI	45	0.49	0.21	0.43	0.03	0.4-0.6
Compliance	45	1.1	0.48	0.96	0.07	0.98-1.3
Rate after opening	45	0.46	0.10	0.43	0.01	0.43-0.49

Table 8b.2 summary data for distensibility measures during 0-50ml distension

	Median	5th percentile	95th percentile
Opening pressure (mmHg)	47	22	75
Opening volume (ml)	38	30	45
Distensibility index (cm^2/mmHg)	0.4	0.3	1.0
Compliance (ml/mmHg)	1.0	0.7	2.3
Distension rate after opening (ml/mmHg)	0.4	0.4	0.7

Table 8b.3 Normal ranges for distensibility parameters measured during inflation

Distensibility at rest

The resting period [mean duration 15 sec (95% CI 13.7-16.8)] was analysed in 45/46 participants (**Table 8b.4**). In one subject the procedure was abondoned just after reaching 50 ml inflation at the participant's request. There were no significant differences between groups of men, nulliparous and parous women for the CSA (p=0.213), pressure (p=0.583), or DI (p=0.244). Normal ranges are presented in **Table 8b.5.**

	n	Mean	sd	Median	se	95% CI
CSA (mm^2)	45	135	68.8	115	10.3	114-156
Pressure (mmHg)	45	68	10.8	68	1.6	64-71
DI (mm^2/mmHg)	45	2.1	1.42	1.9	0.21	1.7-2.6

Table 8b.4 Summary data for distensibility measures at rest

	Median	5th percentile	95th percentile
CSA (cm^2)	115	51	292
Pressure (mmHg)	68	48	89
Distensibility index (cm^2/mmHg)	1.9	0.8	4.8

Table 8b.5 Normal ranges for distensibility parameters measured during rest

There was a statistically significant (p=0.02) linear association between DI at rest and age. The total variation explained by the model was 11.8% (R^2). Based on the model, as age increases by 10 years, resting DI increases by an estimated 0.4 units (95% CI = 0.1-0.7; **Figure 8b.2**). There was no statistically significant linear association between DI at rest and BMI (p=0.518, R^2= 0.01; 95% CI -0.07 to 0.14).

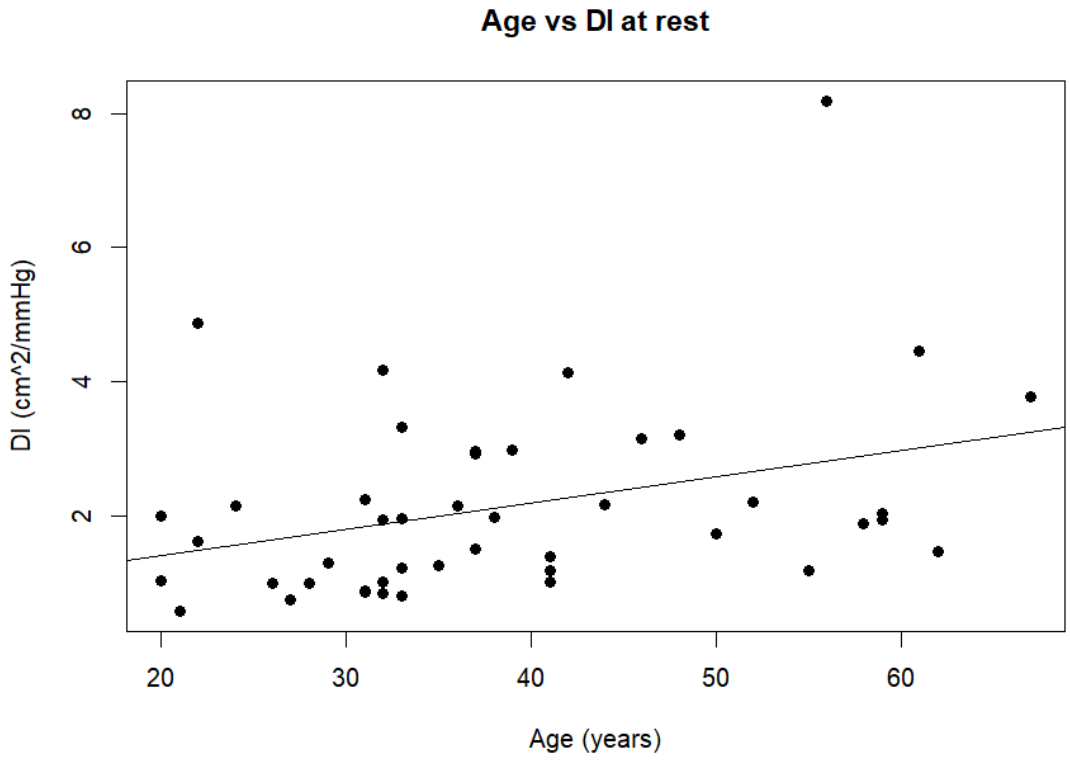


Figure 8b.2 Association between age and resting DI (mm^2/mmHg) is given by the equation 0.64 + 0.039*age (R^2= 11.83, 95% CI= 0.006-0.072).

Correlation between raw data analysis and EndovizX

Pressure, volume and distensibility results from manual analysis was compared with data from EndovizX software in 41 subjects (17 nulliparous women, 16 parous women, and 8 males). Traces from the remaining four subjects could not be uploaded into using the software for unknown reasons and hence were not analysed. There was a statistically significant ($p<0.001$) linear association between raw data analysis and EndovizX analysis for the following inflation parameters:

- Opening pressure, slope= 1.029, R^2= 97.5%, 95% CI: 0.975 to 1.083
- Opening volume, slope= 1.093, R^2= 91.4%, 95% CI 0.983 to 1.203
- Opening DI, slope= 1.016, R^2= 87.3%, 95% CI 0.889 to 1.143

From the colour topography plot, the expected transition of the anal canal from a uniform cylindrical configuration to an hourglass shape during progressive filling was apparent in all subjects. Differences in anal canal diameter along the length of the anal

canal at any given volume (or pressure) indicate differences in distensibility at various points along the anal canal (**Figure 8b.3**). A statistically significant ($p<0.001$) linear association between raw data analysis and EndovizX analysis was also observed for resting pressure (R^2= 98.3%, 95% CI: 0.953 to 1.038) and resting DI (R^2= 92.8%, 95% CI 0.869 to 1.041).

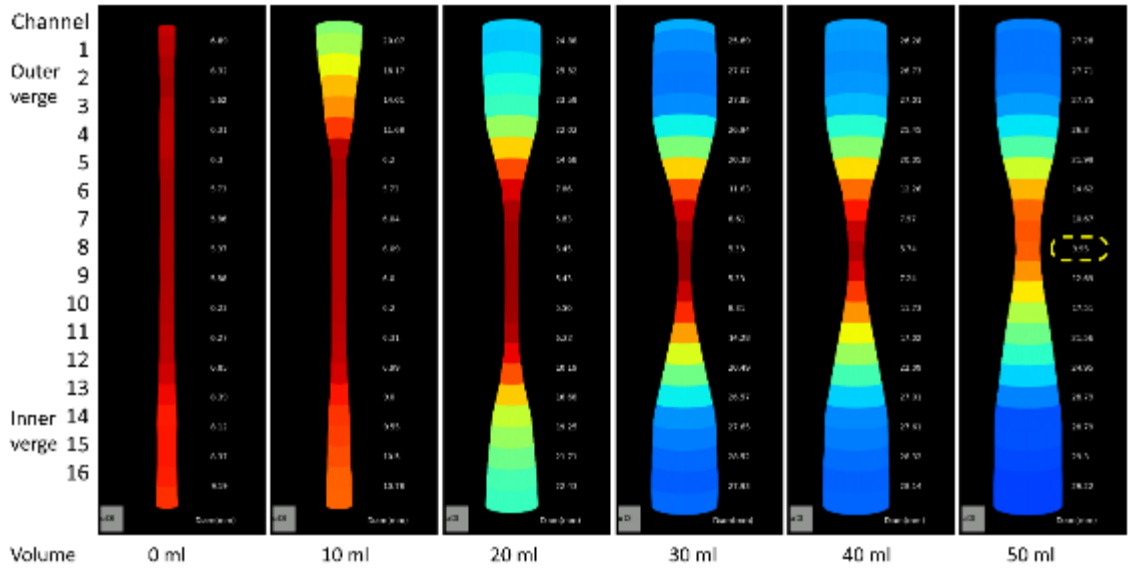


Figure 8b.3 Representative plot of anal canal opening with progressive filling (0-50 ml) showing hourglass shape. Channel diameter is displayed on the right of the image. Minimum diameter at the end of filling is highlighted.

Squeeze

Squeeze parameters were measured in 41 individuals (17 nulliparous women, 16 parous women, and 8 males) using EndovizX software. Inspection of the colour topography plots showed that the anal canal diameter decreased in all participants during squeeze coinciding with an increase in pressure (**Figure 8b.4**).

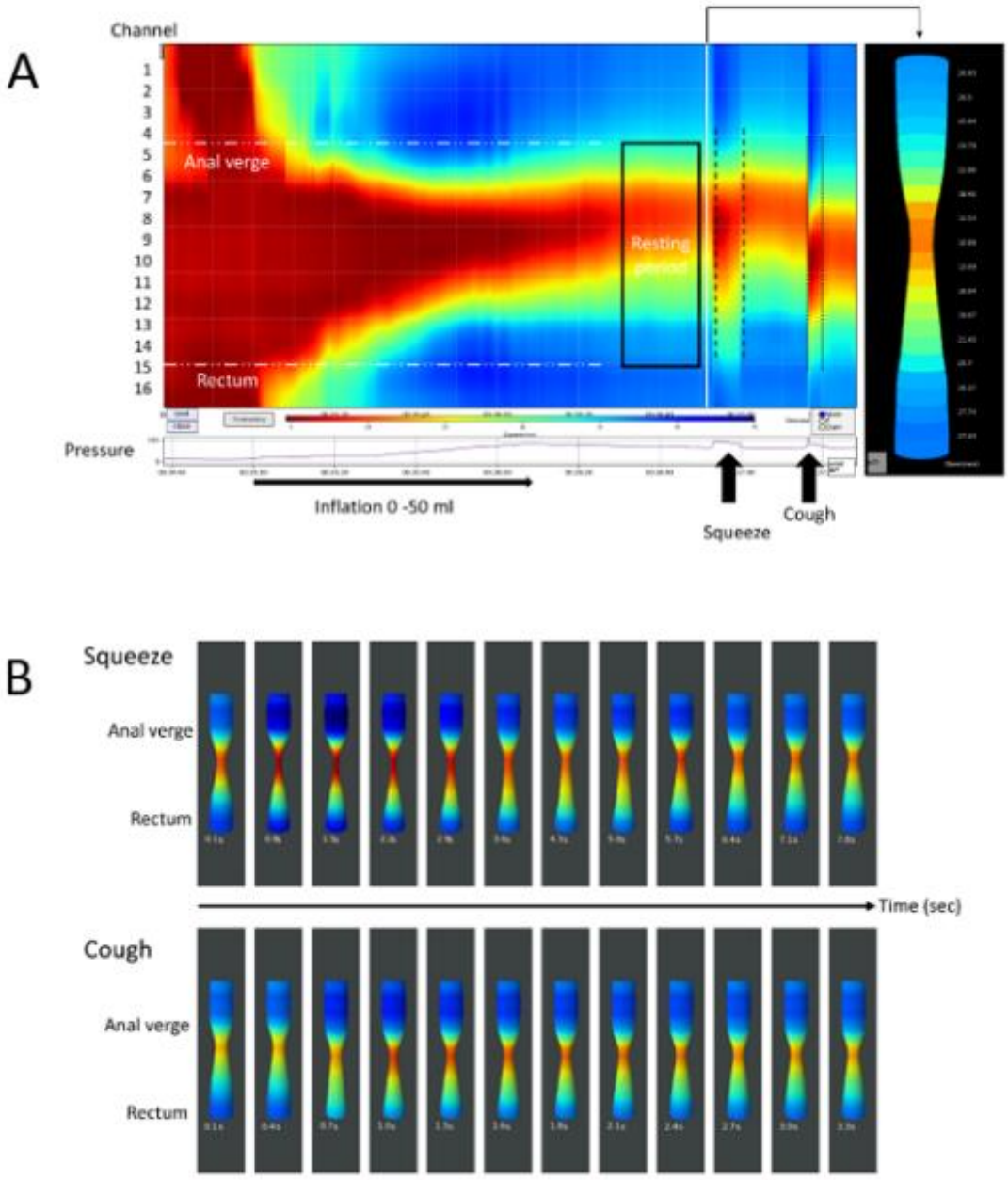


Figure 8b.4 Representative colour topography plot during inflation (0-50 ml) and during static and dynamic manoeuvres at 50 ml. A decrease in anal canal diameter is indicated by a change of colours from yellow/green to orange/red during voluntary contraction (squeeze) and coughing (A). Anal canal narrowing during squeezing and coughing can be observed as changes in the hourglass configuration of the EndoFLIP probe (B).

The mean maximum squeeze pressure was 102 mmHg (95 % CI 94.8-109, range= 68 - 179 mmHg). On average, pressure increased by 58.1% (range 25-190 %) during voluntary contraction (compared with pressure immediately before squeeze). When squeeze pressure was compared between groups of males, nulliparous and parous women (**Figure 8b.5**), ANOVA showed a statistically significant difference between

groups (p= 0.0015, **Appendix 12**). At 125 mmHg, males had the highest mean squeeze pressure of the three groups which was, on average, 27 mmHg higher than in nulliparous (Bonferroni adjusted 95% CI: 6.3 to 48 mmHg, p=0.007) and 32 mmHg higher than in parous (Bonferroni adjusted 95% CI: 11 - 53 mmHg, p=0.0014) women. There was no significant difference in mean squeeze pressure between nulliparous and parous females (mean difference 5 mmHg, SE estimate= 6.7, p>1.0).

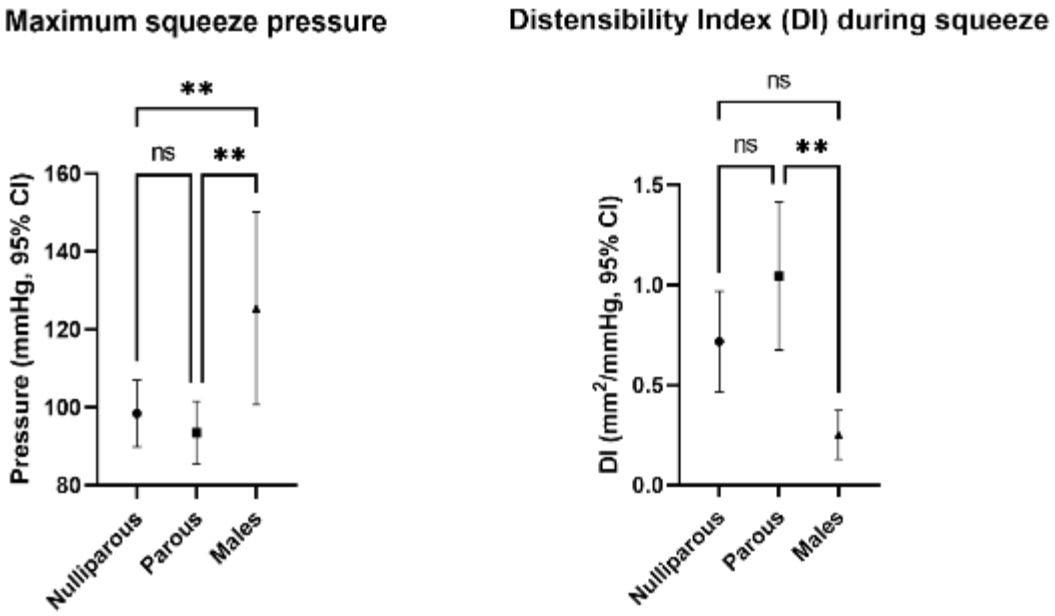


Figure 8b.5 Maximum squeeze pressure and DI during voluntary contraction in nulliparous and parous women and men.

The average DI measured at the minimum diameter was 0.75 mm^2/mmHg (95 % CI 0.56 - 0.95, range= 0.15-2.38). Squeeze DI was significantly lower in males (0.3 mm^2/mmHg) than in parous women (p=0.0053) with a mean difference of 0.8 mm^2/mmHg (Bonferroni adjusted 95% CI: 0.2 - 1.4 mm^2/mmHg) between groups. There was no significant difference in mean DI between males and nulliparous females (mean difference 0.47, SE estimate= 0.23, p=0.1608), or between nulliparous and parous women (mean diff -32.8, SE estimate= 0.19, p=0.2759). Squeeze DI was not associated with age (p=0.6645, R^2= 0.48%; 95% CI -0.019 to 0.012).

Based on these analyses, normal values for squeeze DI are presented for the group as a whole and for males and females (Table 8b.6).

	All (n=41)		Males (n=8)		Female (n=33)	
	5th	95th	5th	95th	5th	95th
Squeeze pressure (mmHg)	71	141	81	179	70	131
Squeeze DI (mm^2/mmHg)	0.17	2.2	0.15	0.57	0.21	2.29

Table 8b.6 Normal ranges for squeeze manoeuvre

Cough

Cough parameters were measured in 31 individuals (13 nulliparous women, 10 parous women, and 8 males) using EndovizX software. Four traces could not be analysed due to software (as described above). No cough had been performed during the remaining excluded studies due to restrictions put in place by infection control during the Covid-19 pandemic. On average cough increased bag pressure by 82.8% (range= 33-232%) compared with pre-cough pressure. The average maximum cough pressure was 103 mmHg (95% CI 92 - 113, range 68-201). When cough pressure was compared between groups of males, nulliparous and parous women, ANOVA showed a statistically significant difference between groups (p= 0.0045, **Figure 8b.6**; see **Appendix 13** for tabulated results). At 130 mmHg, males had the highest mean cough pressure of the three groups, which exceeded the mean of nulliparous females by 36 mmHg (Bonferroni adjusted 95% CI: 8 - 64 mmHg, p=0.0085) and parous females by 37 mmHg (Bonferroni adjusted 95% CI of 7-66 mmHg, p=0.0104). There was no significant difference in mean pressure between nulliparous and parous females (mean difference 1 mmHg, SE estimate= 10.2, p=0.9999).

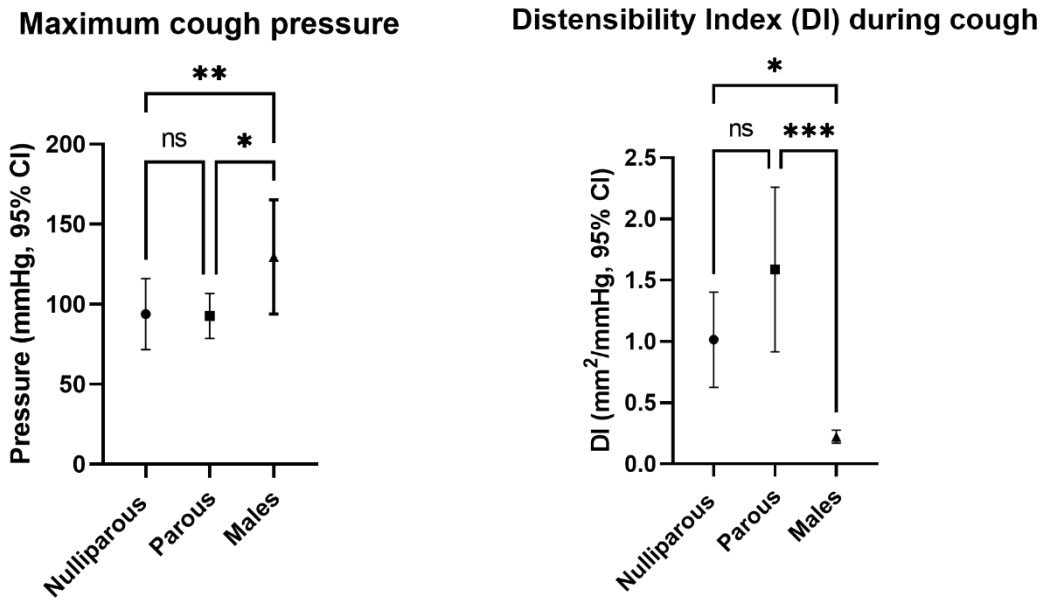


Figure 8b.6 Maximum cough pressure and DI during cough in nulliparous and parous women and men.

Inspection of the colour topography plots showed that the anal canal diameter decreased in all participants during cough, however, unlike during rest and squeeze, bag migration was common during cough (**Figure 8b.7**) (qualitative observation). The average minimum diameter was 9.1 mm (95% CI= 7.8-10.4, range 5.1-15.7). The average DI at the minimum diameter was 1.0 (95% CI 0.7-1.3, range= 0.1-3.0). When cough DI was compared between groups males were found to have the lowest cough DI (0.2 mm^2/mmHg), which was significantly lower than the mean of nulliparous females by 0.8 mm^2/mmHg (Bonferroni adjusted 95% CI: 0.02-1.6 mm^2/mmHg, p=0.0445) and parous females by 1.4 mm^2/mmHg (Bonferroni adjusted 95% CI of 0.5-2.2 mmHg, p=0.0007). There was no significant difference in mean DI between nulliparous and parous females (mean difference -0.6 mm^2/mmHg, SE estimate= 0.3, p=0.1632). There was no statistically significant linear association between DI during cough and age (p=0.6470, R^2=0.73%; 95% CI -0.028 to 0.018).

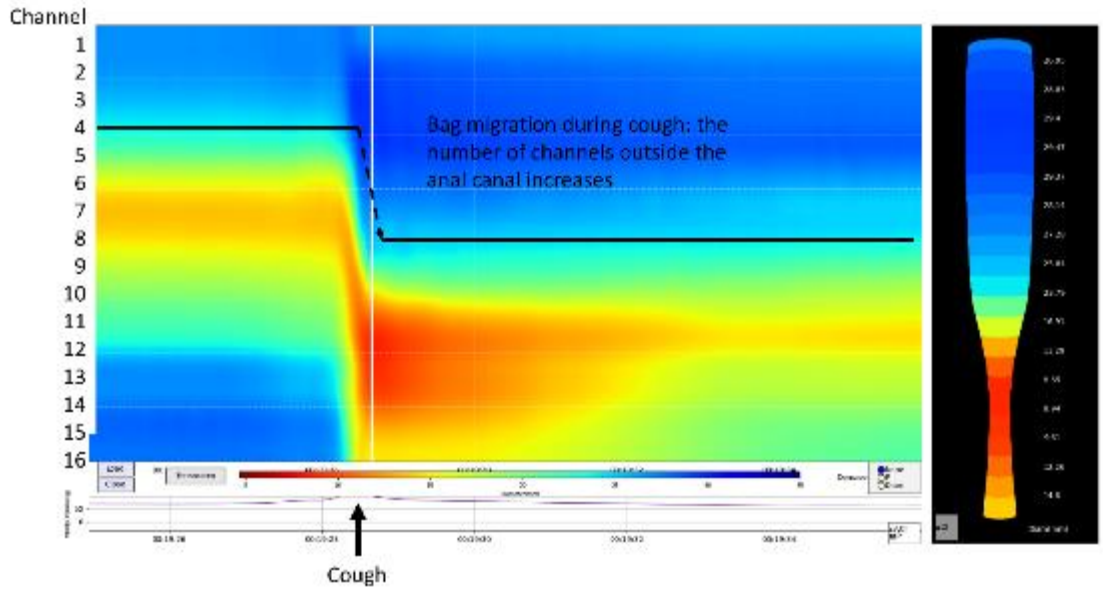


Figure 8b.7 Bag migration during cough occurs because of the bag being 'pushed out' by the force of the cough.

Visual assessment of pressure-radius plots during cough indicated that the minimum diameter at the narrowest part of the anal canal often occurred *after* maximum pressure was reached. Therefore, average DI was also calculated at the narrowest location during maximal pressure rise (DI= 1.0 mm^2/mmHg (95% CI 0.71-1.34, range= 0.11-3.07), however there was no significant difference compared with DI at the narrowest diameter (p=0.8837).

Discussion

Altered stool consistency, rectal reservoir function, and anal sphincter muscle function play important roles in the genesis of FI [327]. Evaluation of the biomechanical properties of gastrointestinal sphincters using FLIP technology to delineate function is gaining popularity [456,462]. Several studies using EndoFLIP in the anal canal have already been published (See Chapter 8a), but normal referance ranges for DI have only been described in women [141]. This chapter described anal canal resistance to opening (during inflation) and DI at rest in a group of 46 HV - the largest HV cohort, including both sexes, studied to date. In addition, the effects of voluntary and involuntary EAS contraction on distensibility were investigated using commercial analytical software. Normal cut-offs were defined based on 5th and 95th percentiles in health, rather than discriminatory ability [141], accounting for gender where appropriate. Consistent with findings by

Gourcerol et al [141] , there was no significant impact of parity on sphincter distensibility in women.

Normal distensibility in HV was characterised by anal canal opening at volumes ≥30 ml and pressures ≥22 mmHg. At 50 ml distension volume, normal resting pressure was ≥48 mmHg with a DI between 0.8 to 4.8 mm^2/mmHg. Squeeze pressure (absolute) should exceed 80 mmHg in men and 69 mmHg in women with distensibility indexes ≤0.6 mm^2/mmHg and ≤2.29 mm^2/mmHg in males and females respectively.

The hourglass configuration of the EndoFLIP bag observed during gradual filling from 0-50 ml is reflective of differences in the visco-elastic and contractile properties of the different muscles which surround the anal canal [462]. We evaluated distensibility at the narrowest location because it is consistently identifiable between participants and has, in theory, the greatest relavance in terms of resistence to flow [461]. Mean anal canal opening volume was 38 ml (95% CI 37 ml – 40 ml), consistent with previous studies which reported anal canal opening at volumes between 30-50 ml in most HV [303,458]. In contrast with previous studies, which saw the anal canal maintain closure at 50 ml in a significant number of HV and FI patients [141,457,458], we were able to determine yield pressure in 98% (45/46) of subjects. In the remaining subject, anal canal opening occurred shortly after inflation was stopped, suggesting that the subject may have been voluntarily contracting the sphincter during inflation. Prolonged squeeze during inflation has been shown to increase the anal canal stiffness [141].

Mean yield pressure in this study was 49 mmHg (95% CI: 45-54 mmHg). Theoretically, low yield pressure increases the potential for gas or faecal leakage to occur during anal sampling (RAIR) or prematurely in response to urge, or during periodic increases in rectal pressure associated with transient anal sphincter relaxations (TASRs) and events like coughing or exercise. The average yield pressure observed in this study was greater than the average rise in rectal pressure (29 mmHg) associated with TASRs [393] and the average rectal pressure associated with coughing (see results in Chapter 6). However, compared with previous studies, yield pressures in this cohort were higher than previously reported in HV [8,303,458]. Furthermore, unlike Alqudah et al [303] who reported opening pressures of 5 and 11 mmHg in men and women, respectively ($p<0.001$), we did not observe a significant difference in opening pressures between males and

females (p=0.270). Yield pressures reported in HV (28.0 ± 1.9 mmHg) and anal cancer patients (15.5 ± 1.3 mmHg) in another study [458] were also below the values observed in our study. However participants in that study were older (~61-62 years on average) and measurements were taken in the middle of the anal canal due to failure of the anal canal to open in many HV; as such reported yield pressures in previous studies were not reflective of results in the stiffest part of the anal canal.

Similarly, we observed higher mean resting pressure (68 mmHg, 95% CI 64-70) than previously reported in older, parous HV (median approx. 50-55 mmHg) [141] using FLIP. Older age is typically associated with a weaker IAS, typified by lower resting tone and muscle thinning [2]. Although resting pressures measured by EndoFLIP and 3D-HRAM were significantly correlated in a previous study [245], it is unclear whether they can be used interchangeably [141,246]. In this cohort, average resting pressure measured by HR-ARM (66 mmHg, 95% CI 64 – 75) was remarkably similar however (see chapter 6). Resting DI (median 1.9 mm^2/mmHg, 5^{th}-95^{th} percentile 0.8-4.8) was slightly higher, but less variable than in HV in the study by Groucerol et al [141] (median 1.5 mm2/mmHg, range 0.3–10.4). Compared with the results of FI patients (3.9 mm2/mmHg, range 0.7–12.1) DI in the HV in this study was lower consistent with previous studies [243-245]. FI patients tend to be older with lower resting pressures [167]. We observed a statistically significant (p=0.02) impact of age on resting DI with an average increase of 0.4 units (95% CI 0.1-0.7) for every 10 years of age. At 40 ml distension volume, no such association between resting DI and age was observed in the study by Gourcerol et al [141]. Given these conflicting findings, further studies are required.

Both voluntary squeeze and coughing resulted in an increase in EndoFLIP bag pressure (102 mmHg and 103 mmHg, respectively), which was associated with narrowing of the anal canal in all subjects. However, because the EndoFLIP does not provide any anatomical reference for bag placement, the active muscle (EAS, puborectalis, or both [243,461]) affecting distensibility cannot be reliably determined. Nevertheless, compared to rest, voluntary and involuntary EAS contraction increased anal canal stiffness. Squeeze DI in males was significantly lower than that in parous females, but not nulliparous women. Squeeze DI in parous women (1.1 mm^2/mmHg, 5^{th} to 95^{th} percentiles 0.2- 2.4) in this study was lower than that reported previously in older

healthy subjects (1.7 mm^2/mmHg, range 0.2–6.2) [141], but did not differ significantly from nulliparous women of a similar age.

We evaluated the effects of cough on DI in those participants whose studies were not affected by restrictions due to the Covid-19 pandemic. While results indicate cut-offs for normal DI (95^{th} percentile) of 0.3 in men and 2.9 in women ($p<0.05$), the clinical relevance of cough DI has not been established. We have also demonstrated previously that the muscular (pressure) response to coughing is impacted by cough effort, which was not controlled for during EndoFLIP. Therefore the increased anal canal stiffness observed in males compared to females may have occurred as a result of greater cough strength, differences in baseline resting tone, or greater pelvic floor contractile strength. Furthermore, bag migration during coughing at 50 ml was present in a significant number of subjects. While the use of analytical software enabled easy identification of changes in narrow zone location, we suggest that simpler measures, such as the degree of anal canal closure (expressed as a percentage of the maximum possible closure at a given location i.e. pre-cough diameter minus probe width) may be more useful to demonstrate the impact/effects of reflex activity on the anal canal than DI.

In this chapter, data analysis was performed using a combination of manual processing of raw data and commercial EndovizX software (Motilityviz). The main benefit of the EndovizX software compared to manual analysis is the ability to very quickly visualise the overall impression of the study (with regard to the protocol performed) and to observe changes in diameter across the full length of the anal canal at once. Analyses of different anal canal regions (proximal, mid/narrowest, distal) can therefore be more easily performed. The main benefit of EndoVizX was in identifying problems with study quality, pressure or diameter artefacts, and bag migration (e.g. during coughing). While distensibility during inflation and at rest yielded comparable results irrespective of the analytical method (manual or software) used, the use of commercial software was faster and less prone copy-paste errors while handling data in Excel. However, care must be taken in selecting the appropriate field of interest. To avoid bias, analysis was based on minimum/maximum DI rather than average DI. This may have resulted in small individual differences between software and manual approaches, but did not affect

results overall. With more experience using the software, its functionality will become more familiar; grasping the basics was straighforward and, based on my own experience, the program was user friendly albeit a bit slow.

Limitations

The limitations pertaining to testing performed in HV overall has been discussed in previous chapters. Regarding analysis of EndoFLIP, we based the assessment of DI on the narrowest location for reasons already discussed. To evaluate both opening pressure and DI, the 50 ml distension volume was evaluated. Normal cut-offs for DI (1 mm^2/mmHg at rest and 0.7 mm^2/mmHg during squeeze) been reported previously for 40 ml distension volume as sensitivity and specificity for differentiating between health and FI were greatest at this volume [141]. However, opening pressures could not be determined in many participants. Meanwhile others have shown that using a combination of distensibility parameters measured at 40 and 50 ml yielded best results [246].

Our results indicated that ageing may be related to an increase in DI. However, participants in this study were relatively young compared with the typical age when FI occurs and we were only able to recruit a small number of elderly HV (for reasons discussed in previous chapters). Despite the number of HV included being the largest ever published overall, the number of individuals in groups males, nulliparous and parous females was small. Further recruitment is needed to expand these data. Extreme values may have had a large effect on some measurements.

Resting pressure and DI was evaluated in the last 15 seconds before squeeze with the participant relaxed. During this period, resting pressure continued to decrease suggesting that a period of stabilisation is required before the measurement; both pressure and diameters should be stable before evaluating DI. In the current study, measurements were made after diameters were considered stable. For this reason, DI was calculated using median CSA and median pressure throughout the resting period.

Finally, our analysis of squeeze and cough manouevres leaves room for improvement. Recently, Zifan et al [465] demonstrated that pressure radius loops in FI patients are located more to the right that HV. Such a shift may be interpreted as muscle weakness,

similar to the interpretation of cardiac loops in heart disease. Anecdotally, we also observed a shift in pressure-radius loops to the right at channels closer to the proximal and distal edges of the catheter compared to the middle/narrowest channels; areas with higher distensibility thus tended to shift to the left. However, we were unable to quantify these observations and present them in a meaningful way at 'group level' using currently available tools at our disposal. It is likely that data from EndoFLIP can be manipulated in many ways to further describe anal canal biomechanics, however not all considered measures are transferable into clinical practice according to the aims of this chapter.

Conclusion

The DI measured at the narrowest anal canal diameter has been proposed as viable alternative for assessing anal sphincter competence with comparable ability to differentiate between HV and FI patients to 3D-HRAM [141,461,465]. This chapter described anal canal distensibility in 46 HV and considered the effects of participant demographics on DI; cut-offs for normal function are also described. Based on our findings, participant age, but not gender, parity or BMI, influenced resting DI. However, the impact of age was small and subject to high variability. Conversely, male gender, but not parity, age or BMI influenced DI during voluntary (and involuntary) contraction. Anal canal yield pressure and volume were similar between all groups and could be determined in most of the participants. Actively resisting anal canal opening increases anal canal stiffness and may prevent flow in this way [141] however, these effects may be shortlived and may not result in the desired action (of pushing contents orally). During inflation, participants should be actively reminded to relax the anal canal to avoid false representation of the pressure required to enable flow. Future studies in FI patients are required to evaluate the clinical utility of DI using the cut-offs presented herein.

Discussion

Thesis overview

This thesis focussed on the evaluation of anorectal function in healthy volunteers (HV) and in patients with faecal incontinence (FI).

The aims of this thesis were:

- to investigate the importance of traditional manometric variables/diagnoses in different patient groups with particular focus on gender, parity, and less recognised forms of FI;
- to further understanding of anorectal function by contemporary and novel investigation tools through expansion of normal ranges and development of novel metrics;
- to develop understanding of (the role of) parity on anorectal function in health and FI;
- to consider the interaction between components of continence in health, with a view of furthering understanding of the multifactorial pathophysiological nature of FI.

The specific objectives of this thesis were:

- to determine the relative prevalence of major disorders of anorectal function (hypotonia and hypocontractility, hyper- and hyposensitivity) in men and women;
- to describe the prevalence, symptoms, and pathophysiology of stress FI;
- to evaluate the role of the cough response in health and FI using HR-ARM;
- to generate novel measures of function and assess the impact of parity on previously under-reported measures of anal sphincter function (cough and anal slow waves);
- to generate or expand knowledge of normal ranges in health with regards to gender, age and parity using novel and established investigation methods, with view of adopting them into clinical practice;
- to investigate the interactions between tests of anorectal function in health.

Summary of findings

Chapter 2

A systematic review of literature was performed to determine the number of adequately controlled studies reporting on the prevalence of major classes of anal and rectal dysfunction. Meta-analysis was performed to establish pooled prevalence of anal hypotonia/hypocontractility and rectal hyper-/hyposensitivity in males and females with FI.

These results conveyed clear gender disparity in the rates of sphincter barrier and rectal sensory dysfunction. Poor voluntary sphincter control was the most prevalent abnormality observed, especially in women. However, the number of appropriately controlled studies was small and few were judged as having low risk of bias. Consistent technique and definition of normal improved certainty of diagnosis (e.g. hyposensitivity), but overall wide confidence intervals and high levels of heterogeneity were observed. This indicates the need for large-scale prospective studies to be performed using a standardised protocol (e.g. the IAPWG protocol [146]) and calls for a collective effort to harmonise practice.

Chapter 3

Based on clinical experience, at least a proportion of patients with FI complain of leakage associated with coughing, sneezing, or exercise. However, stress FI has received little attention in FI literature. To describe the number and type of studies on stress FI available in the literature, explore the definition and prevalence of stress FI reported, describe what was already known about risk factors and pathophysiology associated with stress FI, and explore the relationship between stress FI and the cough response, a literature review was performed.

Contrary to the body of literature on SUI, there was a marked paucity of information available pertaining to stress FI. Further research is needed to understand the relationship between anal canal closure, cough response and stress FI. Although there is sound theoretical basis for the occurrence of stress FI in men and women, few studies have addressed the prevalence and pathophysiology of the condition. In studies which have assessed these, the number of stress FI patients was small [281,292,293], and patient

selection was based on unstandardised definitions. Physiological assessment methods were often poorly described.

Investigation of phenotypes, such as stress FI, is important for understanding FI as the pathophysiology may be different from other types of incontinence [293]. Furthermore, optimising characterisation of different types of FI could improve existing treatment outcomes and develop novel treatment modalities tailored specifically to FI phenotypes [293].

Based on these findings, a retrospective case-control study of FI patients with and without stress FI was conducted using a large database of prospectively collected data from patients investigated for bowel symptoms in the GI Physiology Unit, at the Royal London Hospital between January 2004 and March 2016.

Stress FI was present in over a quarter of FI patients and represented a more severe FI phenotype overall (higher SMIS, more frequent FI, longer duration of symptoms, more likely to have mixed symptoms, more flatus incontinence, and more co-existence) compared to non-stress incontinent controls. Although stress FI patients had more hypocontractility, pathophysiology was only modestly different to controls overall. Based on the importance of a neurologically intact pelvic floor in SUI, it was suggested that future studies should determine anal sphincter response to coughing by incorporating electrophysiological measurements with manometry.

Chapter 4

Chapter 4 describes the findings of a retrospective cohort study which aimed to qualitatively and quantitatively study differences in cough response measured by HR-ARM in health and FI and to investigate the effects of parity on findings.

Using existing data from HV studies performed in previous years and routinely collected patient data, the results of this study presented a promising basis for interpreting cough clinically, recognising that future prospective studies are needed to fully understand its potential. Furthermore, we observed that in-depth analysis of the cough-anorectal reflex, an under-utilised yet routinely performed manoeuvre, appears to have the potential to identify subclinical sphincter dysfunction in parous and in faecally incontinent women compared to asymptomatic nulliparous women. These results present the opportunity to reconsider HR-ARM not only as an "expensive hobby"[392], but as an important tool for identification of at risk individuals in whom preventive measures may serve to halt progression of subclinical anal dysfunction into life-altering disease. Where FI symptoms are already established, evaluation of sphincter function with a dynamic manoevre like cough, which challenges the sphincter barrier response, may be more clinically valid than static measures.

Chapter 5

In chapter 5, a similar retrospective cohort study (to above) was performed to compare anal slow-wave (SW) amplitude at various frequencies between healthy nulliparous and parous women and patients with FI using wavelet analysis. In addition, the direction of propagation at each frequency was determined and feasibility to distinguish between study groups using manometric measures of anal tone other than resting pressure was evaluated.

Wavelet analysis represents a novel, computational method for analysing anal SW captured by HR-ARM. The dominant frequency demonstrated in this study (16 cpm) agreed with findings from conventional manometry [192] and animal studies[406]. This analysis indicated that SW amplitude was reduced in female FI patients compared to both healthy nulliparous and parous women. Childbirth also appears to reduce SW

amplitude in comparison to nulliparous women. Retrograde propagation observed at ~16 cpm may represent an important physiological mechanism to 'clean' the anal canal and help maintain continence.

Chapter 6

Chapter 6 described the major prospective research project undertaken as part of the this thesis. This single centre, observational study in health aimed to define normal ranges for emerging techniques (EndoFLIP, Rapid Barostat) based on a study of asymptomatic volunteers and to expand existing normal datasets for HR-ARM and tests of anal and rectal sensation. Further aims included investigation of the effects of gender, parity and age on anorectal function studies, a comparision of rapid barostat with gold standard electromechanical barostat, and to qualitatively/quantitatively decribe previously undocumented physiological phenomena/novel functional parameters in health.

A description of the study design and investigations performed was provided as well as the results of 'established' tests of anorectal function including HR-ARM, surface EMG, endoanal ultrasound, anal electromucosal sensitivity, and the balloon expulsion test on prelinary data available in 51 HV. These data will contribute to existing normative datasets. If the study is able to resume recruitment following stoppage due to COVID-19, greater use of social media platforms may help drive numbers as well as promoting research into bowel function, an often stigmatised research field. Based on acceptability scores from participants, there were no issues with the study design/protocol, which need addressing. In general, once through the door, partcipants were happy to take part and not bothered by the procedures (a source of hesitation for many).

Chapter 7

The first part of Chapter 7 (7a), compared measures of compliance, capacity and sensory thresholds to distension in health using the RBB pump (Mui Scientific, Canada) and the standard electromechanical barostat.

The RBB pump, was developed to facilitate routine measurement of compliance, capacity, and rectal sensation. Despite clinically insignificant differences between mean

measurements by standard barostat and RBB pump, we demonstrated that wide LoA suggesting that the two methods do not provide entirely comparable measures of compliance (i.e. elasticity). Barostat assessment (of compliance) complements the clinical investigation of FI. However, the term "compliance" is sometimes used more simply to imply a functional measurement made on the rectum [207]. In this sense, we proposed that the RBB showed excellent intra- and inter-rater variability supporting its routine clinical use to measure rectal function so long as the 'manual slope' method was used.

Measurement of capacity and sensory thresholds using the RBB pump showed similar results when compared with standard barostat, but wide CI of the LoA indicated that, once again, the methods (and any existing normal ranges based on standard barostat) were not interchangeable. While the RBB may be clinically useful as an alternative for balloon distension, sensory thresholds are clearly markedly different to previously reported thresholds supporting the need to define normal cut-offs for clinical purposes and further assessment of how sex, age, parity, and body mass index may increase inter-individual variability.

Therefore, in the second part of the Chapter (7b), normal ranges for rectal sensorimotor function in health using the RBB pump (and accounting for any influence of gender, parity, age, and BMI on values) were established. Using the 'manual slope' method to assess compliance based on raw data analysis, we compared these results with automated processes (RBB pump reported values). Finally, the repeatability of compliance and capacity was assessed based on the conditioning and index rounds of filling.

Current methods to assess rectal biomechanical properties and sensory perception are subject to either limited availability and feasibility in the clinical setting (standard barostat) or alternatively, have inherent limitations owing to the material characteristics of equipment and limited procedural control (balloon distension). The RBB pump has been introduced recently to fulfil the need for routine, bed-side assessment of compliance and capacity and represents the 'middle ground' between existing tools. From a usability point of view, the RBB pump is simple to use and provides the opportunity for rapid analysis and reporting. In this Chapter, we presented normal

ranges for compliance, capacity, and sensory thresholds in 50 HV, the largest study of health to be performed to our knowledge. While these initial results require validation in future studies and by others, they represent the first step toward implementation of this technology within routine clinical practice.

Chapter 8

The final chapter of this thesis concerned the results of endoanal application of the functional lumen imaging probe (EndoFLIP), the principles and analysis of which were described in Chapter 8a based a review of existing literature. Published studies have consistently shown that anal canal function, measured using EndoFLIP, differs between FI patients and HV. For example, FI patients appear to have higher distensibility [141,243,244,246,454,465], earlier anal canal opening [463], and greater degrees of muscular damage [465]. Compared to manometry, EndoFLIP has been shown to have greater sensitivity in differentiating between HV and FI patients [141] and has greater potential to identify positive changes following treatment [458]. Resting and squeeze pressures measured by EndoFLIP have been shown to correlate well with manometry [141,245] with EndoFLIP correctly identifying anal weakness in up to 70% of subjects leading some to suggest that manometry and EndoFLIP may be used interchangeably [245]. Others have stressed that while 3D-HDAM and EndoFLIP perform in a similar manner for diagnosing FI, they are not complementary [246]. However, with up to 80% of female FI patients shown to have an abnormally distensible anal sphincter in one study [245], we asked whether the existing normal cut-offs, based on thresholds which best differentiated between health and DI were too forgiving?

Thus in Chapter 8b, we aimed to describe anal canal distensibility during inflation, at rest and during voluntary contraction in prospectively collected data from healthy subjects and determined normal ranges, based on calculation of the 5^{th} and 95^{th} percentiles and accounting for any differences in participant demographics. In addition to analysing raw date manually, these analyses were supported by and compared with analysis performed with the aid of commercial software (EndovizX, Motilityviz).

The distensibility index (DI) was analysed in 46 HV and the effect of effect of participant demographics on DI explored describing cut-offs for normal function for the group as a while because gender, parity and BMI were not found to influence resting DI; a

significant but small effect of age was observed but subject to high variability. Conversely, male gender, but not parity, age or BMI influenced DI during voluntary (and involuntary) contraction. Anal canal yield pressure and volume were similar between all groups and could be determined in most of the participants. During inflation, participants should be actively reminded to relax the anal canal to avoid false representation of the pressure required to enable flow. Future studies in FI patients are required to evaluate the clinical utility of DI using the cut-offs presented.

Future studies

It goes without saying that future research using the novel technologies and metrics evaluated in this thesis should be performed in FI patients and other forms of GI dysfunction (e.g. constipation and IBS). Only then can we understand the utility of the normative data presented throughout this thesis. Thanks to the recently published IAWPG consensus and London classification for disorders of anorectal function, great steps forward toward collaborative, standardised, international research have already been taken. The multimodal study design for evaluating anorectal function in health successfully implemented in this thesis could provide a blue-print for future studies. While the extensive time and equipment required may not be available to all, the materials are provided and data processing (albeit time-consuming) are sufficiently simple to be repeated without the need for custom code or specialist software. For some of these tests (HR-ARM, electromucosal sensitivity, BET), the results from the current study should be amalgamated with existing HV datasets to begin compiling the large normative datasets, stratified or not, called for numerously in the past [2,167].

One of the principle aims of this thesis was to to consider the interaction between components of continence in health, with a view of furthering understanding of the multifactorial pathophysiological nature of FI. Given the unexpected amount of time required to manually process raw data and to validate novel metrics/technology, there is much left to be done in terms of fulfilling this aim. In the first instance, analyses to look at associations between resting and squeeze pressure on HR-ARM with EndoFLIP (pressures and DI). Next, we should aim to determine the whether rectal sensory thresholds by RBB, standard barostat and balloon distension correlate with each other as well as bowel sensations associated with the need to open bowels based on the

information in bowel diarries of healthy volunteers. Finally, “continence webs” (spider plots) which describe the results in each individual, perhaps in relation to other’s results (rank) should be constructed. While an individual functional result may fail to differentiate between groups of healthy or diseased subjects, perhaps there are patterns associated with the overall picture of continence; collectively, are the findings in a single individual generally always low/high or do they vary so that one continence mechanism compensates for another?

Concluding thoughts

The diagnosis of FI is made on the basis of presenting symptoms which may range from small amounts of faecal soiling or uncontrolled flatulence to loss of entire solid motions. Often, only those with the “worst” symptoms reach evaluation by a specialist and only in a proportion of those patients are diagnostic investigations performed. Even so, limited functional tests (e.g. those restricted to evaluation of resting and squeeze pressures at a single location in the anal canal) are often entirely normal owing to the wide range of variability observed in both health and disease. However, performing an increasing number of assessments enhances the diagnostic yield of anorectal functional testing [9], often revealing a range of co-existent abnormalities with shared pathoaetiology. Due to the multifactorial nature of FI, multiple tests are often required to identify or exclude suitable surgical targets (only the starting point of decision making). Nevertheless, the clinical utility of functional tests remains questionable and and we criticise studies that have been conducted for being poorly controlled and performed in limited numbers. However, the paucity of data comes as no surprise; as I have learned over the course of this PhD, performing original research in this field, especially in healthy volunteers is incredibly difficult.

Incontinence, whether urinary, faecal, or flatus in nature, is still a taboo in society much like other symptoms related to bowel function. Often suffered in silence, many patients lack the words or courage to seek assistance for their symptoms and many clinicians lack the knowledge of treatment options to provide care, or to refer on early [469]. The mystery and stigma related to the lower bowel are such that even those without any problems with continence seem to worry about faeces almost as much as they worry about exposing such a vulnerable and private part of their anatomy.

Normal bowel function may be an imaginary concept since the range of values (and bathroom expriences) appears to vary widely in health no matter what the analytical target. Identifying quantifiable biomarkers capable of revealing subclinical pathophysiology in individuals at risk of developing FI and expanding on these in future prospective studies was the dream. Childbirth is the biggest risk factor for FI in women attending for investigations in tertiary sector care [6]. In the absence of obstetric anal sphincter injury, women tend to present many years after the initial event, typically a seemingly 'normal' vaginal delivery. Identifying those individuals due to develop bowel dysfunction following enough 'hits' earlier [405], could save time, money, and potentially even lives [470]. It is not known exactly why the novel measures identified as potential biomakers in retrospective analyses failed to show significance prospectively. No doubt, the limited number of nulliparous and parous volunteers recruited played some part, with the number of analysable coughs further reduced in parous women by the sheer irony of trying to study the effect of coughing on anorectal function during a global pandemic.

References

1. O'Connell PR, Knowles CH, Maeda Y, et al. Surgery for Faecal Incontinence. In: Abrams P, Cardozo L, Wagg A, Wein A, eds. *Incontinence.* 6th ed.: ICUD ICS 2016; 2016:2087-2142.
2. Carrington EV, Scott SM, Bharucha A, et al. Expert consensus document: Advances in the evaluation of anorectal function. *Nature reviews Gastroenterology & hepatology.* 2018;15(5):309-323.
3. Bharucha AE, Dunivan G, Goode PS, et al. Epidemiology, pathophysiology, and classification of fecal incontinence: state of the science summary for the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) workshop. *Am J Gastroenterol.* 2015;110(1):127-136.
4. Rao SS, Committee ACoGPP. Diagnosis and management of fecal incontinence. American College of Gastroenterology Practice Parameters Committee. *Am J Gastroenterol.* 2004;99(8):1585-1604.
5. Carrington EV, Brokjaer A, Craven H, et al. Traditional measures of normal anal sphincter function using high-resolution anorectal manometry (HRAM) in 115 healthy volunteers. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2014;26(5):625-635.
6. Lunniss PJ, Gladman MA, Hetzer FH, Williams NS, Scott SM. Risk factors in acquired faecal incontinence. *J R Soc Med.* 2004;97(3):111-116.
7. Sun WM, Donnelly TC, Read NW. Utility of a combined test of anorectal manometry, electromyography, and sensation in determining the mechanism of 'idiopathic' faecal incontinence. *Gut.* 1992;33(6):807-813.
8. Haas S, Liao D, Gregersen H, Lundby L, Laurberg S, Krogh K. Increased yield pressure in the anal canal during sacral nerve stimulation: a pilot study with the functional lumen imaging probe. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2017;29(2).
9. Scott SM, Gladman MA. Manometric, sensorimotor, and neurophysiologic evaluation of anorectal function. *Gastroenterology clinics of North America.* 2008;37(3):511-538, vii.
10. Thorson AG. Anorectal physiology. *The Surgical clinics of North America.* 2002;82(6):1115-1123.
11. Rasmussen OO. Anorectal function. *Diseases of the colon and rectum.* 1994;37(4):386-403.
12. Knowles CH. Human studies of anorectal sensory function. *Irish journal of medical science.* 2018;187(4):1143-1147.
13. Irving MH, Catchpole B. ABC of colorectal diseases. Anatomy and physiology of the colon, rectum, and anus. *Bmj.* 1992;304(6834):1106-1108.
14. Jorge J, Habr-Gama A. Anatomy and Embryology of the Colon, Rectum and Anus. In: Beck D, Nasseri Y, Hull T, et al., eds. *The ASCRS Manual of the Colon & Rectal Surgery.* New York: Springer New York; 2014:1-25.
15. Christiansen J, Sorensen M, Rasmussen OO, Emblem R, Sjodahl R. Ano-rectal physiology measurements. *Eur J Surg Suppl.* 1991(564):73-74.
16. Salerno G, Sinnatamby C, Branagan G, Daniels IR, Heald RJ, Moran BJ. Defining the rectum: surgically, radiologically and anatomically. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland.* 2006;8 Suppl 3:5-9.

17. Martini F, Bartholomew E. *Essentials of anatomy and physiology.* 7th ed: Askews and Holts; 2016.
18. Knowles CH, Aziz Q. Basic and clinical aspects of gastrointestinal pain. *Pain.* 2009;141(3):191-209.
19. Siri S, Maier F, Santos S, Pierce DM, Feng B. Load-bearing function of the colorectal submucosa and its relevance to visceral nociception elicited by mechanical stretch. *Am J Physiol Gastrointest Liver Physiol.* 2019;317(3):G349-G358.
20. Furness JB. The enteric nervous system and neurogastroenterology. *Nature reviews Gastroenterology & hepatology.* 2012;9(5):286-294.
21. Stoker J. Anorectal and pelvic floor anatomy. *Best practice & research Clinical gastroenterology.* 2009;23(4):463-475.
22. GOLIGHER JC, HUGHES ES. Sensibility of the rectum and colon. Its rôle in the mechanism of anal continence. *Lancet.* 1951;1(6654):543-547.
23. Chan CL, Lunniss PJ, Wang D, Williams NS, Scott SM. Rectal sensorimotor dysfunction in patients with urge faecal incontinence: evidence from prolonged manometric studies. *Gut.* 2005;54(9):1263-1272.
24. Sinnatamby C. *Last's Anatomy.* Churchchill Livingstone; 2011.
25. Lynn PA, Olsson C, Zagorodnyuk V, Costa M, Brookes SJ. Rectal intraganglionic laminar endings are transduction sites of extrinsic mechanoreceptors in the guinea pig rectum. *Gastroenterology.* 2003;125(3):786-794.
26. Ng KS, Brookes SJ, Montes-Adrian NA, Mahns DA, Gladman MA. Electrophysiological characterization of human rectal afferents. *Am J Physiol Gastrointest Liver Physiol.* 2016;311(6):G1047-G1055.
27. Chan CL, Ponsford S, Scott SM, Swash M, Lunniss PJ. Contribution of the pudendal nerve to sensation of the distal rectum. *The British journal of surgery.* 2005;92(7):859-865.
28. Kumar D, Waldron D, Williams NS, Browning C, Hutton MR, Wingate DL. Prolonged anorectal manometry and external anal sphincter electromyography in ambulant human subjects. *Dig Dis Sci.* 1990;35(5):641-648.
29. Swash M. Faecal incontinence. *Bmj.* 1993;307(6905):636-637.
30. Vollebregt PF, Rasijeff AMP, Pares D, et al. Functional anal canal length measurement using high-resolution anorectal manometry to investigate anal sphincter dysfunction in patients with fecal incontinence or constipation. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2019;31(3):e13532.
31. MORGAN CN, THOMPSON HR. Surgical anatomy of the anal canal with special reference to the surgical importance of the internal sphincter and conjoint longitudinal muscle. *Ann R Coll Surg Engl.* 1956;19(2):88-114.
32. Mahadevan V. Anatomy of the Rectum and Anal canal. *Surgery (Oxford).* 2020;38(1):7-11.
33. Thomson H. The anal cushions--a fresh concept in diagnosis. *Postgrad Med J.* 1979;55(644):403-405.
34. DUTHIE HL, GAIRNS FW. Sensory nerve-endings and sensation in the anal region of man. *The British journal of surgery.* 1960;47:585-595.
35. GOLIGHER JC, LEACOCK AG, BROSSY JJ. The surgical anatomy of the anal canal. *The British journal of surgery.* 1955;43(177):51-61.
36. Keef KD, Cobine CA. Control of Motility in the Internal Anal Sphincter. *Journal of neurogastroenterology and motility.* 2019;25(2):189-204.
37. Uz A, Elhan A, Ersoy M, Tekdemir I. Internal anal sphincter: an anatomic study. *Clin Anat.* 2004;17(1):17-20.

38. Nielsen MB, Hauge C, Rasmussen OO, Sørensen M, Pedersen JF, Christiansen J. Anal sphincter size measured by endosonography in healthy volunteers. Effect of age, sex, and parity. *Acta Radiol.* 1992;33(5):453-456.
39. Aronson MP, Lee RA, Berquist TH. Anatomy of anal sphincters and related structures in continent women studied with magnetic resonance imaging. *Obstet Gynecol.* 1990;76(5 Pt 1):846-851.
40. Johnson MA, Polgar J, Weightman D, Appleton D. Data on the distribution of fibre types in thirty-six human muscles. An autopsy study. *J Neurol Sci.* 1973;18(1):111-129.
41. Nockolds CL, Hosker GL, Kiff ES. Fatigue rate of the external anal sphincter. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland.* 2012;14(9):1095-1100.
42. Lexell J, Henriksson-Larsén K, Winblad B, Sjöström M. Distribution of different fiber types in human skeletal muscles: effects of aging studied in whole muscle cross sections. *Muscle Nerve.* 1983;6(8):588-595.
43. Mittal RK, Bhargava V, Sheean G, Ledgerwood M, Sinha S. Purse-string morphology of external anal sphincter revealed by novel imaging techniques. *Am J Physiol Gastrointest Liver Physiol.* 2014;306(6):G505-514.
44. Raizada V, Bhargava V, Karsten A, Mittal RK. Functional morphology of anal sphincter complex unveiled by high definition anal manometery and three dimensional ultrasound imaging. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2011;23(11):1013-1019, e1460.
45. Gagnard C, Godlewski G, Prat D, Lan O, Cousineau J, Maklouf Y. The nerve branches to the external anal sphincter: the macroscopic supply and microscopic structure. *Surg Radiol Anat.* 1986;8(2):115-119.
46. Rao SS. Pathophysiology of adult fecal incontinence. *Gastroenterology.* 2004;126(1 Suppl 1):S14-22.
47. Bharucha AE. Pelvic floor: anatomy and function. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2006;18(7):507-519.
48. Parks AG, Swash M, Urich H. Sphincter denervation in anorectal incontinence and rectal prolapse. *Gut.* 1977;18(8):656-665.
49. Neill ME, Swash M. Increased motor unit fibre density in the external anal sphincter muscle in ano-rectal incontinence: a single fibre EMG study. *J Neurol Neurosurg Psychiatry.* 1980;43(4):343-347.
50. Kiff ES, Swash M. Slowed conduction in the pudendal nerves in idiopathic (neurogenic) faecal incontinence. *The British journal of surgery.* 1984;71(8):614-616.
51. Bannister JJ, Gibbons C, Read NW. Preservation of faecal continence during rises in intra-abdominal pressure: is there a role for the flap valve? *Gut.* 1987;28(10):1242-1245.
52. Bartolo DC, Roe AM, Locke-Edmunds JC, Virjee J, Mortensen NJ. Flap-valve theory of anorectal continence. *The British journal of surgery.* 1986;73(12):1012-1014.
53. Musial F, Crowell MD. Rectal adaptation to distention: implications for the determination of perception thresholds. *Physiol Behav.* 1995;58(6):1145-1148.
54. Arhan P, Faverdin C, Persoz B, et al. Relationship between viscoelastic properties of the rectum and anal pressure in man. *Journal of applied physiology.* 1976;41(5 Pt. 1):677-682.
55. Lunniss P, SM S. Pathophysiology of Anal Incontinence. In: Sultan A, Thakar R, Fenner D, eds. *Perineal and Anal Sphincter Trauma.* London: Springer; 2009:89-101.
56. Broens P, Vanbeckevoort D, Bellon E, Penninckx F. Combined radiologic and manometric study of rectal filling sensation. *Diseases of the colon and rectum.* 2002;45(8):1016-1022.

57. Kendall GP, Thompson DG, Day SJ, Lennard-Jones JE. Inter- and intraindividual variation in pressure-volume relations of the rectum in normal subjects and patients with the irritable bowel syndrome. *Gut.* 1990;31(9):1062-1068.
58. Ihre T. Studies on anal function in continent and incontinent patients. *Scand J Gastroenterol Suppl.* 1974;25:1-64.
59. Sangwan YP, Coller JA, Schoetz DJ, Roberts PL, Murray JJ. Spectrum of abnormal rectoanal reflex patterns in patients with fecal incontinence. *Diseases of the colon and rectum.* 1996;39(1):59-65.
60. Chan CL, Ponsford S, Swash M. The anal reflex elicited by cough and sniff: validation of a neglected clinical sign. *J Neurol Neurosurg Psychiatry.* 2004;75(10):1449-1451.
61. Jorge JM, Wexner SD. Etiology and management of fecal incontinence. *Diseases of the colon and rectum.* 1993;36(1):77-97.
62. Azpiroz F, Enck P, Whitehead WE. Anorectal functional testing: review of collective experience. *Am J Gastroenterol.* 2002;97(2):232-240.
63. Whitehead W, Schuster MM. *Gastrointestinal disorders: behavioral and physiological basis for treatment. .* Orland, Florida, USA: Academic Press; 1985.
64. Perry S, Shaw C, McGrother C, et al. Prevalence of faecal incontinence in adults aged 40 years or more living in the community. *Gut.* 2002;50(4):480-484.
65. Ditah I, Devaki P, Luma HN, et al. Prevalence, trends, and risk factors for fecal incontinence in United States adults, 2005-2010. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association.* 2014;12(4):636-643.e631-632.
66. Sharma A, Yuan L, Marshall RJ, Merrie AE, Bissett IP. Systematic review of the prevalence of faecal incontinence. *The British journal of surgery.* 2016;103(12):1589-1597.
67. Aitola P, Lehto K, Fonsell R, Huhtala H. Prevalence of faecal incontinence in adults aged 30 years or more in general population. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland.* 2010;12(7):687-691.
68. Vollebregt PF, Wiklendt L, Dinning PG, Knowles CH, Scott SM. Coexistent faecal incontinence and constipation: A cross-sectional study of 4027 adults undergoing specialist assessment. *EClinicalMedicine.* 2020;27:100572.
69. Brochard C, Chambaz M, Ropert A, et al. Quality of life in 1870 patients with constipation and/or fecal incontinence: Constipation should not be underestimated. *Clin Res Hepatol Gastroenterol.* 2019;43(6):682-687.
70. Andrews CN, Bharucha AE. The etiology, assessment, and treatment of fecal incontinence. *Nat Clin Pract Gastroenterol Hepatol.* 2005;2(11):516-525.
71. Lestar B, Penninckx F, Kerremans R. The composition of anal basal pressure. An in vivo and in vitro study in man. *International journal of colorectal disease.* 1989;4(2):118-122.
72. Engel AF, Kamm MA, Bartram CI, Nicholls RJ. Relationship of symptoms in faecal incontinence to specific sphincter abnormalities. *International journal of colorectal disease.* 1995;10(3):152-155.
73. Vaizey CJ, Kamm MA, Bartram CI. Primary degeneration of the internal anal sphincter as a cause of passive faecal incontinence. *Lancet.* 1997;349(9052):612-615.
74. Felt-Bersma RJ, Klinkenberg-Knol EC, Meuwissen SG. Anorectal function investigations in incontinent and continent patients. Differences and discriminatory value. *Diseases of the colon and rectum.* 1990;33(6):479-485; discussion 485-476.
75. Prichard D, Harvey DM, Fletcher JG, Zinsmeister AR, Bharucha AE. Relationship Among Anal Sphincter Injury, Patulous Anal Canal, and Anal Pressures in Patients With Anorectal Disorders. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association.* 2015;13(10):1793-1800.e1791.

76. Abrams P, Cardozo L, Wein A. The International Consultation on Incontinence, Research Society (ICI-RS). *Neurourology and urodynamics.* 2010;29(4):596-597.
77. ES K. Faecal Incontinence. *Bmj.* 1992;305:702-704.
78. Deutekom M, Dobben AC, Terra MP, et al. Clinical presentation of fecal incontinence and anorectal function: what is the relationship? *Am J Gastroenterol.* 2007;102(2):351-361.
79. Desprez C, Turmel N, Chesnel C, et al. Comparison of clinical and paraclinical characteristics of patients with urge, mixed, and passive fecal incontinence: a systematic literature review. *International journal of colorectal disease.* 2020.
80. Larsson C, Hedberg CL, Lundgren E, Söderström L, TunÓn K, Nordin P. Anal incontinence after caesarean and vaginal delivery in Sweden: a national population-based study. *Lancet.* 2019;393(10177):1233-1239.
81. Whitehead WE, Simren M, Busby-Whitehead J, et al. Fecal Incontinence Diagnosed by the Rome IV Criteria in the United States, Canada, and the United Kingdom. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association.* 2020;18(2):385-391.
82. Rømmen K, Schei B, Rydning A, H Sultan A, Mørkved S. Prevalence of anal incontinence among Norwegian women: a cross-sectional study. *BMJ open.* 2012;2(4).
83. Woolley SB, CA; Pocock, J; Foutch, GL. Rheological modelling of fresh human faeces. *Journal of Water, Sanitation and Hygiene for Development.* 2014;4(3):484-489.
84. Farag A. The use of flow equation in functional coloproctology: a new theory in anorectal physiology. *Pelviperineology.* 2009;28:17-23.
85. Scott SM, Lunniss PJ. Risk factors in faecal incontinence. In: Ratto C, Doglietto GB, eds. *Fecal incontinence : diagnosis and treatment.* Milan ; [London]: Springer; 2007:43-66.
86. Rao SS, Read NW, Davison PA, Bannister JJ, Holdsworth CD. Anorectal sensitivity and responses to rectal distention in patients with ulcerative colitis. *Gastroenterology.* 1987;93(6):1270-1275.
87. Herbst F, Kamm MA, Morris GP, Britton K, Woloszko J, Nicholls RJ. Gastrointestinal transit and prolonged ambulatory colonic motility in health and faecal incontinence. *Gut.* 1997;41(3):381-389.
88. Rodger CJ, Nicol L, Anderson JH, McKee RF, Finlay IG. Abnormal colonic motility: a possible association with urge fecal incontinence. *Diseases of the colon and rectum.* 2010;53(4):409-413.
89. Chatoor DR, Taylor SJ, Cohen CR, Emmanuel AV. Faecal incontinence. *The British journal of surgery.* 2007;94(2):134-144.
90. Bharucha AE, Fletcher JG, Harper CM, et al. Relationship between symptoms and disordered continence mechanisms in women with idiopathic faecal incontinence. *Gut.* 2005;54(4):546-555.
91. Santoro GA, Eitan BZ, Pryde A, Bartolo DC. Open study of low-dose amitriptyline in the treatment of patients with idiopathic fecal incontinence. *Diseases of the colon and rectum.* 2000;43(12):1676-1681; discussion 1681-1672.
92. Chan CL, Scott SM, Williams NS, Lunniss PJ. Rectal hypersensitivity worsens stool frequency, urgency, and lifestyle in patients with urge fecal incontinence. *Diseases of the colon and rectum.* 2005;48(1):134-140.
93. Rasmussen O, Christensen B, Sørensen M, Tetzschner T, Christiansen J. Rectal compliance in the assessment of patients with fecal incontinence. *Diseases of the colon and rectum.* 1990;33(8):650-653.
94. Burgell R. *The role of rectal hyposensitivity in the development of functional hindgut disorders : clinical significance and pathophysiology*, Queen Mary, University of London; 2014.

95. Burgell RE, Scott SM. Rectal hyposensitivity. *Journal of neurogastroenterology and motility.* 2012;18(4):373-384.
96. Townsend DC, Carrington EV, Grossi U, et al. Pathophysiology of fecal incontinence differs between men and women: a case-matched study in 200 patients. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2016;28(10):1580-1588.
97. Remes-Troche JM, De-Ocampo S, Valestin J, Rao SS. Rectoanal reflexes and sensorimotor response in rectal hyposensitivity. *Diseases of the colon and rectum.* 2010;53(7):1047-1054.
98. Buser WD, Miner PB. Delayed rectal sensation with fecal incontinence. Successful treatment using anorectal manometry. *Gastroenterology.* 1986;91(5):1186-1191.
99. Gladman MA, Aziz Q, Scott SM, Williams NS, Lunniss PJ. Rectal hyposensitivity: pathophysiological mechanisms. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2009;21(5):508-516, e504-505.
100. Loening-Baucke VA, Cruikshank BM. Abnormal defecation dynamics in chronically constipated children with encopresis. *J Pediatr.* 1986;108(4):562-566.
101. Nurko S, Scott SM. Coexistence of constipation and incontinence in children and adults. *Best practice & research Clinical gastroenterology.* 2011;25(1):29-41.
102. Read NW, Abouzekry L, Read MG, Howell P, Ottewell D, Donnelly TC. Anorectal function in elderly patients with fecal impaction. *Gastroenterology.* 1985;89(5):959-966.
103. Vollebregt PF, Hooper RL, Farmer AD, Miller J, Knowles CH, Scott SM. Association between opioid usage and rectal dysfunction in constipation: A cross-sectional study of 2754 patients. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2020;32(7):e13839.
104. Miller R, Bartolo DC, Cervero F, Mortensen NJ. Anorectal sampling: a comparison of normal and incontinent patients. *The British journal of surgery.* 1988;75(1):44-47.
105. Felt-Bersma RJ, Poen AC, Cuesta MA, Meuwissen SG. Anal sensitivity test: what does it measure and do we need it? Cause or derivative of anorectal complaints. *Diseases of the colon and rectum.* 1997;40(7):811-816.
106. Chaliha C, Sultan AH, Bland JM, Monga AK, Stanton SL. Anal function: effect of pregnancy and delivery. *Am J Obstet Gynecol.* 2001;185(2):427-432.
107. Sultan AH, Nugent K. Pathophysiology and nonsurgical treatment of anal incontinence. *BJOG.* 2004;111 Suppl 1:84-90.
108. Rao SSC, Yan Y, Xiang X, Sharma A, Ayyala D, Hamdy S. Effects of Translumbosacral Neuromodulation Therapy on Gut and Brain Interactions and Anorectal Neuropathy in Fecal Incontinence: A Randomized Study. *Neuromodulation.* 2021.
109. Xiang X, Patcharatrakul T, Sharma A, Parr R, Hamdy S, Rao SSC. Cortico-anorectal, Spino-anorectal, and Cortico-spinal Nerve Conduction and Locus of Neuronal Injury in Patients With Fecal Incontinence. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association.* 2019;17(6):1130-1137 e1132.
110. Bharucha AE, Daube J, Litchy W, et al. Anal sphincteric neurogenic injury in asymptomatic nulliparous women and fecal incontinence. *Am J Physiol Gastrointest Liver Physiol.* 2012;303(2):G256-262.
111. Snooks SJ, Setchell M, Swash M, Henry MM. Injury to innervation of pelvic floor sphincter musculature in childbirth. *Lancet.* 1984;2(8402):546-550.
112. Kamm MA. Obstetric damage and faecal incontinence. *Lancet.* 1994;344(8924):730-733.
113. Ryhammer AM, Laurberg S, Bek KM. Age and anorectal sensibility in normal women. *Scandinavian journal of gastroenterology.* 1997;32(3):278-284.

114. Lubowski DZ, Swash M, Nicholls RJ, Henry MM. Increase in pudendal nerve terminal motor latency with defaecation straining. *The British journal of surgery.* 1988;75(11):1095-1097.
115. Olsen AL, Rao SS. Clinical neurophysiology and electrodiagnostic testing of the pelvic floor. *Gastroenterology clinics of North America.* 2001;30(1):33-54, v-vi.
116. Bharucha AE, Zinsmeister AR, Schleck CD, Melton LJ. Bowel disturbances are the most important risk factors for late onset fecal incontinence: a population-based case-control study in women. *Gastroenterology.* 2010;139(5):1559-1566.
117. Otto SD, Burmeister S, Buhr HJ, Kroesen A. Sacral nerve stimulation induces changes in the pelvic floor and rectum that improve continence and quality of life. *J Gastrointest Surg.* 2010;14(4):636-644.
118. Ehrenpreis ED, Chang D, Eichenwald E. Pharmacotherapy for fecal incontinence: a review. *Diseases of the colon and rectum.* 2007;50(5):641-649.
119. Omar MI, Alexander CE. Drug treatment for faecal incontinence in adults. *The Cochrane database of systematic reviews.* 2013(6):CD002116.
120. Enck P, Van der Voort IR, Klosterhalfen S. Biofeedback therapy in fecal incontinence and constipation. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2009;21(11):1133-1141.
121. Norton C. Behavioral management of fecal incontinence in adults. *Gastroenterology.* 2004;126(1 Suppl 1):S64-70.
122. Norton C, Chelvanayagam S, Wilson-Barnett J, Redfern S, Kamm MA. Randomized controlled trial of biofeedback for fecal incontinence. *Gastroenterology.* 2003;125(5):1320-1329.
123. Zutshi M, Salcedo L, Hammel J, Hull T. Anal physiology testing in fecal incontinence: is it of any value? *International journal of colorectal disease.* 2010;25(2):277-282.
124. Vaizey CJ, Kamm MA, Turner IC, Nicholls RJ, Woloszko J. Effects of short term sacral nerve stimulation on anal and rectal function in patients with anal incontinence. *Gut.* 1999;44(3):407-412.
125. Liberman H, Faria J, Ternent CA, Blatchford GJ, Christensen MA, Thorson AG. A prospective evaluation of the value of anorectal physiology in the management of fecal incontinence. *Diseases of the colon and rectum.* 2001;44(11):1567-1574.
126. Chiarioni G, Bassotti G, Stanganini S, Vantini I, Whitehead WE, Stegagnini S. Sensory retraining is key to biofeedback therapy for formed stool fecal incontinence. *Am J Gastroenterol.* 2002;97(1):109-117.
127. Altomare DF, Rinaldi M, Petrolino M, et al. Reliability of electrophysiologic anal tests in predicting the outcome of sacral nerve modulation for fecal incontinence. *Diseases of the colon and rectum.* 2004;47(6):853-857.
128. Knowles CH, Horrocks EJ, Bremner SA, et al. Percutaneous tibial nerve stimulation versus sham electrical stimulation for the treatment of faecal incontinence in adults (CONFIDeNT): a double-blind, multicentre, pragmatic, parallel-group, randomised controlled trial. *Lancet.* 2015;386(10004):1640-1648.
129. Horrocks EJ, Chadi SA, Stevens NJ, Wexner SD, Knowles CH. Factors Associated With Efficacy of Percutaneous Tibial Nerve Stimulation for Fecal Incontinence, Based on Post-Hoc Analysis of Data From a Randomized Trial. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association.* 2017;15(12):1915-1921.e1912.
130. Malouf AJ, Norton CS, Engel AF, Nicholls RJ, Kamm MA. Long-term results of overlapping anterior anal-sphincter repair for obstetric trauma. *Lancet.* 2000;355(9200):260-265.
131. Matzel KE, Kamm MA, Stosser M, et al. Sacral spinal nerve stimulation for faecal incontinence: multicentre study. *Lancet.* 2004;363(9417):1270-1276.

132. Matzel KE, Stadelmaier U, Hohenfellner M, Gall FP. Electrical stimulation of sacral spinal nerves for treatment of faecal incontinence. *Lancet.* 1995;346(8983):1124-1127.
133. Wexner SD, Coller JA, Devroede G, et al. Sacral nerve stimulation for fecal incontinence: results of a 120-patient prospective multicenter study. *Ann Surg.* 2010;251(3):441-449.
134. Graf W, Mellgren A, Matzel KE, et al. Efficacy of dextranomer in stabilised hyaluronic acid for treatment of faecal incontinence: a randomised, sham-controlled trial. *Lancet.* 2011;377(9770):997-1003.
135. Norton C, Thomas L, Hill J, Group GD. Management of faecal incontinence in adults: summary of NICE guidance. *Bmj.* 2007;334(7608):1370-1371.
136. Anandam JL. Surgical management for fecal incontinence. *Clinics in colon and rectal surgery.* 2014;27(3):106-109.
137. Altomare DF, Binda GA, Dodi G, et al. Disappointing long-term results of the artificial anal sphincter for faecal incontinence. *The British journal of surgery.* 2004;91(10):1352-1353.
138. Baeten CG, Konsten J, Spaans F, et al. Dynamic graciloplasty for treatment of faecal incontinence. *Lancet.* 1991;338(8776):1163-1165.
139. Dudding TC, Parés D, Vaizey CJ, Kamm MA. Predictive factors for successful sacral nerve stimulation in the treatment of faecal incontinence: a 10-year cohort analysis. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland.* 2008;10(3):249-256.
140. Altomare DF, De Fazio M, Giuliani RT, Catalano G, Cuccia F. Sphincteroplasty for fecal incontinence in the era of sacral nerve modulation. *World journal of gastroenterology.* 2010;16(42):5267-5271.
141. Gourcerol G, Granier S, Bridoux V, Menard JF, Ducrotte P, Leroi AM. Do endoflip assessments of anal sphincter distensibility provide more information on patients with fecal incontinence than high-resolution anal manometry? *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2016;28(3):399-409.
142. Grossi U, Di Tanna GL, Heinrich H, Taylor SA, Knowles CH, Scott SM. Systematic review with meta-analysis: defecography should be a first-line diagnostic modality in patients with refractory constipation. *Alimentary pharmacology & therapeutics.* 2018;48(11-12):1186-1201.
143. Grossi U, Heinrich H, Di Tanna GL, et al. Systematic Characterization of Defecographic Abnormalities in a Consecutive Series of 827 Patients with Chronic Constipation. *Diseases of the colon and rectum.* 2021.
144. Schuster MM, Hookman P, Hendrix TR, Mendeloff AI. Simultaneous Manometric Recording of Internal and External Anal Sphincteric Reflexes. *Bull Johns Hopkins Hosp.* 1965;116:79-88.
145. Rao SS, Azpiroz F, Diamant N, Enck P, Tougas G, Wald A. Minimum standards of anorectal manometry. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2002;14(5):553-559.
146. Carrington EV, Heinrich H, Knowles CH, et al. The international anorectal physiology working group (IAPWG) recommendations: Standardized testing protocol and the London classification for disorders of anorectal function. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2020;32(1):e13679.
147. Dinning PG, Carrington EV, Scott SM. The use of colonic and anorectal high-resolution manometry and its place in clinical work and in research. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2015;27(12):1693-1708.

148. Carrington EV, Heinrich H, Knowles CH, et al. Methods of anorectal manometry vary widely in clinical practice: Results from an international survey. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2017;29(8):e13016.
149. Noelting J, Ratuapli SK, Bharucha AE, Harvey DM, Ravi K, Zinsmeister AR. Normal values for high-resolution anorectal manometry in healthy women: effects of age and significance of rectoanal gradient. *Am J Gastroenterol.* 2012;107(10):1530-1536.
150. Sauter M, Heinrich H, Fox M, et al. Toward more accurate measurements of anorectal motor and sensory function in routine clinical practice: validation of high-resolution anorectal manometry and Rapid Barostat Bag measurements of rectal function. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2014;26(5):685-695.
151. Rasijeff AMP, Withers M, Burke JM, Jackson W, Scott SM. High-resolution anorectal manometry: A comparison of solid-state and water-perfused catheters. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2017;29(11).
152. Cuong LM, Van Quyet H, Hung TM, et al. Normal values for high-resolution anorectal manometry in healthy young adults: evidence from Vietnam. *BMC Gastroenterol.* 2021;21(1):295.
153. Mion F, Garros A, Brochard C, et al. 3D High-definition anorectal manometry: Values obtained in asymptomatic volunteers, fecal incontinence and chronic constipation. Results of a prospective multicenter study (NOMAD). *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2017;29(8).
154. Coss-Adame E, Rao SS, Valestin J, Ali-Azamar A, Remes-Troche JM. Accuracy and Reproducibility of High-definition Anorectal Manometry and Pressure Topography Analyses in Healthy Subjects. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association.* 2015;13(6):1143-1150.e1141.
155. Li Y, Yang X, Xu C, Zhang Y, Zhang X. Normal values and pressure morphology for three-dimensional high-resolution anorectal manometry of asymptomatic adults: a study in 110 subjects. *International journal of colorectal disease.* 2013;28(8):1161-1168.
156. Chaliha C, Sultan AH, Emmanuel AV. Normal ranges for anorectal manometry and sensation in women of reproductive age. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland.* 2007;9(9):839-844.
157. Felt-Bersma RJ, Meuwissen SG. Anal manometry. *International journal of colorectal disease.* 1990;5(3):170-173.
158. Diamant NE, Kamm MA, Wald A, Whitehead WE. AGA technical review on anorectal testing techniques. *Gastroenterology.* 1999;116(3):735-760.
159. Jones MP, Post J, Crowell MD. High-resolution manometry in the evaluation of anorectal disorders: a simultaneous comparison with water-perfused manometry. *Am J Gastroenterol.* 2007;102(4):850-855.
160. Heinrich H, Fruehauf H, Sauter M, et al. The effect of standard compared to enhanced instruction and verbal feedback on anorectal manometry measurements. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2013;25(3):230-237, e163.
161. Fang JC, Hilden K, Tuteja AK, Peterson KA. Comparison of air-coupled balloon esophageal and anorectal manometry catheters with solid-state esophageal manometry and water-perfused anorectal manometry catheters. *Dig Dis Sci.* 2004;49(10):1657-1663.

162. Simpson RR, Kennedy ML, Nguyen MH, Dinning PG, Lubowski DZ. Anal manometry: a comparison of techniques. *Diseases of the colon and rectum.* 2006;49(7):1033-1038.
163. Vitton V, Ben Hadj Amor W, Baumstarck K, Grimaud JC, Bouvier M. Water-perfused manometry vs three-dimensional high-resolution manometry: a comparative study on a large patient population with anorectal disorders. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland.* 2013;15(12):e726-731.
164. Kang HR, Lee JE, Lee JS, et al. Comparison of High-resolution Anorectal Manometry With Water-perfused Anorectal Manometry. *Journal of neurogastroenterology and motility.* 2015;21(1):126-132.
165. Thekkinkattil DK, Lim MK, Nicholls MJ, Sagar PM, Finan PJ, Burke DA. Contribution of posture to anorectal manometric measurements: are the measurements in left-lateral position physiologic? *Diseases of the colon and rectum.* 2007;50(12):2112-2119.
166. Grossi U, Carrington EV, Bharucha AE, Horrocks EJ, Scott SM, Knowles CH. Diagnostic accuracy study of anorectal manometry for diagnosis of dyssynergic defecation. *Gut.* 2016;65(3):447-455.
167. Heinrich H, Misselwitz B. High-Resolution Anorectal Manometry - New Insights in the Diagnostic Assessment of Functional Anorectal Disorders. *Visc Med.* 2018;34(2):134-139.
168. Telford KJ, Ali AS, Lymer K, Hosker GL, Kiff ES, Hill J. Fatigability of the external anal sphincter in anal incontinence. *Diseases of the colon and rectum.* 2004;47(5):746-752; discussion 752.
169. Maeda Y, Vaizey CJ, Hollington P, Stern J, Kamm MA. Physiological, psychological and behavioural characteristics of men and women with faecal incontinence. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland.* 2009;11(9):927-932.
170. Zbar AP, Kmiot WA, Aslam M, et al. Use of vector volume manometry and endoanal magnetic resonance imaging in the adult female for assessment of anal sphincter dysfunction. *Diseases of the colon and rectum.* 1999;42(11):1411-1418.
171. Lee HJ, Jung KW, Han S, et al. Normal values for high-resolution anorectal manometry/topography in a healthy Korean population and the effects of gender and body mass index. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2014;26(4):529-537.
172. Lee HR, Lim SB, Park JY. Anorectal manometric parameters are influenced by gender and age in subjects with normal bowel function. *International journal of colorectal disease.* 2014;29(11):1393-1399.
173. Jameson JS, Chia YW, Kamm MA, Speakman CT, Chye YH, Henry MM. Effect of age, sex and parity on anorectal function. *The British journal of surgery.* 1994;81(11):1689-1692.
174. Oblizajek NR, Gandhi S, Sharma M, et al. Anorectal pressures measured with high-resolution manometry in healthy people-Normal values and asymptomatic pelvic floor dysfunction. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2019;31(7):e13597.
175. James-Stevenson T, Xu H, Heit M, Shin A. Age and Dyssynergia Subtypes Associated With Normal Sphincter Pressures in Women With Fecal Incontinence. *Female pelvic medicine & reconstructive surgery.* 2018;24(3):247-251.
176. Rao SS, Hatfield R, Soffer E, Rao S, Beaty J, Conklin JL. Manometric tests of anorectal function in healthy adults. *Am J Gastroenterol.* 1999;94(3):773-783.
177. McHugh SM, Diamant NE. Effect of age, gender, and parity on anal canal pressures. Contribution of impaired anal sphincter function to fecal incontinence. *Dig Dis Sci.* 1987;32(7):726-736.

178. Mimura T, Kaminishi M, Kamm MA. Diagnostic evaluation of patients with faecal incontinence at a specialist institution. *Dig Surg.* 2004;21(3):235-241; discussion 241.
179. Lam TJ, Kuik DJ, Felt-Bersma RJ. Anorectal function evaluation and predictive factors for faecal incontinence in 600 patients. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland.* 2012;14(2):214-223.
180. Fowler AL, Mills A, Virjee J, Callaway M, Durdey P, Thomas MG. Comparison of ultrasound and manometric sphincter length and incontinence scores. *Diseases of the colon and rectum.* 2003;46(8):1078-1082.
181. Sun WM, Read NW, Donnelly TC. Anorectal function in incontinent patients with cerebrospinal disease. *Gastroenterology.* 1990;99(5):1372-1379.
182. Rao SS. Dyssynergic defecation and biofeedback therapy. *Gastroenterology clinics of North America.* 2008;37(3):569-586, viii.
183. Neshatian L, Fosso C, Quigley E. Abnormal Balloon Expulsion Test in Patients with Fecal Incontinence. *J Gastrointestin Liver Dis.* 2020;29(3):345-351.
184. Rao SS, Welcher KD, Leistikow JS. Obstructive defecation: a failure of rectoanal coordination. *Am J Gastroenterol.* 1998;93(7):1042-1050.
185. Chiarioni G, Kim SM, Vantini I, Whitehead WE. Validation of the balloon evacuation test: reproducibility and agreement with findings from anorectal manometry and electromyography. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association.* 2014;12(12):2049-2054.
186. Carter D, Bardan E, Maradey-Romero C. Clinical and physiological risk factors for fecal incontinence in chronically constipated women. *Techniques in coloproctology.* 2019;23(5):429-434.
187. Rao SSC, Kavlock R, Rao S. Influence of body position and stool characteristics on defecation in humans. *The American journal of gastroenterology.* 2006;101(12):2790-2796.
188. Rasijeff AMP, Garcia-Zermeno K, Carrington EV, Knowles C, Scott SM. Systematic evaluation of cough-anorectal pressure responses in health and in fecal incontinence: A high-resolution anorectal manometry study. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2020:e13999.
189. Opazo A, Aguirre E, Saldaña E, Fantova MJ, Clavé P. Patterns of impaired internal anal sphincter activity in patients with anal fissure. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland.* 2013;15(4):492-499.
190. Carrington EV, Grossi U, Knowles CH, Scott SM. Normal values for high-resolution anorectal manometry: a time for consensus and collaboration. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2014;26(9):1356-1357.
191. Vollebregt PF, Wiklendt L, Ang D, et al. Altered anal slow-wave pressure activity in low anterior resection syndrome: short case series in two independent specialist centres provide new mechanistic insights. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland.* 2021;23(2):444-450.
192. Sun WM, Rao SS. Manometric assessment of anorectal function. *Gastroenterology clinics of North America.* 2001;30(1):15-32.
193. Cobine CA, Hannigan KI, McMahon M, Ni Bhraonain EP, Baker SA, Keef KD. Rhythmic calcium transients in smooth muscle cells of the mouse internal anal sphincter. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2020;32(3):e13746.
194. Aaronson I, Nixon HH. A clinical evaluation of anorectal pressure studies in the diagnosis of Hirschsprung's disease. *Gut.* 1972;13(2):138-146.

195. Xu X, Pasricha PJ, Sallam HS, Ma L, Chen JD. Clinical significance of quantitative assessment of rectoanal inhibitory reflex (RAIR) in patients with constipation. *J Clin Gastroenterol.* 2008;42(6):692-698.
196. Zbar AP, Beer-Gabel M, Chiappa AC, Aslam M. Fecal incontinence after minor anorectal surgery. *Diseases of the colon and rectum.* 2001;44(11):1610-1619; discussion 1619-1623.
197. Rao SS. Manometric evaluation of defecation disorders: Part II. Fecal incontinence. *The Gastroenterologist.* 1997;5(2):99-111.
198. Farthing MJ, Lennard-jones JE. Sensibility of the rectum to distension and the anorectal distension reflex in ulcerative colitis. *Gut.* 1978;19(1):64-69.
199. Sun WM, Read NW, Miner PB. Relation between rectal sensation and anal function in normal subjects and patients with faecal incontinence. *Gut.* 1990;31(9):1056-1061.
200. Whitehead WE, Schuster MM. Anorectal physiology and pathophysiology. *Am J Gastroenterol.* 1987;82(6):487-497.
201. Gladman MA, Dvorkin LS, Lunniss PJ, Williams NS, Scott SM. Rectal hyposensitivity: a disorder of the rectal wall or the afferent pathway? An assessment using the barostat. *Am J Gastroenterol.* 2005;100(1):106-114.
202. Gladman MA, Dvorkin LS, Scott SM, Lunniss PJ, Williams NS. A novel technique to identify patients with megarectum. *Diseases of the colon and rectum.* 2007;50(5):621-629.
203. Camilleri M. Testing the sensitivity hypothesis in practice: tools and methods, assumptions and pitfalls. *Gut.* 2002;51 Suppl 1:i34-40.
204. Devroede G, Lamarche J. Functional importance of extrinsic parasympathetic innervation to the distal colon and rectum in man. *Gastroenterology.* 1974;66(2):273-280.
205. Gladman MA, Lunniss PJ, Scott SM, Swash M. Rectal hyposensitivity. *Am J Gastroenterol.* 2006;101(5):1140-1151.
206. Sørensen M, Rasmussen OO, Tetzschner T, Christiansen J. Physiological variation in rectal compliance. *The British journal of surgery.* 1992;79(10):1106-1108.
207. Madoff RD, Orrom WJ, Rothenberger DA, Goldberg SM. Rectal compliance: a critical reappraisal. *International journal of colorectal disease.* 1990;5(1):37-40.
208. Lefaucheur JP. Neurophysiological testing in anorectal disorders. *Muscle Nerve.* 2006;33(3):324-333.
209. Merletti R, Bottin A, Cescon C, et al. Multichannel surface EMG for the non-invasive assessment of the anal sphincter muscle. *Digestion.* 2004;69(2):112-122.
210. Scott S, Lunniss P. Risk factors in fecal incontinence In: Ratto C, Doglietto G, eds. *Fecal Incontinence : Diagnosis and Treatment.* Springer-Verlag London Ltd: Springer-Verlag London Ltd; 2007:43–66.
211. Vasudevan SP, Scott SM, Gladman MA, Lunniss PJ. Rectal hyposensitivity: evaluation of anal sensation in female patients with refractory constipation with and without faecal incontinence. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2007;19(8):660-667.
212. Roe AM, Bartolo DC, Mortensen NJ. New method for assessment of anal sensation in various anorectal disorders. *The British journal of surgery.* 1986;73(4):310-312.
213. Rogers J. Testing for and the role of anal and rectal sensation. *Baillieres Clin Gastroenterol.* 1992;6(1):179-191.
214. Miller R. The Measurement of anorectal sensation. In: Kumar D, Waldron D, Williams NS, eds. *Clinical measurement in coloproctology.* London: Springer-Verlag; 1991:60-66.
215. Abdool Z, Sultan AH, Thakar R. Ultrasound imaging of the anal sphincter complex: a review. *The British journal of radiology.* 2012;85(1015):865-875.

216. Steensma AB. The Central and Posterior Compartments. In: Dietz HPH, L.P; Steensma, A.B., ed. *Atlas of Pelvic Floor Ultrasound.* UK: Springer-Verlag London; 2008:63-75.
217. Harvey MA, Pierce M, Alter JE, et al. Obstetrical Anal Sphincter Injuries (OASIS): Prevention, Recognition, and Repair. *J Obstet Gynaecol Can.* 2015;37(12):1131-1148.
218. Karmarkar R, Bhide A, Digesu A, Khullar V, Fernando R. Mode of delivery after obstetric anal sphincter injury. *Eur J Obstet Gynecol Reprod Biol.* 2015;194:7-10.
219. Stoker J, Rociu E, Wiersma TG, Lameris JS. Imaging of anorectal disease. *The British journal of surgery.* 2000;87(1):10-27.
220. Bharucha AE, Fletcher JG, Melton LJ, Zinsmeister AR. Obstetric trauma, pelvic floor injury and fecal incontinence: a population-based case-control study. *Am J Gastroenterol.* 2012;107(6):902-911.
221. Williams AB, Bartram CI, Halligan S, Spencer JA, Nicholls RJ, Kmiot WA. Anal sphincter damage after vaginal delivery using three-dimensional endosonography. *Obstet Gynecol.* 2001;97(5 Pt 1):770-775.
222. Albuquerque A, Macedo G. Idiopathic internal anal sphincter degeneration: how common is it? Does size really matter? *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland.* 2017;19(4):396-397.
223. Dvorkin LS, Chan CL, Knowles CH, Williams NS, Lunniss PJ, Scott SM. Anal sphincter morphology in patients with full-thickness rectal prolapse. *Diseases of the colon and rectum.* 2004;47(2):198-203.
224. Halligan S, Sultan A, Rottenberg G, Bartram CI. Endosonography of the anal sphincters in solitary rectal ulcer syndrome. *International journal of colorectal disease.* 1995;10(2):79-82.
225. Marshall M, Halligan S, Fotheringham T, Bartram C, Nicholls RJ. Predictive value of internal anal sphincter thickness for diagnosis of rectal intussusception in patients with solitary rectal ulcer syndrome. *The British journal of surgery.* 2002;89(10):1281-1285.
226. Mahony R, Behan M, Daly L, Kirwan C, O'Herlihy C, O'Connell PR. Internal anal sphincter defect influences continence outcome following obstetric anal sphincter injury. *Am J Obstet Gynecol.* 2007;196(3):217 e211-215.
227. Sultan AH, Kamm MA, Hudson CN, Thomas JM, Bartram CI. Anal-sphincter disruption during vaginal delivery. *N Engl J Med.* 1993;329(26):1905-1911.
228. Pinsk I, Brown J, Phang PT. Assessment of sonographic quality of anal sphincter muscles in patients with faecal incontinence. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland.* 2009;11(9):933-940.
229. Titi MA, Jenkins JT, Urie A, Molloy RG. Correlation between anal manometry and endosonography in females with faecal incontinence. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland.* 2008;10(2):131-137.
230. Hainsworth AJ, Solanki D, Hamad A, Morris SJ, Schizas AM, Williams AB. Integrated total pelvic floor ultrasound in pelvic floor defaecatory dysfunction. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland.* 2017;19(1):O54-O65.
231. Valsky DV, Cohen SM, Lipschuetz M, et al. Third- or Fourth-Degree Intrapartum Anal Sphincter Tears Are Associated With Levator Ani Avulsion in Primiparas. *J Ultrasound Med.* 2016;35(4):709-715.
232. Fox M, Thumshirn M, Fried M, Schwizer W. Barostat measurement of rectal compliance and capacity. *Diseases of the colon and rectum.* 2006;49(3):360-370.
233. Delvaux M. Role of visceral sensitivity in the pathophysiology of irritable bowel syndrome. *Gut.* 2002;51 Suppl 1:i67-71.

234. Whitehead WE, Delvaux M. Standardization of barostat procedures for testing smooth muscle tone and sensory thresholds in the gastrointestinal tract. The Working Team of Glaxo-Wellcome Research, UK. *Dig Dis Sci.* 1997;42(2):223-241.
235. Hammer HF, Phillips SF, Camilleri M, Hanson RB. Rectal tone, distensibility, and perception: reproducibility and response to different distensions. *The American journal of physiology.* 1998;274(3):G584-590.
236. Bharucha AE, Novak V, Camilleri M, Zinsmeister AR, Hanson RB, Low PA. Alpha 2-adrenergic modulation of colonic tone during hyperventilation. *The American journal of physiology.* 1997;273(5):G1135-1140.
237. Cremonini F, Houghton LA, Camilleri M, et al. Barostat testing of rectal sensation and compliance in humans: comparison of results across two centres and overall reproducibility. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2005;17(6):810-820.
238. Gladman MA, Scott SM, Chan CL, Williams NS, Lunniss PJ. Rectal hyposensitivity: prevalence and clinical impact in patients with intractable constipation and fecal incontinence. *Diseases of the colon and rectum.* 2003;46(2):238-246.
239. van der Schaar PJ, Lamers CB, Masclee AA. The role of the barostat in human research and clinical practice. *Scand J Gastroenterol Suppl.* 1999;230:52-63.
240. Bharucha AE. Outcome measures for fecal incontinence: anorectal structure and function. *Gastroenterology.* 2004;126(1 Suppl 1):S90-98.
241. Gregersen H, Kassab G. Biomechanics of the gastrointestinal tract. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 1996;8(4):277-297.
242. Kwiatek MA, Kahrilas K, Soper NJ, et al. Esophagogastric junction distensibility after fundoplication assessed with a novel functional luminal imaging probe. *J Gastrointest Surg.* 2010;14(2):268-276.
243. Fynne L, Luft F, Gregersen H, et al. Distensibility of the anal canal in patients with systemic sclerosis: a study with the functional lumen imaging probe. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland.* 2013;15(1):e40-47.
244. Sorensen G, Liao D, Lundby L, et al. Distensibility of the anal canal in patients with idiopathic fecal incontinence: a study with the Functional Lumen Imaging Probe. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2014;26(2):255-263.
245. Leroi AM, Melchior C, Charpentier C, et al. The diagnostic value of the functional lumen imaging probe versus high-resolution anorectal manometry in patients with fecal incontinence. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2018;30(6):e13291.
246. Zifan A, Sun C, Gourcerol G, Leroi AM, Mittal RK. Endoflip vs high-definition manometry in the assessment of fecal incontinence: A data-driven unsupervised comparison. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2018;30(12):e13462.
247. Hirano I, Pandolfino JE, Boeckxstaens GE. Functional Lumen Imaging Probe for the Management of Esophageal Disorders: Expert Review From the Clinical Practice Updates Committee of the AGA Institute. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association.* 2017;15(3):325-334.
248. Ahuja NK, Agnihotri A, Lynch KL, et al. Esophageal distensibility measurement: impact on clinical management and procedure length. *Diseases of the esophagus : official journal of the International Society for Diseases of the Esophagus.* 2017;30(8):1-8.

249. Carlson DA, Kahrilas PJ, Ritter K, Lin Z, Pandolfino JE. Mechanisms of repetitive retrograde contractions in response to sustained esophageal distension: a study evaluating patients with postfundoplication dysphagia. *Am J Physiol Gastrointest Liver Physiol.* 2018;314(3):G334-G340.
250. Vaizey CJ, Kamm MA. Prospective assessment of the clinical value of anorectal investigations. *Digestion.* 2000;61(3):207-214.
251. Andrews C, Bharucha AE, Seide B, Zinsmeister AR. Rectal sensorimotor dysfunction in women with fecal incontinence. *Am J Physiol Gastrointest Liver Physiol.* 2007;292(1):G282-289.
252. Gundling F, Seidl H, Scalercio N, Schmidt T, Schepp W, Pehl C. Influence of gender and age on anorectal function: normal values from anorectal manometry in a large caucasian population. *Digestion.* 2010;81(4):207-213.
253. Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *J Clin Epidemiol.* 2009;62(10):1006-1012.
254. Rajindrajith S, Devanarayana NM, Benninga MA. Review article: faecal incontinence in children: epidemiology, pathophysiology, clinical evaluation and management. *Alimentary pharmacology & therapeutics.* 2013;37(1):37-48.
255. Athanasakos E, Cleeve S, Thapar N, et al. Anorectal manometry in children with defecation disorders BSPGHAN Motility Working Group consensus statement. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2020;32(6):e13797.
256. Carrington EV, Knowles CH, Grossi U, Scott SM. High-resolution Anorectal Manometry Measures Are More Accurate Than Conventional Measures in Detecting Anal Hypocontractility in Women With Fecal Incontinence. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association.* 2019;17(3):477-485.e479.
257. Paramor KA, Ibrahim QI, Sadowski DC. Clinical parameters and symptom severity in males with fecal leakage and incontinence. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2014;26(3):361-367.
258. Hotouras A, Thaha MA, Allison ME, Currie A, Scott SM, Chan CL. Percutaneous tibial nerve stimulation (PTNS) in females with faecal incontinence: the impact of sphincter morphology and rectal sensation on the clinical outcome. *International journal of colorectal disease.* 2012;27(7):927-930.
259. Burgell RE, Bhan C, Lunniss PJ, Scott SM. Fecal incontinence in men: coexistent constipation and impact of rectal hyposensitivity. *Diseases of the colon and rectum.* 2012;55(1):18-25.
260. Nyaga VN, Arbyn M, Aerts M. Metaprop: a Stata command to perform meta-analysis of binomial data. *Arch Public Health.* 2014;72(1):39.
261. Gooneratne ML, Scott SM, Lunniss PJ. Unilateral pudendal neuropathy is common in patients with fecal incontinence. *Diseases of the colon and rectum.* 2007;50(4):449-458.
262. Pehl C, Seidl H, Scalercio N, et al. Accuracy of anorectal manometry in patients with fecal incontinence. *Digestion.* 2012;86(2):78-85.
263. Raza N, Bielefeldt K. Discriminative value of anorectal manometry in clinical practice. *Dig Dis Sci.* 2009;54(11):2503-2511.
264. Munoz Yague T, Alvarez Sanchez V, Ibanez Pinto A, Solis-Herruzo J. Clinical, anorectal manometry and surface electromyography in the study of patients with fecal incontinence. *Rev Esp Enferm Dig.* 2003;95(9):635-639, 629-634.
265. Lacima G, Pera M, Amador A, Escaramís G, Piqué JM. Long-term results of biofeedback treatment for faecal incontinence: a comparative study with untreated controls.

Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland. 2010;12(8):742-749.
266. Heitmann PT, Rabbitt P, Schloithe A, et al. Relationships between the results of anorectal investigations and symptom severity in patients with faecal incontinence. *International journal of colorectal disease.* 2019;34(8):1445-1454.
267. Delechenaut P, Leroi AM, Weber J, Touchais JY, Czernichow P, Denis P. Relationship between clinical symptoms of anal incontinence and the results of anorectal manometry. *Diseases of the colon and rectum.* 1992;35(9):847-849.
268. Haylen BT, de Ridder D, Freeman RM, et al. An International Urogynecological Association (IUGA)/International Continence Society (ICS) joint report on the terminology for female pelvic floor dysfunction. *International urogynecology journal.* 2010;21(1):5-26.
269. Gee AS, Durdey P. Urge incontinence of faeces is a marker of severe external anal sphincter dysfunction. *The British journal of surgery.* 1995;82(9):1179-1182.
270. Irwin DE, Kopp ZS, Agatep B, Milsom I, Abrams P. Worldwide prevalence estimates of lower urinary tract symptoms, overactive bladder, urinary incontinence and bladder outlet obstruction. *BJU Int.* 2011;108(7):1132-1138.
271. Milsom I, Gyhagen M. The prevalence of urinary incontinence. *Climacteric.* 2019;22(3):217-222.
272. Delancey JO, Ashton-Miller JA. Pathophysiology of adult urinary incontinence. *Gastroenterology.* 2004;126(1 Suppl 1):S23-32.
273. Snooks SJ, Barnes PR, Swash M. Damage to the innervation of the voluntary anal and periurethral sphincter musculature in incontinence: an electrophysiological study. *J Neurol Neurosurg Psychiatry.* 1984;47(12):1269-1273.
274. Leroi AM, Weber J, Menard JF, Touchais JY, Denis P. Prevalence of anal incontinence in 409 patients investigated for stress urinary incontinence. *Neurourology and urodynamics.* 1999;18(6):579-590.
275. Alnaif B, Drutz HP. The prevalence of urinary and fecal incontinence in Canadian secondary school teenage girls: questionnaire study and review of the literature. *Int Urogynecol J Pelvic Floor Dysfunct.* 2001;12(2):134-137; discussion138.
276. Ross JW. Laparoscopic Burch colposuspension and overlapping sphincteroplasty for double incontinence. *JSLS.* 2001;5(3):203-209.
277. Lacima G, Espuña M, Pera M, Puig-Clota M, Quintó L, García-Valdecasas JC. Clinical, urodynamic, and manometric findings in women with combined fecal and urinary incontinence. *Neurourology and urodynamics.* 2002;21(5):464-469.
278. D'Ancona C, Haylen B, Oelke M, et al. The International Continence Society (ICS) report on the terminology for adult male lower urinary tract and pelvic floor symptoms and dysfunction. *Neurourology and urodynamics.* 2019;38(2):433-477.
279. Remes-Troche JM, Rao SS. Neurophysiological testing in anorectal disorders. *Expert Rev Gastroenterol Hepatol.* 2008;2(3):323-335.
280. Swash M. Neurogenic hypothesis of stress incontinence. In: G B, J W, eds. *Neurobiology of incontinence.* John Wiley and Sons; 1990:156-175.
281. Dessie SG, Modest AM, Von Bargen E, Hacker M, Rosenblatt PL. Characterizing fecal incontinence [Abst]. *Female pelvic medicine & reconstructive surgery.* 2014;20(4):S156.
282. Elgendy K. Anal sphincters contraction during cough: manometric and electromyographic study in normal subjects and patients with stress fecal incontinence [Abst]. *Neurogastroenterology & Motility.* 2014;26:48-49.
283. Marecki M, Seo JY. Perinatal urinary and fecal incontinence: suffering in silence. *J Perinat Neonatal Nurs.* 2010;24(4):330-340.
284. Lacima G, Pera M. Combined fecal and urinary incontinence: an update. *Curr Opin Obstet Gynecol.* 2003;15(5):405-410.

285. Kumar N. Neurogastroenterology. *Continuum Lifelong Learning in Neurology.* 2008;14(1):13-52.
286. Ash D. Sustaining safe and acceptable bowel care in spinal cord injured patients. *Nurs Stand.* 2005;20(8):55-64, quiz 66.
287. Marziale L, Lucarini G, Mazzocchi T, Ricotti L, Menciassi A. Comparative analysis of occlusion methods for artificial sphincters. *Artif Organs.* 2020;44(9):995-1005.
288. Schwabegger AH, Kronberger P, Obrist P, Brath E, Miko I. Functional sphincter ani externus reconstruction for treatment of fecal stress incontinence using free latissimus dorsi muscle transfer with coaptation to the pudendal nerve: preliminary experimental study in dogs. *J Reconstr Microsurg.* 2007;23(2):79-85.
289. Benezech A, Desmazes-Dufeu N, Baumstarck K, et al. Prevalence of Fecal Incontinence in Adults with Cystic Fibrosis. *Dig Dis Sci.* 2018;63(4):982-988.
290. White D, Stiller K, Roney F. The Prevalence and severity of symptoms of incontinence in adult cystic fibrosis patients. *Physiotherapy Theory and Practice.* 2000;16:35-42.
291. JW R. Treatment of Stress and Fecal Incontinence. 1999.
292. Shafik A, El Sibai O, Shafik IA, Shafik AA. Stress, urge, and mixed types of partial fecal incontinence: pathogenesis, clinical presentation, and treatment. *Am Surg.* 2007;73(1):6-9.
293. Hoke TP, Meyer I, Blanchard CT, Szychowski JM, Richter HE. Characterization of symptom severity and impact on four fecal incontinence phenotypes in women presenting for evaluation. *Neurourology and urodynamics.* 2021;40(1):237-244.
294. Swash M. The neurogenic hypothesis of stress incontinence. *Ciba Found Symp.* 1990;151:156-170; discussion 170-155.
295. N K. Neurogastroenterology. *Continuum Lifelong Learning Neurol.* 2008;14:13-52.
296. van Meegdenburg MM, Trzpis M, Broens PM. Fecal incontinence and parity in the Dutch population: A cross-sectional analysis. *United European gastroenterology journal.* 2018;6(5):781-790.
297. Littlewood JM. Gastrointestinal complications in cystic fibrosis. *J R Soc Med.* 1992;85 Suppl 19:13-19.
298. Badalian SS, Sagayan E, Simonyan H, Minassian VA, Isahakian A. The prevalence of pelvic floor disorders and degree of bother among women attending primary care clinics in Armenia. *Eur J Obstet Gynecol Reprod Biol.* 2020;246:106-112.
299. Kuzniar TJ, Morgenthaler TI, Afessa B, Lim KG. Chronic cough from the patient's perspective. *Mayo Clin Proc.* 2007;82(1):56-60.
300. Polley L, Yaman N, Heaney L, et al. Impact of cough across different chronic respiratory diseases: comparison of two cough-specific health-related quality of life questionnaires. *Chest.* 2008;134(2):295-302.
301. Varma MG, Brown JS, Creasman JM, et al. Fecal incontinence in females older than aged 40 years: who is at risk? *Diseases of the colon and rectum.* 2006;49(6):841-851.
302. Yuan Y, Qiu L, Li ZY, et al. An epidemiology study of fecal incontinence in adult Chinese women living in urban areas. *Chin Med J (Engl).* 2020;133(3):262-268.
303. Alqudah MM, Gregersen H, Drewes AM, McMahon BP. Evaluation of anal sphincter resistance and distensibility in healthy controls using EndoFLIP (c). *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2012;24(12):e591-599.
304. Arumugam PJ, Patel B, Rieck G, et al. Are obstetric risk factors and bowel symptoms associated with defaecographic and manometric abnormalities in women awaiting hysterectomy? *J Obstet Gynaecol.* 2004;24(3):274-278.
305. Deffieux X, Raibaut P, Rene-Corail P, et al. External anal sphincter contraction during cough: not a simple spinal reflex. *Neurourology and urodynamics.* 2006;25(7):782-787.

306. Mazor Y, Prott G, Jones M, Kellow J, Ejova A, Malcolm A. Anorectal physiology in health: A randomized trial to determine the optimum catheter for the balloon expulsion test. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2019;31(4):e13552.
307. Seong M-K, Kim K-, Yoo Y-B. Evaluation of anal continence function by advanced anal manometric parameters. *J Korean Soc Coloproctol.* 2009;25(1):20-25.
308. Tantiphlachiva K, Pattana-Arun J, Sahakitrungrueng C, Rojanasakul A. Comprehensive evaluation of fecal incontinence: A preliminary report of anatomical neurophysiologic study [Abst]. *Journal of the Medical Association of Thailand.* 2018;101(4):S29-S37.
309. Papachrysostomou MC, Smith AN, Stevenson AJM. Does electrical stimulation of the pelvic floor alter the proctographic parameters in faecal incontinence? *European Journal of Gastroenterology and Hepatology.* 1994;6:139-144.
310. Meagher AP, Lubowski DZ, King DW. The cough response of the anal sphincter. *International journal of colorectal disease.* 1993;8(4):217-219.
311. Amarenco G, Ismael SS, Lagauche D, et al. Cough anal reflex: strict relationship between intravesical pressure and pelvic floor muscle electromyographic activity during cough. Urodynamic and electrophysiological study. *J Urol.* 2005;173(1):149-152.
312. Vallès M, Mearin F. Pathophysiology of bowel dysfunction in patients with motor incomplete spinal cord injury: comparison with patients with motor complete spinal cord injury. *Diseases of the colon and rectum.* 2009;52(9):1589-1597.
313. Mion F, Garros A, Subtil F, Damon H, Roman S. Anal sphincter function as assessed by 3D high definition anorectal manometry. *Clin Res Hepatol Gastroenterol.* 2018;42(4):378-381.
314. Seong MK, Kim TW. Significance of defecographic parameters in diagnosing pelvic floor dyssynergia. *Journal of the Korean Surgical Society.* 2013;84(4):225-230.
315. Farag A. Use of the Hagen-Poiseuille law: a new mathematical approach for the integration and evaluation of anorectal physiological testing in patients with faecal incontinence and pelvic dyschezia and in normal controls. *Eur Surg Res.* 1998;30(4):279-289.
316. Mazor Y, Jones M, Andrews A, Kellow JE, Malcolm A. Novel insights into fecal incontinence in men. *Am J Physiol Gastrointest Liver Physiol.* 2017;312(1):G46-G51.
317. Mulak A, Paradowski L. Anorectal function and dyssynergic defecation in different subgroups of patients with irritable bowel syndrome. *International journal of colorectal disease.* 2010;25(8):1011-1016.
318. Sung HY, Choi MG, Kim YI, Lee KS, Kim JS. Anorectal manometric dysfunctions in newly diagnosed, early-stage Parkinson's disease. *J Clin Neurol.* 2012;8(3):184-189.
319. Miah I. The Clinical value of performing cough assessment in patients with faecal incontinence during anal manometry [Abst]. *Gut.* 2019;68:A212.
320. Lazarescu A, Turnbull GK, Vanner S. Investigating and treating fecal incontinence: when and how. *Can J Gastroenterol.* 2009;23(4):301-308.
321. Nygaard IE, Shaw JM. Physical activity and the pelvic floor. *Am J Obstet Gynecol.* 2016;214(2):164-171.
322. Rao SS. Advances in diagnostic assessment of fecal incontinence and dyssynergic defecation. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association.* 2010;8(11):910-919.
323. Duthie HL, Watts JM. Contribution of the External Anal Sphincter to the Pressure Zone in the Anal Canal. *Gut.* 1965;6:64-68.
324. Townsend MK, Matthews CA, Whitehead WE, Grodstein F. Risk factors for fecal incontinence in older women. *Am J Gastroenterol.* 2013;108(1):113-119.

325. Read NW, Bartolo DC, Read MG. Differences in anal function in patients with incontinence to solids and in patients with incontinence to liquids. *The British journal of surgery.* 1984;71(1):39-42.
326. Menees SB, Almario CV, Spiegel BMR, Chey WD. Prevalence of and Factors Associated With Fecal Incontinence: Results From a Population-Based Survey. *Gastroenterology.* 2018;154(6):1672-1681.e1673.
327. Rao SS, Bharucha AE, Chiarioni G, et al. Functional Anorectal Disorders. *Gastroenterology.* 2016.
328. Meinds RJ, van Meegdenburg MM, Trzpis M, Broens PM. On the prevalence of constipation and fecal incontinence, and their co-occurrence, in the Netherlands. *International journal of colorectal disease.* 2017;32(4):475-483.
329. Luber KM. The definition, prevalence, and risk factors for stress urinary incontinence. *Rev Urol.* 2004;6 Suppl 3:S3-9.
330. Vollebregt PF, Burgell RE, Hooper RL, Knowles CH, Scott SM. Clinical Impact of Rectal Hyposensitivity: A Cross-Sectional Study of 2,876 Patients With Refractory Functional Constipation. *Am J Gastroenterol.* 2020.
331. Mohammed SD, Lunniss PJ, Zarate N, et al. Joint hypermobility and rectal evacuatory dysfunction: an etiological link in abnormal connective tissue? *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2010;22(10):1085-e1283.
332. Vaizey CJ, Carapeti E, Cahill JA, Kamm MA. Prospective comparison of faecal incontinence grading systems. *Gut.* 1999;44(1):77-80.
333. Agachan F, Chen T, Pfeifer J, Reissman P, Wexner SD. A constipation scoring system to simplify evaluation and management of constipated patients. *Diseases of the colon and rectum.* 1996;39(6):681-685.
334. Hinton JM, Lennard-Jones JE, Young AC. A ne method for studying gut transit times using radioopaque markers. *Gut.* 1969;10(10):842-847.
335. Palit S, Bhan C, Lunniss PJ, et al. Evacuation proctography: a reappraisal of normal variability. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland.* 2014;16(7):538-546.
336. Dvorkin LS, Knowles CH, Scott SM, Williams NS, Lunniss PJ. Rectal intussusception: characterization of symptomatology. *Diseases of the colon and rectum.* 2005;48(4):824-831.
337. Adusumilli S, Gosselink MP, Fourie S, et al. Does the presence of a high grade internal rectal prolapse affect the outcome of pelvic floor retraining in patients with faecal incontinence or obstructed defaecation? *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland.* 2013;15(11):e680-685.
338. Roberts JP, Newell MS, Deeks JJ, Waldron DW, Garvie NW, Williams NS. Oral [111In]DTPA scintigraphic assessment of colonic transit in constipated subjects. *Dig Dis Sci.* 1993;38(6):1032-1039.
339. Mearin F, Lacy BE, Chang L, et al. Bowel Disorders. *Gastroenterology.* 2016.
340. Cornacchia M, Zenorini A, Perobelli S, Zanolla L, Mastella G, Braggion C. Prevalence of urinary incontinence in women with cystic fibrosis. *BJU Int.* 2001;88(1):44-48.
341. Lien KC, Morgan DM, Delancey JO, Ashton-Miller JA. Pudendal nerve stretch during vaginal birth: a 3D computer simulation. *Am J Obstet Gynecol.* 2005;192(5):1669-1676.
342. Deffieux X, Hubeaux K, Faivre E, et al. Sacral reflexes and urinary incontinence in women: new concepts. *Ann Phys Rehabil Med.* 2009;52(3):256-268.
343. PARKS AG, PORTER NH, MELZAK J. Experimental study of the reflex mechanism controlling the muscle of the pelvic floor. *Diseases of the colon and rectum.* 1962;5:407-414.

344. Deffieux X, Hubeaux K, Porcher R, Ismael SS, Raibaut P, Amarenco G. Abnormal pelvic response to cough in women with stress urinary incontinence. *Neurourology and urodynamics.* 2008;27(4):291-296.
345. GARRY RC, ROBERTS TD, TODD JK. Reflexes involving the external urethral sphincter in the cat. *J Physiol.* 1959;149:653-665.
346. Fowler CJ, Griffiths D, de Groat WC. The neural control of micturition. *Nat Rev Neurosci.* 2008;9(6):453-466.
347. Park JM, Bloom DA, McGuire EJ. The guarding reflex revisited. *Br J Urol.* 1997;80(6):940-945.
348. AJ W. Neuromuscular dysfunction of the lower urinary tract. In: PC W, AB R, TA S, ED V, eds. *Campbell's urology.* Vol 2. 6th edn ed.: Saunders Co.; 1992:573-642.
349. Kiff ES, Barnes PR, Swash M. Evidence of pudendal neuropathy in patients with perineal descent and chronic straining at stool. *Gut.* 1984;25(11):1279-1282.
350. Brandt FT, Lorenzato F, Albuquerque CD, Junior AeS, de Carvalho Poça A, Viana RA. Tension-free vaginal tape versus lata fascia sling: The importance of transvulvar ultrasound in the assessment of relevant anatomical parameters in treatment of women with stress urinary incontinence. *Indian J Urol.* 2009;25(1):62-67.
351. Yin Y, Xia Z, Feng X, Luan M, Qin M. Three-Dimensional Transperineal Ultrasonography for Diagnosis of Female Occult Stress Urinary Incontinence. *Med Sci Monit.* 2019;25:8078-8083.
352. Patel PD, Amrute KV, Badlani GH. Pelvic organ prolapse and stress urinary incontinence: A review of etiological factors. *Indian J Urol.* 2007;23(2):135-141.
353. DeLancey JO. Structural support of the urethra as it relates to stress urinary incontinence: the hammock hypothesis. *Am J Obstet Gynecol.* 1994;170(6):1713-1720; discussion 1720-1713.
354. ENHORNING G. Functional sphincterometry-a test for stress incontinence. *Urol Int.* 1960;10:129-136.
355. de Vries AM, Heesakkers JPFA. Contemporary diagnostics and treatment options for female stress urinary incontinence. *Asian J Urol.* 2018;5(3):141-148.
356. DeLancey JO, Trowbridge ER, Miller JM, et al. Stress urinary incontinence: relative importance of urethral support and urethral closure pressure. *J Urol.* 2008;179(6):2286-2290; discussion 2290.
357. Delancey JO. Why do women have stress urinary incontinence? *Neurourology and urodynamics.* 2010;29 Suppl 1:S13-17.
358. Johnson JD, Lamensdorf H, Hollander IN, Thurman AE. Use of transvaginal endosonography in the evaluation of women with stress urinary incontinence. *J Urol.* 1992;147(2):421-425.
359. Parks AG, Porter NH, Hardcastle J. The syndrome of the descending perineum. *Proc R Soc Med.* 1966;59(6):477-482.
360. Pinna Pintor M, Zara GP, Falletto E, et al. Pudendal neuropathy in diabetic patients with faecal incontinence. *International journal of colorectal disease.* 1994;9(2):105-109.
361. Cook IJ, Talley NJ, Benninga MA, Rao SS, Scott SM. Chronic constipation: overview and challenges. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2009;21 Suppl 2:1-8.
362. Bampton PA, Dinning PG, Kennedy ML, Lubowski DZ, deCarle D, Cook IJ. Spatial and temporal organization of pressure patterns throughout the unprepared colon during spontaneous defecation. *Am J Gastroenterol.* 2000;95(4):1027-1035.
363. Bassotti G, Gaburri M. Manometric investigation of high-amplitude propagated contractile activity of the human colon. *The American journal of physiology.* 1988;255(5 Pt 1):G660-664.

364. Rao SS, Sadeghi P, Beaty J, Kavlock R, Ackerson K. Ambulatory 24-h colonic manometry in healthy humans. *Am J Physiol Gastrointest Liver Physiol.* 2001;280(4):G629-639.
365. Di Lorenzo C, Flores AF, Hyman PE. Age-related changes in colon motility. *J Pediatr.* 1995;127(4):593-596.
366. Scott SM. Manometric techniques for the evaluation of colonic motor activity: current status. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2003;15(5):483-513.
367. Connell AM, Hilton C, Irvine G, Lennard-Jones JE, Misiewicz JJ. Variation of bowel habit in two population samples. *Br Med J.* 1965;2(5470):1095-1099.
368. Palit S, Lunniss PJ, Scott SM. The physiology of human defecation. *Dig Dis Sci.* 2012;57(6):1445-1464.
369. Hampel C, Wienhold D, Benken N, Eggersmann C, Thüroff JW. Definition of overactive bladder and epidemiology of urinary incontinence. *Urology.* 1997;50(6A Suppl):4-14; discussion 15-17.
370. Gunnarsson M, Mattiasson A. Female stress, urge, and mixed urinary incontinence are associated with a chronic and progressive pelvic floor/vaginal neuromuscular disorder: An investigation of 317 healthy and incontinent women using vaginal surface electromyography. *Neurourology and urodynamics.* 1999;18(6):613-621.
371. Bump RC, Norton PA. Epidemiology and natural history of pelvic floor dysfunction. *Obstet Gynecol Clin North Am.* 1998;25(4):723-746.
372. D'Agata EM. Methodologic issues of case-control studies: a review of established and newly recognized limitations. *Infect Control Hosp Epidemiol.* 2005;26(4):338-341.
373. Pirro N, Sastre B, Sielezneff I. [What are the risk factors of anal incontinence after vaginal delivery?]. *J Chir (Paris).* 2007;144(3):197-202.
374. Brochard C, Bouguen G, Bodere A, et al. Prospective cohort study of phenotypic variation based on an anal sphincter function in adults with fecal incontinence. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2016;28(10):1554-1560.
375. Cauley CE, Savitt LR, Weinstein M, et al. A Quality-of-Life Comparison of Two Fecal Incontinence Phenotypes: Isolated Fecal Incontinence Versus Concurrent Fecal Incontinence With Constipation. *Diseases of the colon and rectum.* 2019;62(1):63-70.
376. Liu J, Guaderrama N, Nager CW, Pretorius DH, Master S, Mittal RK. Functional correlates of anal canal anatomy: puborectalis muscle and anal canal pressure. *Am J Gastroenterol.* 2006;101(5):1092-1097.
377. Cheeney G, Remes-Troche JM, Attaluri A, Rao SS. Investigation of anal motor characteristics of the sensorimotor response (SMR) using 3-D anorectal pressure topography. *Am J Physiol Gastrointest Liver Physiol.* 2011;300(2):G236-240.
378. Mundet L, Cabib C, Ortega O, et al. Defective Conduction of Anorectal Afferents Is a Very Prevalent Pathophysiological Factor Associated to Fecal Incontinence in Women. *Journal of neurogastroenterology and motility.* 2019;25(3):423-435.
379. Kumar D. In: *Clinical Measurement in Coloproctology.* Great Britain: Springer-Verlag; 1991:37-42.
380. Barnett JL, Hasler WL, Camilleri M. American Gastroenterological Association medical position statement on anorectal testing techniques. American Gastroenterological Association. *Gastroenterology.* 1999;116(3):732-760.
381. M S, SJ S. Electromyography in pelvic floor disorders. In: *Coloproctology and the Pelvic floor: Pathophysiology and management.* London: Butterworth and Co; 1985:99.
382. Taverner D, Smiddy FG. An electromyographic study of the normal function of the external anal sphincter and pelvic diaphragm. *Diseases of the colon and rectum.* 1959;2(2):153-160.
383. Gowers R. The automatic action of the sphincter ani. 1877:77-84.

384. Gosling J, Plumb A, Taylor SA, Cohen R, Emmanuel AV. High-resolution anal manometry: Repeatability, validation, and comparison with conventional manometry. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2019;31(6):e13591.
385. Read NW, Harford WV, Schmulen AC, Read MG, Santa Ana C, Fordtran JS. A clinical study of patients with fecal incontinence and diarrhea. *Gastroenterology.* 1979;76(4):747-756.
386. Neill ME, Parks AG, Swash M. Physiological studies of the anal sphincter musculature in faecal incontinence and rectal prolapse. *The British journal of surgery.* 1981;68(8):531-536.
387. Cali RL, Blatchford GJ, Perry RE, Pitsch RM, Thorson AG, Christensen MA. Normal variation in anorectal manometry. *Diseases of the colon and rectum.* 1992;35(12):1161-1164.
388. Leo CA, Cavazzoni E, Thomas GP, Hodgkison J, Murphy J, Vaizey CJ. Evaluation of 153 Asymptomatic Subjects Using the Anopress Portable Anal Manometry Device. *Journal of neurogastroenterology and motility.* 2018;24(3):431-436.
389. MELZAK J, PORTER NH. STUDIES OF THE REFLEX ACTIVITY OF THE EXTERNAL SPHINCTER ANI IN SPINAL MAN. *Paraplegia.* 1964;1:277-296.
390. Chaliha C, Kalia V, Stanton SL, Monga A, Sultan AH. Antenatal prediction of postpartum urinary and fecal incontinence. *Obstet Gynecol.* 1999;94(5 Pt 1):689-694.
391. Laurberg S, Swash M, Henry MM. Delayed external sphincter repair for obstetric tear. *The British journal of surgery.* 1988;75(8):786-788.
392. Basilisco G, Bharucha AE. High-resolution anorectal manometry: An expensive hobby or worth every penny? *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2017;29(8).
393. Carrington EV. *Studies of anorectal function using high resolution anorectal manometry in health and faecal incontinence*, Queen Mary, University of London; 2017.
394. Orkin BA, Hanson RB, Kelly KA, Phillips SF, Dent J. Human anal motility while fasting, after feeding, and during sleep. *Gastroenterology.* 1991;100(4):1016-1023.
395. Hancock BD. Measurement of anal pressure and motility. *Gut.* 1976;17(8):645-651.
396. Ho YH, Tan M, Leong AF, Seow-Choen F. Ambulatory manometry in patients with colonic J-pouch and straight coloanal anastomoses: randomized, controlled trial. *Diseases of the colon and rectum.* 2000;43(6):793-799.
397. Vassilakis JS, Pechlivanides G, Zoras OJ, et al. Anorectal function after low anterior resection of the rectum. *International journal of colorectal disease.* 1995;10(2):101-106.
398. Wankling WJ, Brown BH, Collins CD, Duthie HL. Basal electrical activity in the anal canal in man. *Gut.* 1968;9(4):457-460.
399. Yoshino H, Kayaba H, Hebiguchi T, et al. Multiple clinical presentations of anal ultra slow waves and high anal pressure: megacolon, hemorrhoids and constipation. *Tohoku J Exp Med.* 2007;211(2):127-132.
400. Wiklendt L, Costa M, Brookes S, Dinning PG. Bayesian Functional Mixed-Effects Model with Gaussian Process Responses for Wavelet Spectra of Spatiotemporal Colonic Manometry Signals. 2019:arXiv:1912.02389. https://ui.adsabs.harvard.edu/abs/2019arXiv191202389W. Accessed December 01, 2019.
401. Wiklendt L, Brookes SJH, Costa M, Travis L, Spencer NJ, Dinning PG. A Novel Method for Electrophysiological Analysis of EMG Signals Using MesaClip. *Front Physiol.* 2020;11:484.
402. Williamson ME, Lewis WG, Holdsworth PJ, Finan PJ, Johnston D. Decrease in the anorectal pressure gradient after low anterior resection of the rectum. A study using

continuous ambulatory manometry. *Diseases of the colon and rectum.* 1994;37(12):1228-1231.
403. Bharucha AE, Zinsmeister AR, Locke GR, Schleck C, McKeon K, Melton LJ. Symptoms and quality of life in community women with fecal incontinence. *Clinical gastroenterology and hepatology : the official clinical practice journal of the American Gastroenterological Association.* 2006;4(8):1004-1009.
404. Staller K, Townsend MK, Khalili H, et al. Menopausal Hormone Therapy Is Associated With Increased Risk of Fecal Incontinence in Women After Menopause. *Gastroenterology.* 2017;152(8):1915-1921 e1911.
405. Bharucha AE, Zinsmeister AR, Locke GR, et al. Prevalence and burden of fecal incontinence: a population-based study in women. *Gastroenterology.* 2005;129(1):42-49.
406. Hall KA, Ward SM, Cobine CA, Keef KD. Spatial organization and coordination of slow waves in the mouse anorectum. *J Physiol.* 2014;592(17):3813-3829.
407. Whitehead WE, Borrud L, Goode PS, et al. Fecal incontinence in US adults: epidemiology and risk factors. *Gastroenterology.* 2009;137(2):512-517, 517.e511-512.
408. Scott SM, Knowles CH, Wang D, et al. The nocturnal jejunal migrating motor complex: defining normal ranges by study of 51 healthy adult volunteers and meta-analysis. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2006;18(10):927-935.
409. Wang YT, Mohammed SD, Farmer AD, et al. Regional gastrointestinal transit and pH studied in 215 healthy volunteers using the wireless motility capsule: influence of age, gender, study country and testing protocol. *Alimentary pharmacology & therapeutics.* 2015;42(6):761-772.
410. Wiklendt L, Mohammed SD, Scott SM, Dinning PG. Classification of normal and abnormal colonic motility based on cross-correlations of pancolonic manometry data. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2013;25(3):e215-223.
411. Knowles AM, Knowles CH, Scott SM, Lunniss PJ. Effects of age and gender on three-dimensional endoanal ultrasonography measurements: development of normal ranges. *Techniques in coloproctology.* 2008;12(4):323-329.
412. Frank L, Kleinman L, Farup C, Taylor L, Miner P, Jr. Psychometric validation of a constipation symptom assessment questionnaire. *Scandinavian journal of gastroenterology.* 1999;34(9):870-877.
413. Smits-Engelsman B, Klerks M, Kirby A. Beighton score: a valid measure for generalized hypermobility in children. *J Pediatr.* 2011;158(1):119-123, 123 e111-114.
414. Lewis SJ, Heaton KW. Stool form scale as a useful guide to intestinal transit time. *Scandinavian journal of gastroenterology.* 1997;32(9):920-924.
415. O'Donnell P, Beck C, Doyle R, Eubanks C. Surface electrodes in perineal electromyography. *Urology.* 1988;32(4):375-379.
416. Houghton LA, Fell C, Whorwell PJ, Jones I, Sudworth DP, Gale JD. Effect of a second-generation alpha2delta ligand (pregabalin) on visceral sensation in hypersensitive patients with irritable bowel syndrome. *Gut.* 2007;56(9):1218-1225.
417. Houghton LA, Cremonini F, Camilleri M, et al. Effect of the NK(3) receptor antagonist, talnetant, on rectal sensory function and compliance in healthy humans. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2007;19(9):732-743.
418. Algladi T, Harris M, Whorwell PJ, Paine P, Hamdy S. Modulation of human visceral sensitivity by noninvasive magnetoelectrical neural stimulation in health and irritable bowel syndrome. *Pain.* 2015;156(7):1348-1356.

419. Rao SSC, Yan Y, Erdogan A, et al. Barostat or syringe-assisted sensory biofeedback training for constipation with rectal hyposensitivity: A randomized controlled trial. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2021:e14226.
420. Felt-Bersma RJ, Sloots CE, Poen AC, Cuesta MA, Meuwissen SG. Rectal compliance as a routine measurement: extreme volumes have direct clinical impact and normal volumes exclude rectum as a problem. *Diseases of the colon and rectum.* 2000;43(12):1732-1738.
421. Gladman MA, Scott SM, Williams NS, Lunniss PJ. Clinical and physiological findings, and possible aetiological factors of rectal hyposensitivity. *The British journal of surgery.* 2003;90(7):860-866.
422. Scott SM, van den Berg MM, Benninga MA. Rectal sensorimotor dysfunction in constipation. *Best practice & research Clinical gastroenterology.* 2011;25(1):103-118.
423. Scientific M. Rapid Barostat Bag Pump User Manual. In:2021.
424. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet.* 1986;1(8476):307-310.
425. Carkeet A. Exact parametric confidence intervals for Bland-Altman limits of agreement. *Optom Vis Sci.* 2015;92(3):e71-80.
426. Carkeet A. A Review of the Use of Confidence Intervals for Bland-Altman Limits of Agreement in Optometry and Vision Science. *Optom Vis Sci.* 2020;97(1):3-8.
427. Awad RA. The transcendence of assess rectal sensitivity with a barostat. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2020;32(1):e13760.
428. Krogh K, Ryhammer AM, Lundby L, Gregersen H, Laurberg TS. Comparison of methods used for measurement of rectal compliance. *Diseases of the colon and rectum.* 2001;44(2):199-206.
429. Fox M, Thumshirn M, Frühauf H, Fried M, Schwizer W. Determinants of fecal continence in healthy, continent subjects: a comprehensive analysis by anal manometry, rectal barostat and a stool substitute retention test. *Digestion.* 2011;83(1-2):46-53.
430. Giavarina D. Understanding Bland Altman analysis. *Biochem Med (Zagreb).* 2015;25(2):141-151.
431. Krouwer JS. Why Bland-Altman plots should use X, not (Y+X)/2 when X is a reference method. *Stat Med.* 2008;27(5):778-780.
432. Lagier E, Delvaux M, Vellas B, et al. Influence of age on rectal tone and sensitivity to distension in healthy subjects. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 1999;11(2):101-107.
433. Siproudhis L, Bellissant E, Juguet F, Allain H, Bretagne JF, Gosselin M. Perception of and adaptation to rectal isobaric distension in patients with faecal incontinence. *Gut.* 1999;44(5):687-692.
434. Malcolm A, Camilleri M. Coloanal motor coordination in association with high-amplitude colonic contractions after pharmacological stimulation. *Am J Gastroenterol.* 2000;95(3):715-719.
435. Lembo T, Munakata J, Mertz H, et al. Evidence for the hypersensitivity of lumbar splanchnic afferents in irritable bowel syndrome. *Gastroenterology.* 1994;107(6):1686-1696.
436. Anonymous. The NLIN procedure. In: SAS/STAT User's Guide (version 6). In. Cary, NC, USA: SAS Inst, Inc; 1989:1135–1194.
437. Mertz H, Naliboff B, Mayer EA. Symptoms and physiology in severe chronic constipation. *Am J Gastroenterol.* 1999;94(1):131-138.
438. Crowell MD. Pathogenesis of slow transit and pelvic floor dysfunction: from bench to bedside. *Rev Gastroenterol Disord.* 2004;4 Suppl 2:S17-27.

439. Saldana Ruiz NK, AM. Fecal incontinence - Challenges and solutions. *World journal of gastroenterology.* 2017;23(1):11-24.
440. Loening-Baucke V, Pringle KC, Ekwo EE. Anorectal manometry for the exclusion of Hirschsprung's disease in neonates. *Journal of pediatric gastroenterology and nutrition.* 1985;4(4):596-603.
441. Meunier P, Marechal JM, de Beaujeu MJ. Rectoanal pressures and rectal sensitivity studies in chronic childhood constipation. *Gastroenterology.* 1979;77(2):330-336.
442. Salvioli B, Bharucha AE, Rath-Harvey D, Pemberton JH, Phillips SF. Rectal compliance, capacity, and rectoanal sensation in fecal incontinence. *Am J Gastroenterol.* 2001;96(7):2158-2168.
443. Boeckxstaens GE, Drug V, Dumitrascu D, et al. Phenotyping of subjects for large scale studies on patients with IBS. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2016;28(8):1134-1147.
444. Bouin M, Plourde V, Boivin M, et al. Rectal distention testing in patients with irritable bowel syndrome: sensitivity, specificity, and predictive values of pain sensory thresholds. *Gastroenterology.* 2002;122(7):1771-1777.
445. Kuiken SD, Lindeboom R, Tytgat GN, Boeckxstaens GE. Relationship between symptoms and hypersensitivity to rectal distension in patients with irritable bowel syndrome. *Alimentary pharmacology & therapeutics.* 2005;22(2):157-164.
446. Morel Cerda EC, Garcia-Zermeno K, Amieva-Balmori M, Aquino-Ruiz A, Remes-Troche JM. Sensitivity and rectal capacity in patients with anorectal disorders. A study using novel Rapid Barostat Bag (RBB) pump. 2020.
447. Rosier EM, Iadarola MJ, Coghill RC. Reproducibility of pain measurement and pain perception. *Pain.* 2002;98(1-2):205-216.
448. Reeve D. Anatomy of the Sphincters of the Alimentary Canal. In: Thomas PMC, ed. *Alimentary Sphincters and their Disorders* UK: Palgrave Macmillan; 1981:1-26.
449. Harris LD, Winans CS, Pope CE, 2nd. Determination of yield pressures: a method for measuring anal sphincter competence. *Gastroenterology.* 1966;50(6):754-760.
450. Harris LD, Pope CE, 2nd. "Squeeze" Vs. Resistance: An Evaluation of the Mechanism of Sphincter Competence. *The Journal of clinical investigation.* 1964;43:2272-2278.
451. McMahon BP, Frokjaer JB, Kunwald P, et al. The functional lumen imaging probe (FLIP) for evaluation of the esophagogastric junction. *Am J Physiol Gastrointest Liver Physiol.* 2007;292(1):G377-384.
452. Kwiatek MA, Pandolfino JE, Hirano I, Kahrilas PJ. Esophagogastric junction distensibility assessed with an endoscopic functional luminal imaging probe (EndoFLIP). *Gastrointest Endosc.* 2010;72(2):272-278.
453. Gregersen H, Djurhuus JC. Impedance planimetry: a new approach to biomechanical intestinal wall properties. *Digestive diseases.* 1991;9(6):332-340.
454. Luft F, Fynne L, Gregersen H, et al. Functional luminal imaging probe: a new technique for dynamic evaluation of mechanical properties of the anal canal. *Techniques in coloproctology.* 2012;16(6):451-457.
455. Pandolfino JE, de Ruigh A, Nicodeme F, Xiao Y, Boris L, Kahrilas PJ. Distensibility of the esophagogastric junction assessed with the functional lumen imaging probe (FLIP) in achalasia patients. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2013;25(6):496-501.
456. Desprez C, Roman S, Leroi AM, Gourcerol G. The use of impedance planimetry (Endoscopic Functional Lumen Imaging Probe, EndoFLIP((R))) in the gastrointestinal tract: A systematic review. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2020;32(9):e13980.

457. Gronlund D, Poulsen JL, Krogh K, et al. The impact of naloxegol on anal sphincter function - Using a human experimental model of opioid-induced bowel dysfunction. *Eur J Pharm Sci.* 2018;117:187-192.
458. Haas S, Faaborg P, Liao D, et al. Anal sphincter dysfunction in patients treated with primary radiotherapy for anal cancer: a study with the functional lumen imaging probe. *Acta Oncol.* 2018;57(4):465-472.
459. Desprez C, Melchior C, Gourcerol G, et al. Assessment of anal sphincter distensibility following the STARR procedure: a pilot study. *Acta Chir Belg.* 2020;120(3):198-201.
460. Brusa T, Abler D, Tutuian R, et al. Anatomy and mechanical properties of the anal sphincter muscles in healthy senior volunteers. *Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society.* 2018.
461. Brusa T, Abler D, Tutuian R, et al. MR-FLIP: A new method that combines FLIP with anatomical information for the spatial compliance assessment of the anal sphincter muscles. *Colorectal disease : the official journal of the Association of Coloproctology of Great Britain and Ireland.* 2016.
462. Tuttle LJ, Zifan A, Sun C, Swartz J, Roalkvam S, Mittal RK. Measuring length-tension function of the anal sphincters and puborectalis muscle using the functional luminal imaging probe. *Am J Physiol Gastrointest Liver Physiol.* 2018;315(5):G781-G787.
463. Chen SC, Futaba K, Leung WW, et al. Functional Lumen Imaging Probe Assessment of Anal Canal Distensibility [Abst]. 2018.
464. Bredenoord AJ, Smout AJ. Advances in motility testing--current and novel approaches. *Nature reviews Gastroenterology & hepatology.* 2013;10(8):463-472.
465. Zifan A, Mittal RK, Kunkel DC, Swartz J, Barr G, Tuttle LJ. Loop analysis of the anal sphincter complex in fecal incontinent patients using functional luminal imaging probe. *Am J Physiol Gastrointest Liver Physiol.* 2020;318(1):G66-G76.
466. Kumar L. *Investigating the utility of EndoFLIP, a novel diagnostic modality for assessing anal canal function*, University College London (University of London); 2020.
467. Jung SA, Pretorius DH, Weinstein M, Nager CW, Den-Boer D, Mittal RK. Closure mechanism of the anal canal in women: assessed by three-dimensional ultrasound imaging. *Diseases of the colon and rectum.* 2008;51(6):932-939.
468. Chen HM, Li BW, Li LY, et al. Functional lumen imaging probe in gastrointestinal motility diseases. *J Dig Dis.* 2019;20(11):572-577.
469. Brett L. *PMSL: Or How I Literally Pissed Myself Laughing.* London, UK: Green Tree, Bloomsbury Publishing Plc; 2020.
470. Rubin EB, Buehler AE, Halpern SD. States Worse Than Death Among Hospitalized Patients With Serious Illnesses. *JAMA Internal Medicine.* 2016;176(10):1557-1559.

List of Appendices

Appendix 1

Chapter 2 Systematice review: detailed search strategy

AND	"fecal incontinence"[All Fields] OR "faecal incontinence"[All Fields] OR "urge incontinence"[All Fields] OR "pelvic floor dysfunction"[All Fields] OR "overflow incontinence"[All Fields] OR "involuntary loss of stool"[All Fields] OR "soiling"[All Fields] OR "functional anorectal disorders"[All Fields] OR "anal sphincter weakness"[All Fields] OR "fecal seepage"[All Fields] OR "faecal seepage"[All Fields] OR "sphincteric motor dysfunction"[All Fields] OR "flatus incontinence"[All Fields] OR "anatomic disturbance of the pelvic floor"[All Fields] OR "anal incontinence"[All Fields] OR "anorectal incontinence"[All Fields] OR "bowel incontinence"[All Fields] OR "passive incontinence"[All Fields] OR "post-defaecation leakage"[All Fields] OR "feces incontinence"[All Fields] OR "anus incontinence"[All Fields] OR "encopresis"[All Fields] OR "anal sphincter dysfunction"[All Fields]
AND	"anal manometry"[All Fields] OR "anorectal manometry"[All Fields] OR "conventional manometry"[All Fields] OR "high resolution manometry"[All Fields] OR "high-resolution anorectal manometry"[All Fields] OR "high definition manometry"[All Fields] or "high-definition anorectal manometry"[All Fields] OR "rectal sensory testing"[All Fields] OR "rectal sensation"[All Fields] OR "rectal sensitivity"[All Fields] OR "rectal sensibility"[All Fields] OR "sensation test"[All Fields] OR "balloon distension"[All Fields] OR "balloon expulsion"[All Fields] OR "balloon catheter"[All Fields] OR "rectal distension"[All Fields]
AND	"resting pressure"[All Fields] OR "resting tone" [All Fields] OR "rest" [All Fields] OR "squeeze"[All Fields] OR "contraction" [All Fields] OR "voluntary contraction" OR "sensation"[All Fields] OR "sensibility"[All Fields] OR "sensory"[All Fields] OR "first sensation"[All Fields] OR "desire to defecate"[All Fields] OR "urgency to defecate"[All Fields] OR "first constant sensation"[All Fields] OR "urge sensation"[All Fields] OR "first urge sensation"[All Fields]OR "desire to defaecate"[All Fields] OR "urgency to defaecate"[All Fields] OR "urge to defecate"[All Fields] OR "urge to defaecate"[All Fields] OR "volume"[All Fields] OR "maximum tolerable volume"[All Fields]

	Sources of bias addressed	McHugh 1987	Felt-Bersma 1990	Sun 1990	Delechenaut 1992	Bharucha 2005	Hotouras 2012	Burgell 2012	Paramor 2014	Townsend 2016	Vollebregt 2018	Leroi 2018	Carrington 2018	Heitmann 2019
1	Was the research question or objective in this paper clearly stated and appropriate?	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2	Was the study population clearly specified and defined?	Yes	Yes	Yes	No	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3	Did the authors include a sample size justification?	No	No	No	No	No	No	No	No	No	No	No	No	No
4	Were controls selected or recruited from the same or similar population that gave rise to the cases (including the same time frame?)	Yes	Yes	Yes	No	Yes	No	Yes	No	Yes	No	Yes	Yes	No
5	Were the definitions, inclusion and exclusion criteria, algorithms or processes used to identify or select cases and controls valid, reliable, and implemented consistently across all study participants?	Yes	No	Yes	No	Yes	No	Yes	Yes	Yes	Yes	No	Yes	No

6	Were the cases clearly defined and differentiated from controls?	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No
7	If less than 100 percent of eligible cases and/or controls were selected for the study, were the cases and/or controls randomly selected from those eligible?	No	NA	No	No	No	No	No	No	No	Yes	No	No	No
8	Was there use of concurrent controls?	Yes	No	Yes	No	Yes	No	No	No	No	No	Yes	No	No
9	Were the investigators able to confirm that the exposure/risk occurred prior to the development of the condition or event that defined a participant as a case? We're patients consented prior to the study?	CD	CD	Yes	No	Yes	CD	CD	CD	CD	CD	No	Yes	CD
10	Were the measures of exposure/risk clearly defined, valid, reliable, and implemented consistently (including the same time period) across all study participants?	Yes	No	No	No	Yes	No	Yes	Yes	Yes	Yes	No	Yes	No
11	Were the assessors of exposure/risk blinded to the	No	No	No	No	No	No	No	No	No	No	No	Yes	No

	case or control status of participants?													
12	Were key potential confounding variables measured and adjusted statistically in the analyses? If matching was used, did the investigators account for matching during study analysis?	Yes	Yes	Yes	No	Yes	No	Yes	No	No	Yes	Yes	Yes	No
	Total Yes	**8**	**4**	**7**	**2**	**9**	**2**	**7**	**5**	**6**	**7**	**6**	**9**	**2**
	Total No	**3**	**6**	**5**	**10**	**3**	**8**	**4**	**6**	**5**	**4**	**6**	**3**	**9**
	Total CD	**1**	**1**	**0**	**0**	**0**	**1**	**1**	**1**	**1**	**0**	**0**	**0**	**1**
	Total NA	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
	Max possible	**11**	**10**	**12**	**12**	**12**	**11**	**11**	**11**	**11**	**12**	**12**	**12**	**11**
	Total score from possible maximum (%)	**73%**	**40%**	**58%**	**17%**	**75%**	**18%**	**64%**	**45%**	**55%**	**58%**	**50%**	**75%**	**18%**

Appendix 3

This table shows the number of subjects desired and achieved for case-control matching of stress and non-stress FI subjects for gender, parity, and age.

Age group	Controls					
	Males		Nulliparous females		Parous females	
	Desired	Achieved	Desired	Achieved	Desired	Achieved
18-25	4	4	24	24	4	4
26-30	12	11	22	17	22	22
31-35	14	14	14	10	44	44
36-40	8	8	6	6	54	54
41-45	14	14	6	6	72	72
46-50	24	24	8	8	104	104
51-55	18	18	14	14	126	126
56-60	24	24	10	10	146	146
61-65	16	16	6	6	106	106
66-70	12	12	2	2	106	106
71-75	14	14	4	4	78	78
76-80	12	12	0	0	40	40
Total	**172**	**171**	**116**	**107**	**902**	**902**

Appendix 4

Chapter 6 Prospective methods PIS

Queen Mary
University of London

NEUROGASTROENTEROLOGY GROUP

The Wingate Institute for Neurogastroenterology, 26, Ashfield Street, LONDON E1 2AJ
Neuroscience & Trauma, Blizard, QMUL

Participant Information Sheet

Measurement of anorectal function in healthy volunteers

We would like to invite you to be part of this research project, if you would like to. You should only agree to take part if you want to, it is entirely up to you. If you choose not to take part there won't be any disadvantages for you and you will hear no more about it.

Please read the following information carefully before you decide to take part; this will tell you why the research is being done and what you will be asked to do if you take part. Please ask if there is anything that is not clear or if you would like more information.

If you decide to take part you will be asked to sign the attached form to say that you agree.

You are still free to withdraw at any time and without giving a reason.

WHY IS THIS STUDY NEEDED?

A sound definition of what is normal in health is the basis of understanding any disease and its treatment. Maintaining bowel function is something most people do not worry about. However, some people have difficulties 'holding on' to stool (incontinence), emptying the bowel (constipation) or both. Affected persons may be afraid to leave their home for fear of losing stool, avoid eating, or are troubled by pain and bloating. These problems are often not mentioned to the doctor for fear of social stigma and feelings of embarrassment. How the back passage and the rectum (the storage part of the bowel) work can be assessed using a variety of tests that help us understand why bowel problems might be happening. These tests look at different aspects of bowel function: muscle strength and flexibility of the back passage and awareness of stool in the rectum. To interpret these tests properly, we first need to understand what is considered 'normal'. We do this by completing the tests in healthy individuals without bowel problems.

WHAT ARE THE AIMS OF THE STUDY?

This study aims to measure function of the lower bowel (rectum) and back passage (anus) in healthy people using new technology, to ultimately help people with bowel problems. We also want to assess how well new equipment for testing rectal sensation compares with older techniques.

WHY HAVE I BEEN INVITED?

You have been asked to participate in this study as you have indicated that you are an adult over the age of 18 with no history of bowel problems, or symptoms of incontinence or constipation.

DO I HAVE TO TAKE PART?

No. Participating in this research study is entirely voluntary. You should take as long as you wish to consider the information in this document and, if you wish, discuss it with any family, friends or your GP. You can contact us if you require any clarification or further explanation. You can also contact INVOLVE (http://www.invo.org.uk/), which is a national advisory group that supports greater public involvement in NHS, public health and social care research, who share knowledge and learning on public involvement in research.

Should you decide to participate you will be asked to sign a consent form. You will be given a copy of the Information Sheet and Signed Consent Form for your records. If you do decide to take part you are still free to withdraw at any time and without giving a reason.

WHAT WILL HAPPEN TO ME IF I DECIDE TO TAKE PART?

If you are interested in taking part in the study and with your permission, we will contact you by telephone to check that you fit the study criteria. We will ask you some simple questions about your bowels, your age and whether or not you have had any children. If you are eligible to take part in the study, a study visit will be scheduled.

Your participation will require one study visit to the Wingate Institute for Neurogastroenterology. This visit will last for about 3 hours (see section on expenses and travel below). You will be asked to complete a number of short questionnaires regarding your bowels and undergo tests of bowel

function. After your visit you will be asked to keep a bowel diary for 7 days. This can be sent back using the prepaid envelope provided to you.

On the day of your visit, you will be asked to have nothing to eat or drink (except for water) for at least 3 hours before you attend. When you arrive, you will meet the researcher who will go over the information in this leaflet to make sure you understand what participation in the study will mean. You will then be asked to sign a consent form.

You may also wish to participate in an additional test (Standard Barostat) that will help us compare old and new equipment for measuring rectal sensation. You will be asked if you wish to consent to this 'extra' procedure when you sign the consent form.

You will be asked to complete five short questionnaires about your bowel function and a brief medical history will taken by one of the researchers to confirm that you are fully eligible to take part in the study. If you are a woman, you will be asked about any deliveries (births) that you may have had. We will make a record of your height and weight.

The following tests of bowel function will then be performed (we will provide a chaperone during the tests):

a. The researcher will examine your back passage with a gloved finger. This is a brief test that will take less than one minute.

b. The strength of the anal muscles will then be measured with a flexible tube (**manometry** catheter) that is about half the width of a pencil; there is a flat balloon on the end. This will be inserted through the back passage and secured to the buttock with sticky tape. Small stickers that measures muscle contraction (electrodes) will be placed near the anus. You will be asked to do a series of manoeuvres (squeezing, 'pushing', and coughing) to test the function of the anal muscles.

c. Sensation in the lower bowel will be tested by using the balloon at the end of the manometry catheter. As the balloon is slowly inflated and you will be asked to report on the feeling of the balloon inflating (**balloon distension**). The tube and the balloon will then be removed.

d. The **sensation** of the back passage will be tested using another thin probe. This is inserted just inside the back passage. A very mild current is passed through an electrode at the tip and you will be asked to report when you can feel the pulse. This test is not painful or damaging to the back passage.

e. The muscles of the back passage will be looked at using an **ultrasound** probe that will be passed just inside the back passage. The probe is about the size of a finger. This is a brief test that takes about 2 minutes.

f. The flexibility or elasticity of the anal muscles will then be measured with a flexible tube (**EndoFLIP®** catheter) that is about the width of a pencil and is covered by a balloon. This will be inserted within the back passage and you will once again be asked to squeeze and cough during the procedure. You may feel a feeling of mild pressure, but no pain.

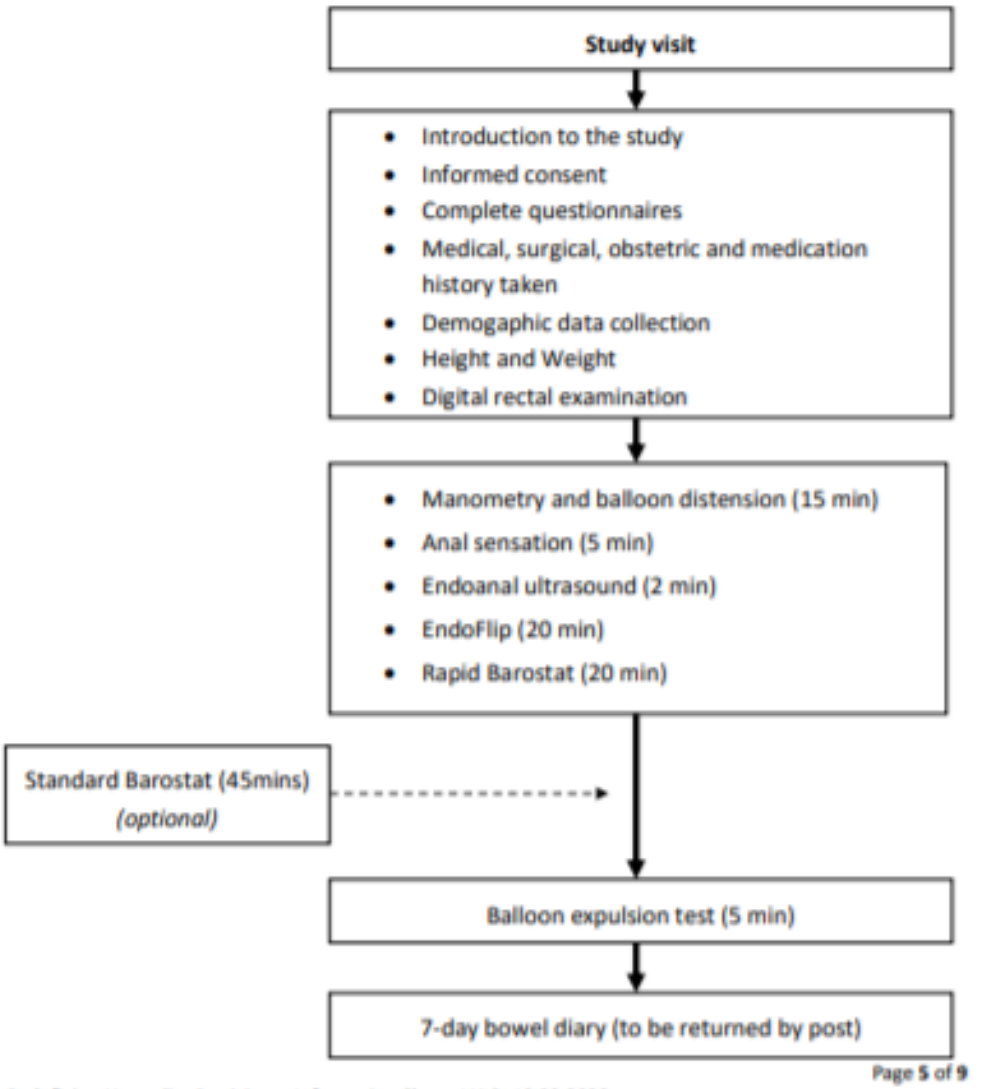
g. The function of your lower bowel will then be tested with a soft non-latex bag, which is placed through the back passage (**Rapid Barostat**). The bag will be inflated slowly using an air pump. You will be asked to report when you feel the sensation of the balloon inflating.

h. Finally, emptying of the back passage will be tested by placing a small balloon into the back passage and filling it with warm water. You will then be asked to evacuate the balloon while sitting on a commode in privacy (**Balloon expulsion test**).

i. After the tests are finished you will be free to go. We will ask you to keep a seven day **bowel diary** detailing your bowel habits during this period and ask you to send this back to us in the post using a prepaid envelope.

A picture of our bowel function lab where tests will take place.

STUDY SCHEME DIAGRAM



WHAT IS THE OPTIONAL 'EXTRA' TEST LIKE (STANDARD BAROSTAT)?

If you agreed to the 'extra' test (**Standard Barostat**), a different non-latex bag will be placed in the back passage before or after the Rapid Barostat rectal sensation test (described above). As before, the bag will be inflated by a pump and you will again be asked to report when you feel the sensation of the balloon inflating. This test is similar to the previous test, but takes a longer time. Your participation will allow us to assess if this slower method can be replaced with the quicker method while still providing the same results.

WILL THE TESTS BE PAINFUL OR UNCOMFORTABLE?

Most of the investigations are routinely performed on patients in many hospitals and are almost always tolerated without any problems. You may feel some discomfort as the measurement tubes are passed into the back passage, but none of the tests should be painful.

WHAT WILL I HAVE TO DO?

If you choose to be part of this study, it is important for you to:

- Attend your visit on the scheduled date
- Follow the instructions you receive prior to and during the visits
- Complete and return a bowel diary after your visit

You will not need to modify your lifestyle in any way and you can continue taking any medications as usual.

EXPENSES AND PAYMENTS

On completion of the study you will be compensated with £75 for your time and travel expenses. If you withdraw from the study early (or are not eligible for the study) you will not receive the above sum. A further £25 compensation will be given to those who take part in the 'extra' Standard Barostat test (£100 in total). You may need to declare this remuneration for tax/benefit purposes.

WHAT ARE THE POTENTIAL BENEFITS OF TAKING PART?

Although this study will not be of any direct benefit to you, we hope that the results of this study will increase our understanding of how the lower bowel functions. This will help in the assessment and treatment of patients with bowel problems in the future and allow further research in the area.

WHAT ARE THE POTENTIAL RISKS AND DISADVANTAGES OF TAKING PART?

There are no serious risks anticipated due to participation in this study. There is the very small possibility that the test may cause a slight irritation or bleeding of the area around the bottom. However, the tests in this study are routinely performed in many hospitals usually without any problems at all. If you suffer from any problems following the tests you will always be able to get in touch with the researcher who performed the test.

WHAT HAPPENS IF THERE IS A PROBLEM?

Queen Mary University of London is sponsoring this study and has agreed that if you are harmed as a result of your participation in the study, you will be compensated, provided that, on the balance of probabilities, an injury was caused as a direct result of the intervention or procedure you received during the course of the study. These special compensation arrangements apply where injury is caused to you that would not have occurred if you were not in the trial. These arrangements do not affect your right to pursue a claim through legal action.

If you have a complaint you should contact the Joint Research Management Office: research-ethics@qmul.ac.uk.

HOW WILL MY INFORMATION BE KEPT CONFIDENTIAL?

All information which is collected about you during the course of this research is kept strictly confidential and is made untraceable to you. All data collected will be stored against a number, not your name, in a password-protected computer that only the investigators of the study will have access to.

It is up to you to decide whether or not to take part. If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form.

Please read Queen Mary's privacy notice for research participants[1] for important information about your personal data and your rights in this respect.

If you have any questions or concerns about the manner in which the study was conducted please, in the first instance, contact the researcher responsible for the study. If this is unsuccessful, or not appropriate, please contact the Secretary at the Queen Mary Ethics of Research Committee, Room W104, Queens' Building, Mile End Campus, Mile End Road, London, E1 4NS or research-ethics@qmul.ac.uk. If you have any questions relating to data protection, please contact Data Protection Officer, Queens' Building, Mile End Road, London, E1 4NS or data-protection@qmul.ac.uk

[1]This is found at: http://www.arcs.qmul.ac.uk/media/arcs/policyzone/Privacy-Notice-for-Research-Participants.pdf

WHAT WILL HAPPEN TO THE RESULTS OF THE STUDY?

The results of this study may be published in a medical journal or be presented at a scientific conference. The data will be anonymous and none of the participants involved in the study will be identified in any report or publication. Should you wish to see the results, or the publication, please ask a member of the research team. A lay summary of the overall results of the study will also be available. If you would like a copy please inform one of the study team.

WHO IS SPONSORING AND FUNDING THE STUDY

The sponsor, who has overall responsibility for this study, is Queen Mary, University of London. The research is being funded by a research grant from Bowel and Cancer Research. Additional funding has been received from Mui Scientific (Ontario, Canada) and Medical Measurement Systems Ltd (Enschede, the Netherlands), who manufacture some of the test equipment used.

WHO HAS REVIEWED THIS STUDY

International guidelines exist to ensure clinical studies are performed properly and ethically. These guidelines are called, 'Good Clinical Practice' and the 'Declaration of Helsinki'. All studies are performed to these standards.

This study has been peer reviewed and also approved by the Joint Research Management Office, Queen Mary University of London. This study has been reviewed and given favourable opinion by Queen Mary Research Ethics Committee.

WILL MY GP BE INFORMED THAT I AM TAKING PART IN THE STUDY?

If you agree to take part in the study, we will write to your GP to let them know about your participation if you wish.

FURTHER INFORMATION AND CONTACT DETAILS

You are encouraged to ask any questions you wish before, during or after you have participated in the study. If you have any questions about the study or any of the procedures involved, please contact a member of the research team:

Principal Investigator:
Dr Mark Scott, PhD
Senior Clinical Scientist/Director of GI Physiology Unit
Barts and The London School of Medicine and Dentistry
Queen Mary University of London
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Tel: +44 (20)78822639
E-mail: a.rasijeff@qmul.ac.uk

Appendix 5

Chapter 6 Pre-screening form and screening form

Queen Mary University of London

Barts and The London Queen Mary's School of Medicine and Dentistry

NEUROGASTROENTEROLOGY GROUP

The Wingate Institute for Neurogastroenterology, 26, Ashfield Street, LONDON E1 2AJ
Neuroscience & Trauma, Blizard, QMUL

Study Number:

Study Title: DEFINING NORMAL RANGES FOR TEST RESULTS USING EXISTING AND EMERGING TECHNOLOGIES FOR THE DIAGNOSTIC ASSESSMENT OF ANORECTAL FUNCTION

Pre-screening Assessment:

Pre-screening Identification Number: _S_____

Sex: Male ☐ Female ☐ Nulliparous ☐
Parous ☐

Pre-screening conducted by:

Verbal Consent:

I have fully informed the individual of all aspects of the study and what the pre-screening involves and provided the opportunity for them to consider the information, ask questions and answered these satisfactorily. ☐

They have been informed that this pre-screening is voluntary ☐

They have agreed to the pre-screening ☐

Signature of person taking consent: _____

Date: _____

Page 1 of 2

NEUROGASTROENTEROLOGY GROUP

The Wingate Institute for Neurogastroenterology, 26, Ashfield Street, LONDON E1 2AJ
Neuroscience & Trauma, Blizard, QMUL

Pre-screening Questions:

Healthy subjects aged between 18-75 years of either gender	Yes/No
Any co-existing acute or chronic disease at the time of recruitment.	Yes/No
Ability to understand subject information sheet and instructions in English and able to provide informed consent.	Yes/No
Any history of GI symptoms in the 3 months prior to the study	Yes/No
Suffers with constipation/ faecal incontinence/ flatus incontinence/ urgency	Yes/No
Taking medication known to affect gastrointestinal function (e.g laxatives)	Yes/No
Pregnant or breastfeeding	Yes/No
Symptoms of prolapse or previous hysterectomy	Yes/No
Allergy to latex	Yes/No
Previous gastrointestinal surgery	Yes/No
Any other medical condition that would make it unsafe for the subject to participate, determined by the treating physician	Yes/No

If 'Yes', details:

NB: If greyed out responses are all circled, then the subject is suitable to proceed to formal screening for the study.

Is the participant interested in taking part in Part B (Standard Barostat) on the day? Yes/No

Study Number:

Study Title: DEFINING NORMAL RANGES FOR TEST RESULTS USING EXISTING AND EMERGING TECHNOLOGIES FOR THE DIAGNOSTIC ASSESSMENT OF ANORECTAL FUNCTION

Screening Assessment: Date:

Pre-screening Identification Number: _S_____

Sex: Male ☐ Female ☐ Nulliparous ☐
Parous ☐

SCREENING

1. Inclusion/exclusion criteria review

Inclusion Criteria:

- Healthy subject aged between 18-75 years of either gender without any co-existing acute or chronic disease at the time of recruitment. Yes/No
- Ability to understand subject information sheet and instructions in English and able to provide informed consent. Yes/No
- No history of any gastrointestinal symptoms in the 3 months prior to the study. Yes/No
- Average Bristol stool type between 2-5 and frequency of 3-21 spontaneous bowel movements per week Yes/No
- Cleveland Clinic Constipation Score (CCCS) of <8 Yes/No
  CCCS score: _____/30
- St Marks Incontinence Score ≤5 Yes/No
  Vaizey score: _____/24

Exclusion criteria:

- Allergy to latex Yes/No

- Pregnant or breast-feeding Yes/No
- Ongoing other diagnosed lower gastrointestinal disease or complication (e.g. IBS, Crohn's disease, Coeliac disease, chronic diarrhoea, etc.) Yes/No
- Any clinically relevant abnormalities or alarm features such as sudden weight loss, rectal bleeding, recent change in bowel habit (<3 months), abdominal pain and stool positive for occult blood Yes/No
- Prior GI surgery (including gastric bypass or laparoscopic banding), except cholecystectomy and appendectomy Yes/No
- Neurologic diseases known to affect lower GI function such as multiple sclerosis, stroke, spinal cord injury, Hirschsprung disease Yes/No
- Ongoing therapy with medications known to affect lower GI function Yes/No
- Illness that may preclude the subject's ability to complete the study or that may confound the study outcomes or any other serious illness resulting in >2 weeks inability to work in the 3 months before the study start Yes/No
- Subjects with co-morbid illnesses such as cardiovascular, endocrine, renal or other chronic disease likely to affect gut motility or limit normal functions (e.g. reduced mobility or increased fragility Yes/No
- Self-reported symptoms of pelvic organ prolapse and previous hysterectomy Yes/No
- History of severe haemorrhoids, chronic anal fissures or other anal pathology Yes/No

2. Is the volunteer currently on any medication prescribed or self-medicated

	Name	Dose	Start Date
If 'yes', details:			

3. Alcohol Consumption History:
   Does participant consume alcohol Yes/No

   If 'yes', details: Units/day
   Units/week

(1 unit = half-pint (284 mL) of beer / one (25 mL) measure of spirits / one glass (125 mL) of wine.)

4. Smoking History:
   Current smoker: Yes/No

   If 'yes', details: Number/day Year started

5. Recreational drug history:
   Does participant take recreational/illicit drugs Yes/No

Screening Completed by: _____

Date: _____

Appendix 6

Informed consent form

Queen Mary University of London

Barts and The London Queen Mary's School of Medicine and Dentistry

NEUROGASTROENTEROLOGY GROUP

The Wingate Institute for Neurogastroenterology, 26, Ashfield Street, LONDON E1 2AJ

Neuroscience & Trauma, Blizard, QMUL

Consent form

Please complete this form after you have read the Information Sheet and/or listened to an explanation about the research.

Title of Study: Measurement of anorectal function in healthy volunteers

Queen Mary Ethics of Research Committee Ref: QMREC2017/33

Thank you for considering taking part in this research. The person organizing the research must explain the project to you before you agree to take part.

If you have any questions arising from the Information Sheet or explanation already given to you, please ask the researcher before you decide whether to join in. You will be given a copy of this Consent Form to keep and refer to at any time. If you are willing to participate in this study, please circle the appropriate responses and sign and date the declaration underneath.

Statement	Circle a response
I agree that the research project named above has been explained to me to my satisfaction in verbal and/or written form	YES / NO
I understand that if I decide at any other time during the research that I no longer wish to participate in this project, I can notify the researchers involved and be withdrawn from it immediately	YES / NO
I have read both the notes written above and the Information Sheet about the project, and understand what the research study involves	YES / NO
I agree to take part in the study, which will include use of my personal data	YES / NO
I understand that the information collected about me will be used to support other research in the future, and may be shared anonymously with other researchers.	YES / NO
I agree to my General Practitioner being informed of my participation in the study.	YES / NO

Participant's Signature:_____ Date: _____

Page 1 of 2 When completed: 1 for participant; 1 for researcher site file. Study ID:

NEUROGASTROENTEROLOGY GROUP

The Wingate Institute for Neurogastroenterology, 26, Ashfield Street, LONDON E1 2AJ
Neuroscience & Trauma, Blizard, QMUL

Investigator's Statement:

I _____ confirm that I have carefully explained the nature, demands and any foreseeable risks (where applicable) of the proposed research to the volunteer and provided a copy of this form

Appendix 7

Chapter 6- Questionnaire pack for volunteers

Measurement of anorectal function in healthy volunteers

Questionnaire pack

Study ID:

Date:

This questionnaire pack contains 4 questionnaires. Please complete all questions even if you think that they do not apply to you.

Once you have finished please hand the completed pack back to the researcher

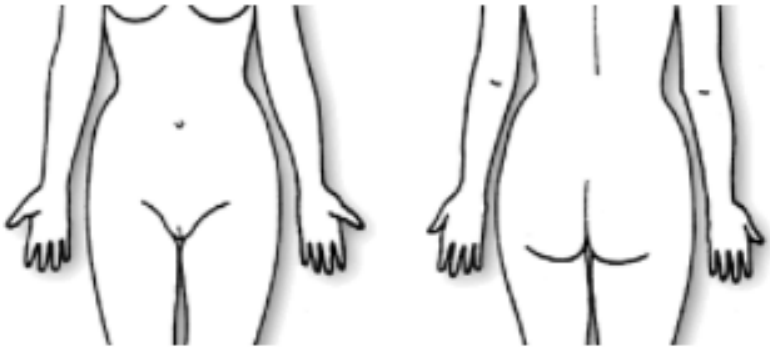
When you visit the toilet, do you have a feeling / sensation that makes you want to go?

☐ Yes – please fill in sections 1 and 2

☐ No – please fill in section 2

SECTION 1

Please carefully shade one or both diagrams below to indicate the location of this sensation



How would you describe this feeling? Please tick *any* applicable words below or add your own

- ☐ Aching
- ☐ Bloating
- ☐ Butterflies/gurgling
- ☐ Colicky/griping
- ☐ Cramping
- ☐ Fullness
- ☐ Heat/burning
- ☐ Heaviness/dragging
- ☐ Irritation
- ☐ Pressure
- ☐ Prickling
- ☐ Sickness/nausea
- ☐ Spasm
- ☐ Squeezing
- ☐ Stabbing
- ☐ Throbbing
- ☐ Tickling
- ☐ Tingling
- ☐ I can't describe the feeling

Other 1_____ Other 2 _____ Other 3_____

In general, how strong is this feeling? Place a vertical mark on the line below to indicate how strong you feel this feeling

No feeling ——————————————— Very strong feeling

Section 2

	No	Somewhat	Yes
Are you usually successful in opening your bowels?	☐	☐	☐
Do you usually strain?	☐	☐	☐

Place a vertical mark on the line below to indicate how complete emptying feels most of the time

Not complete at all — Complete

What does the stool look like? Please circle the appropriate image below

EFFECT OF EMOTIONS ON SYMPTOMS

It is well known that emotions play an important part in gastrointestinal illnesses. This section is designed to help your doctors understand how you are feeling. For each question, please tick the reply that comes closest to how you have been feeling in the last week

I feel tense or 'wound up'

- ☐ Most of the time
- ☐ A lot of the time
- ☐ From time to time, occasionally
- ☐ Not at all

I still enjoy the things I used to enjoy

- ☐ Definitely as much
- ☐ Not quite so much
- ☐ Only a little
- ☐ Hardly at all

I get a sort of frightened feeling as if something awful is about to happen

- ☐ Very definitely and quite badly
- ☐ Yes, but not too badly
- ☐ A little, but it doesn't worry me
- ☐ Not at all

I can laugh and see the funny side of things

- ☐ As much as I always could
- ☐ Not quite as much now
- ☐ Definitely not so much now
- ☐ Not at all

Worrying thoughts go through my mind

- ☐ A great deal of the time
- ☐ A lot of the time
- ☐ Not too often
- ☐ Very little

I feel cheerful

- ☐ Never
- ☐ Not often
- ☐ Sometimes
- ☐ Most of the time

I can sit at ease and feel relaxed

- ☐ Definitely
- ☐ Usually
- ☐ Not often
- ☐ Not at all

I feel as if I am slowed down

- ☐ Nearly all of the time
- ☐ Very often
- ☐ Sometimes
- ☐ Not at all

I get a sort of frightened feeling like 'butterflies' in the stomach

- ☐ Not at all
- ☐ Occasionally
- ☐ Quite often
- ☐ Very often

I have lost interest in my appearance

- ☐ Definitely
- ☐ I don't take as much care as I should
- ☐ I may not take quite as much care
- ☐ I take just as much care as ever

I feel restless as I have to be on the move

- ☐ Very much indeed
- ☐ Quite a lot
- ☐ Not very much
- ☐ Not at all

I look forward with enjoyment to things

- ☐ As much as I ever did
- ☐ Rather less than I used to
- ☐ Definitely less than I used to
- ☐ Hardly at all

I get sudden feelings of panic

- ☐ Very often indeed
- ☐ Quite often
- ☐ Not very often
- ☐ Not at all

I can enjoy a good book of radio or television programme

- ☐ Often
- ☐ Sometimes
- ☐ Not often
- ☐ Very seldom

This survey asks for your views about your health. This information will help you keep track of how you feel and how well you are able to do your usual activities.

Answer every question by selecting the answer as indicated. If you are unsure about how to answer a question, please give the best answer you can.

---

1. In general, would you say your health is:

Excellent	Very good	Good	Fair	Poor
○	○	○	○	○

---

2. Compared to one year ago, how would you rate your health in general now?

Much better now than one year ago	Somewhat better now than one year ago	About the same as one year ago	Somewhat worse now than one year ago	Much worse now than one year ago
○	○	○	○	○

---

3. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

		Yes, limited a lot	Yes, limited a little	No, not limited at all
a	Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	○	○	○
b	Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	○	○	○
c	Lifting or carrying groceries	○	○	○
d	Climbing several flights of stairs	○	○	○
e	Climbing one flight of stairs	○	○	○
f	Bending, kneeling, or stooping	○	○	○
g	Walking more than a mile	○	○	○
h	Walking several hundred yards	○	○	○
i	Walking one hundred yards	○	○	○
j	Bathing or dressing yourself	○	○	○

---

4. **During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of your physical health?**

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a Cut down on the amount of time you spent on work or other activities	○	○	○	○	○
b Accomplished less than you would like	○	○	○	○	○
c Were limited in the kind of work or other activities	○	○	○	○	○
d Had difficulty performing the work or other activities (for example, it took extra effort)	○	○	○	○	○

5. **During the past 4 weeks, how much of the time have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?**

	All of the time	Most of the time	Some of the time	A little of the time	None of the time
a Cut down on the amount of time you spent on work or other activities	○	○	○	○	○
b Accomplished less than you would like	○	○	○	○	○
c Did work or activities less carefully than usual	○	○	○	○	○

6. **During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?**

Not at all	Slightly	Moderately	Quite a bit	Extremely
○	○	○	○	○

7. **How much bodily pain have you had during the past 4 weeks?**

None	Very mild	Mild	Moderate	Severe	Very severe
○	○	○	○	○	○

8. **During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?**

Not at all	A little bit	Moderately	Quite a bit	Extremely
○	○	○	○	○

9. **These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling.**

How much of the time during the past 4 weeks...

		All of the time	Most of the time	Some of the time	A little of the time	None of the time
a	Did you feel full of life?	○	○	○	○	○
b	Have you been very nervous?	○	○	○	○	○
c	Have you felt so down in the dumps that nothing could cheer you up?	○	○	○	○	○
d	Have you felt calm and peaceful?	○	○	○	○	○
e	Did you have a lot of energy?	○	○	○	○	○
f	Have you felt downhearted and depressed?	○	○	○	○	○
g	Did you feel worn out?	○	○	○	○	○
h	Have you been happy?	○	○	○	○	○
i	Did you feel tired?	○	○	○	○	○

10. **During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?**

All of the time	Most of the time	Some of the time	A little of the time	None of the time
○	○	○	○	○

11. **How TRUE or FALSE is each of the following statements for you?**

		Definitely true	Mostly true	Don't know	Mostly false	Definitely false
A	I seem to get sick a little easier than other people	○	○	○	○	○
B	I am as healthy as anybody I know	○	○	○	○	○
C	I expect my health to get worse	○	○	○	○	○
D	My health is excellent	○	○	○	○	○

Thank you for completing these questions!

PAC-SYM

This questionnaire asks you about your constipation in the **past 2 weeks.** Answer each question according to your symptoms, as accurately as possible. There are no right or wrong answers.

For each symptom below, please indicate **how severe** your symptoms have been during the **past 2 weeks.** If you have not had the symptom during the past 2 weeks, check 0. If the symptom seemed mild, check 1. If the symptom seemed moderate, check 2. If the symptom seemed severe, check 3. If the symptom seemed very severe, check 4. Please be sure to answer every question.

How severe have each of these symptoms been in the last 2 weeks	Absent	Mild	Moderate	Severe	Very Severe
	0	1	2	3	4
Discomfort in your abdomen					
Pain in your abdomen					
Bloating in your abdomen					
Stomach cramps					
Painful bowel movements					
Rectal burning during or after a bowel movement					
Incomplete bowel movement, like you didn't "finish"					
Bowel movements that were too hard					
Bowel movements that were too small					
Straining or squeezing to try to pass bowel movements					
Feeling like you have to pass a bowel movement but you couldn't (false alarm)					

Appendix 8

Chapter 6 bowel diary

Visit 1

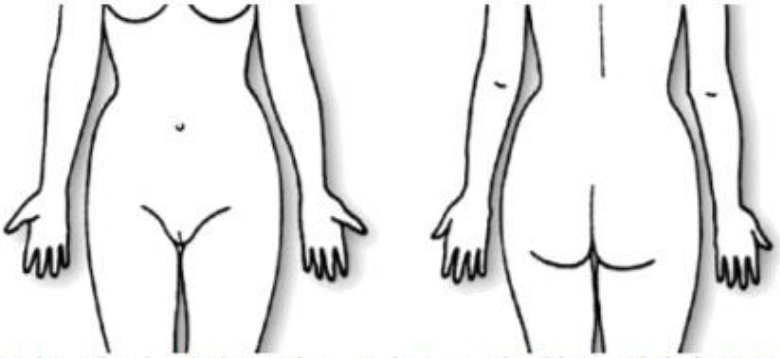
Date: Time:

When you visited the toilet, did you have a feeling / sensation that made you want to go?

☐ Yes – please fill in sections 1 and 2 ☐ No – please fill in section 2

Section 1

Please carefully shade one or both diagrams below to indicate the location of this sensation



How would you describe this feeling? Please tick *any* applicable words below or add your own

☐ Aching	☐ Fullness	☐ Prickling	☐ Throbbing
☐ Bloating	☐ Heat/burning	☐ Sickness/nausea	☐ Tickling
☐ Butterflies/gurgling	☐ Heaviness/dragging	☐ Spasm	☐ Tingling
☐ Colicky/griping	☐ Irritation	☐ Squeezing	☐ I can't describe the feeling
☐ Cramping	☐ Pressure	☐ Stabbing	

Other 1_____ Other 2 _____ Other 3 _____

How strong was this feeling? Place a vertical mark on the line below to indicate how strong you felt this feeling

No feeling ——————————— Very strong feeling

Section 2

	no	somewhat	yes
Were you successful in opening your bowels? Did you strain?	☐	☐	☐

Was it felt to be complete? Place a vertical mark on the line below to indicate how complete the motion felt

Not complete at all ——————————— Complete

What did the stool look like? Please circle the appropriate image below

hard lumps formed lumpy formed cracked formed soft soft blobs mushy liquid

Visit 1

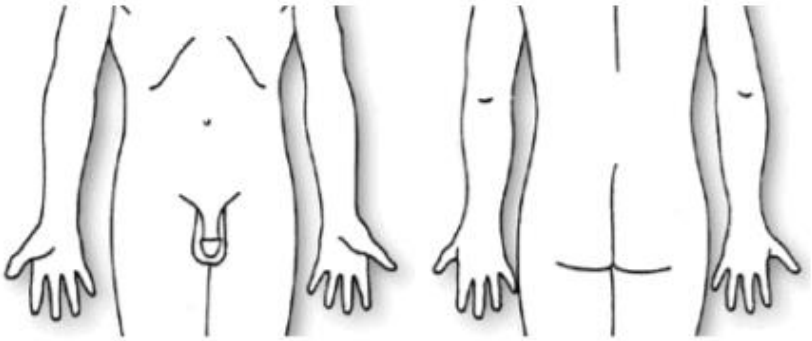
Date: Time:

When you visited the toilet, did you have a feeling / sensation that made you want to go?

☐ Yes – please fill in sections 1 and 2 ☐ No – please fill in section 2

Section 1

Please carefully **shade** one or both diagrams below to indicate the location of this sensation



How would you describe this feeling? Please tick *any* applicable words below or add your own

☐ Aching	☐ Fullness	☐ Prickling	☐ Throbbing
☐ Bloating	☐ Heat/burning	☐ Sickness/nausea	☐ Tickling
☐ Butterflies/gurgling	☐ Heaviness/dragging	☐ Spasm	☐ Tingling
☐ Colicky/griping	☐ Irritation	☐ Squeezing	☐ I can't describe the feeling
☐ Cramping	☐ Pressure	☐ Stabbing	

Other 1_____ Other 2 _____ Other 3 _____

How strong was this feeling? Place a vertical mark on the line below to indicate how strong you felt this feeling

No feeling ——————————— Very strong feeling

Section 2

	no	somewhat	yes
Were you successful in opening your bowels? Did you strain?	☐	☐	☐

Was it felt to be complete? Place a vertical mark on the line below to indicate how complete the motion felt

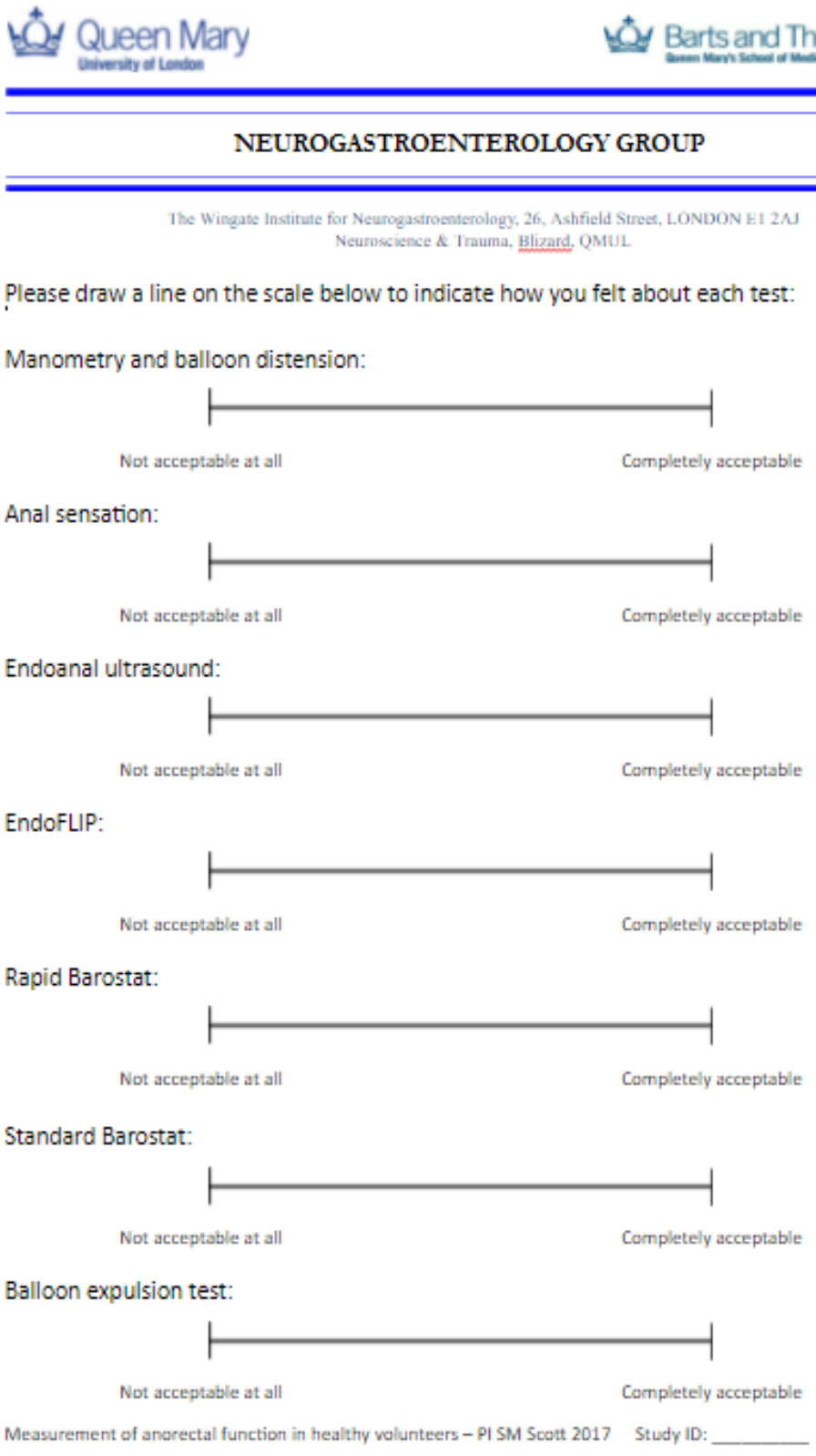
Not complete at all ——————————— Complete

What did the stool look like? Please circle the appropriate image below

hard lumps formed lumpy formed cracked formed soft soft blobs mushy liquid

Appendix 9

VAS score of acceptability



Queen Mary University of London

Barts and The London Queen Mary's School of Medicine and Dentistry

NEUROGASTROENTEROLOGY GROUP

The Wingate Institute for Neurogastroenterology, 26, Ashfield Street, LONDON E1 2AJ
Neuroscience & Trauma, Blizard, QMUL

Please draw a line on the scale below to indicate how you felt about each test:

Manometry and balloon distension:

Not acceptable at all — Completely acceptable

Anal sensation:

Not acceptable at all — Completely acceptable

Endoanal ultrasound:

Not acceptable at all — Completely acceptable

EndoFLIP:

Not acceptable at all — Completely acceptable

Rapid Barostat:

Not acceptable at all — Completely acceptable

Standard Barostat:

Not acceptable at all — Completely acceptable

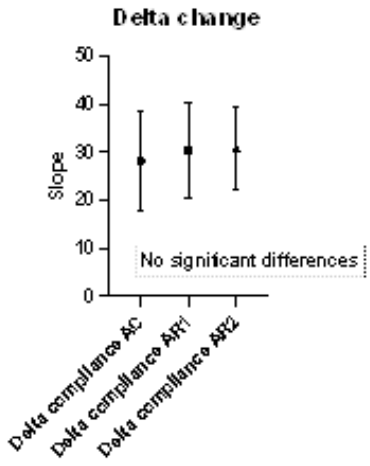
Balloon expulsion test:

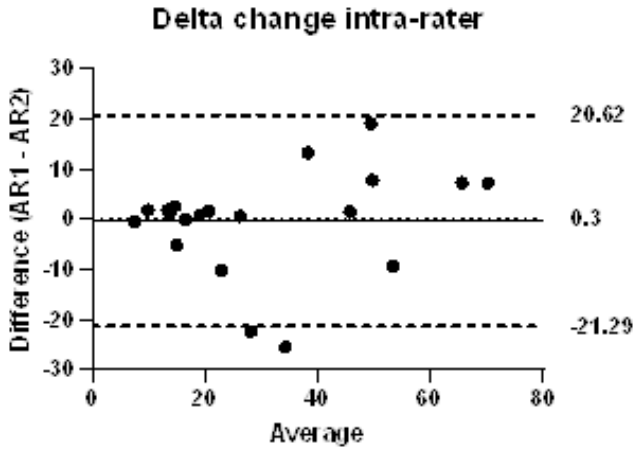
Not acceptable at all — Completely acceptable

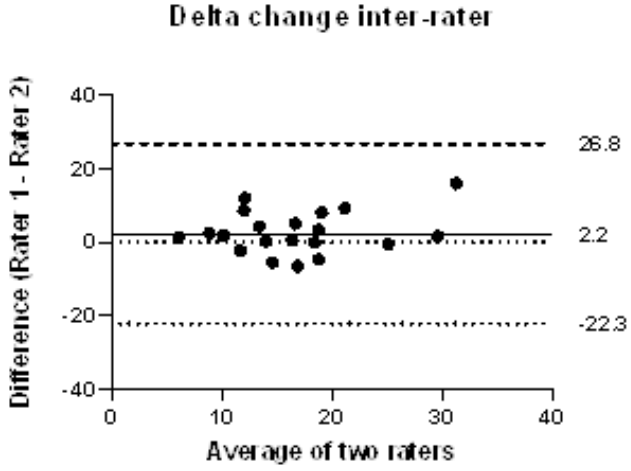
Measurement of anorectal function in healthy volunteers – PI SM Scott 2017 Study ID: _____

Appendix 10

Chapter 7a Results on the reliability of the delta change methods for assessing compliance using stand and rapid barostat







Appendix 11

Chapter 7b Example of an RBB report

Patient:		**Patient ID:**		**Physician:**	
Gender:	Female	**Other ID:**		**Ref. Physician:**	
Date of Birth:		**Insurance:**		**Operator:**	
Age:	34			**Exam Date:**	

Rectal Structure and Motor Function

	Result	Normal	Classification
Rectal Capacity	238 mL	200 - 450 mL	Normal
Pressure @ 50 %	16 mmHg	9 - 16 mmHg	Normal
Compliance	10.0 mL/mmHg	-	-

Rectal Sensitivity

	Threshold	% Capacity	Normal	Classification
Discomfort	258 mL	108%	80 - 100 %	Hypo
Urge	91 mL	38%	40 - 80 %	Hyper
Sensation	24 mL	10%	10 - 30 %	Normal

Disclaimer: The summary findings based on this data analysis should not be interpreted as final diagnosis

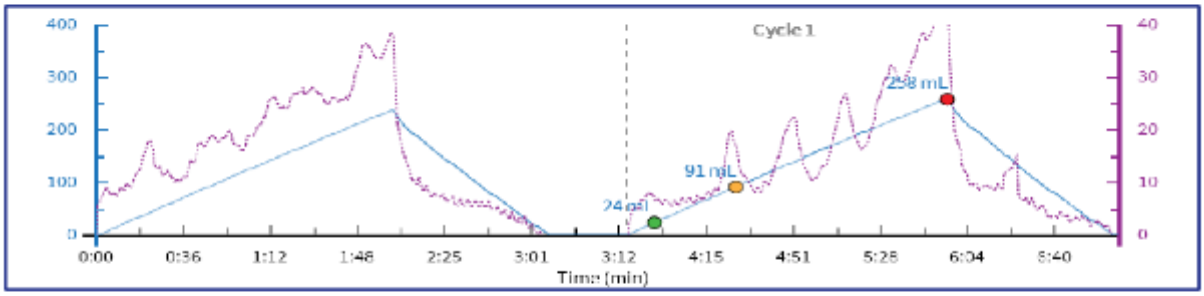
Procedure

Standard RBB Protocol consisting of Capacity Test and Sensation Test. First, the Capacity Test is performed by inflating a **700mL** RBB Catheter Bag in the patient's rectum until **40mmHg** is reached. The capacity is recorded and the Bag is then immediately emptied. Next, the Sensation Test is performed by recording **Sensation**, **Urge** and **Discomfort** as the Bag is inflating in the patient's rectum. Once **Discomfort** is recorded, the Bag is then immediately emptied.

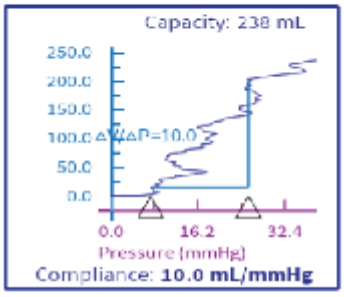
Test Parameters

Inflation Flow Rate: **120 mL/Min** Deflation Flow Rate: **180 mL/Min** Max. Bag Pressure: **40 mmHg** Max. Bag Volume: **700 mL**

RBB Graph



Compliance Graph



Sensation Study Table

	Cycle 1	Cycle 2	Cycle 3	Average	%	Summary
Discomfort	258 mL	-	-	258mL	108%	Hypo
Urge	91 mL	-	-	91mL	38%	Hyper
Sensation	24 mL	-	-	24mL	10%	Normal
Capacity	-	-	-	238 mL	100%	-

Comments

Additional notes on procedure...

	Hyper	Normal	Hypo
Discomfort	<80%	80-100%	>100%
Urge	<40%	40-80%	>80%
Sensation	<10%	10-30%	>30%

Normal Rectal Capacity @ 40mmHg: **200 - 450 mL**
Normal Pressure @ 50% Capacity: **9 - 16 mmHg**

Appendix 12

Chapter 8 Summary and ANOVA tables for EndoFLIP distensibility assessment

Participants

The sex, parity status, age, and BMI were recorded.

Table 1 Table of mean age and BMI:

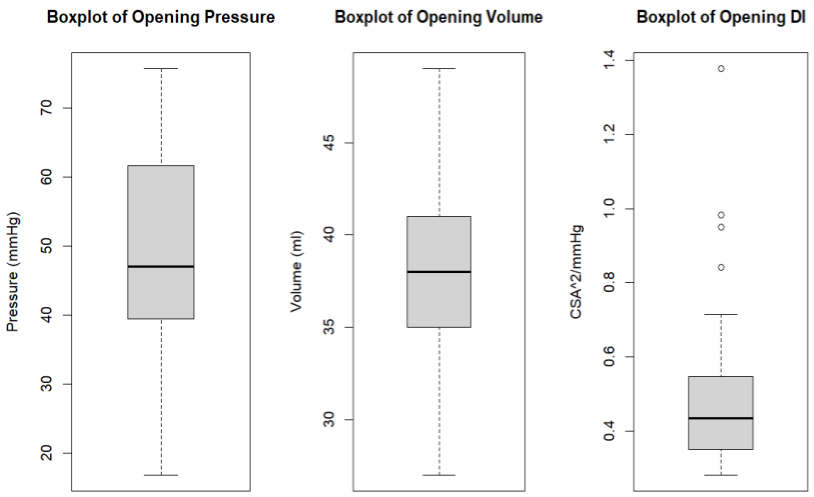
	Total	Males	Females	Nulliparous	Parous
n	46	11	35	19	16
Age (sd)	38.5 (12.4)	38.2 (16.3)	38.5 (11.2)	35.3 (11.8)	42.4 (9.3)
Body Mass Index (sd)	24.1 (4.5)	24.8 (3.8)	23.9 (4.8)	23.7 (5.5)	24.0 (3.8)

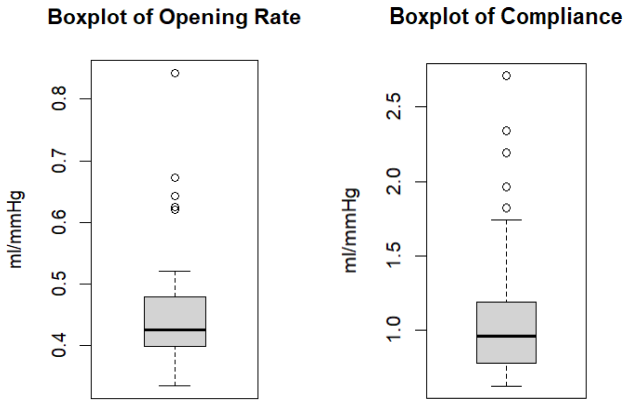
Distensibility during inflation

Summarise distensibility data (opening pressure, opening volume,opening DI, slope of V-P curve and compliance during 0-50ml inflation) using tables and boxplots.

Table 2 summary data for distensibility measures during 0-50ml distension:

	n	Mean	sd	Median	se	95% CI
Opening pressure	45	49	15	47	2.25	45-54
Opening volume	45	38	4.6	38	0.68	37-40
Opening DI	45	0.49	0.21	0.43	0.03	0.43-0.56
Rate after opening	45	0.46	0.10	0.43	0.01	0.43-0.49
Compliance	45	1.1	0.48	0.96	0.07	1-1.3





Compare grouped differences for the variables during distension:

Opening Pressure

		Total	Nulliparous	Parous	Male
N		45	19	16	10
Mean		49.3	52.5	44.4	50.9
Std. Deviation		15.1	13.8	15.4	16.4
Std. Error		2.25	3.16	3.86	5.18
95% Confidence Interval for Mean	Lower Bound	44.8	45.9	36.2	39.2

	Upper Bound	53.8	59.2	52.6	62.6
Minimum		16.8	22.3	16.8	22.3
Maximum		75.7	75.7	75.7	70.7

ANOVA table

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	604.13	2	302.07	1.351	0.270
Residuals	9389.82	42	223.57		
Total	9993.95	44			

Opening Volume

		Total	Nulliparous	Parous	Male
N		45	19	16	10
Mean		38.1	39.2	36.6	38.4
Std. Deviation		4.6	4.3	4.9	4.4
Std. Error		0.68	1.00	1.21	1.38
95% Confidence Interval for Mean	Lower Bound	36.7	37.1	34.0	35.3
	Upper Bound	39.5	41.3	39.2	41.5
Minimum		27.0	32.0	27.0	32.0
Maximum		49.0	49.0	44.0	45.0

ANOVA table

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	62.15	2	31.07	1.511	0.232
Residuals	863.50	42	20.56		
Total	925.64	44			

Opening DI

		Total	Nulliparous	Parous	Male
N		45	19	16	10
Mean		0.49	0.45	0.56	0.48
Std. Deviation		0.2	0.2	0.3	0.2
Std. Error		0.03	0.04	0.07	0.06
95% Confidence Interval for Mean	Lower Bound	0.43	0.37	0.42	0.33
	Upper Bound	0.56	0.53	0.70	0.62
Minimum		0.28	0.28	0.28	0.29
Maximum		1.38	0.98	1.38	0.95

ANOVA table

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	0.106	2	0.053	1.194	0.313
Residuals	1.862	42	0.044		
Total	1.968	44			

Rate of opening

		Total	Nulliparous	Parous	Male
N		45	19	16	10
Mean		0.46	0.45	0.46	0.46
Std. Deviation		0.1	0.1	0.1	0.1
Std. Error		0.01	0.03	0.02	0.02
95% Confidence Interval for Mean	Lower Bound	0.43	0.39	0.42	0.41
	Upper Bound	0.48	0.51	0.51	0.52
Minimum		0.33	0.33	0.36	0.36
Maximum		0.84	0.84	0.67	0.64

ANOVA table

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	0.001	2	0.001	0.073	0.930
Residuals	0.398	42	0.009		
Total	0.399	44			

Compliance

		Total	Nulliparous	Parous	Male
N		45	19	16	10
Mean		1.13	1.03	1.25	1.10
Std. Deviation		0.5	0.5	0.4	0.5
Std. Error		0.07	0.12	0.10	0.16
95% Confidence Interval for Mean	Lower Bound	0.98	0.78	1.04	0.75
	Upper Bound	1.27	1.29	1.46	1.45
Minimum		0.63	0.63	0.72	0.65
Maximum		2.71	2.71	1.96	2.34

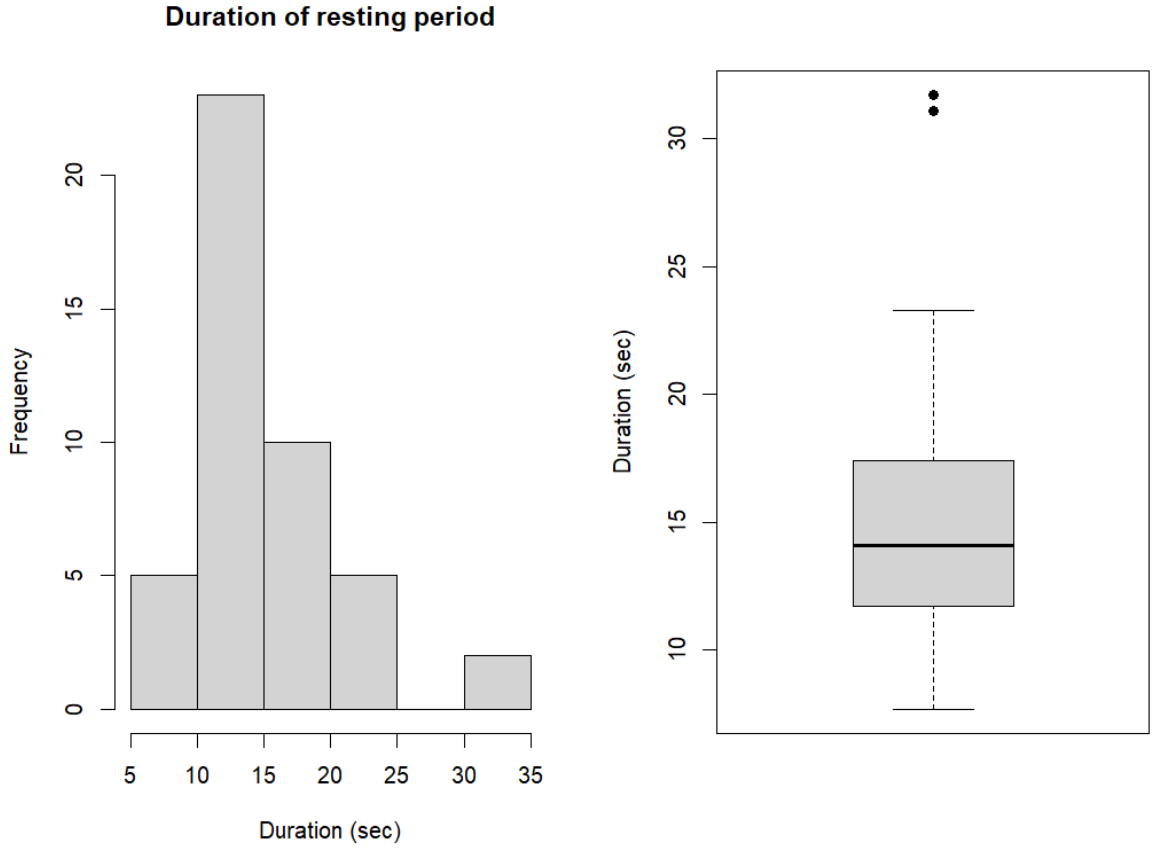
ANOVA table

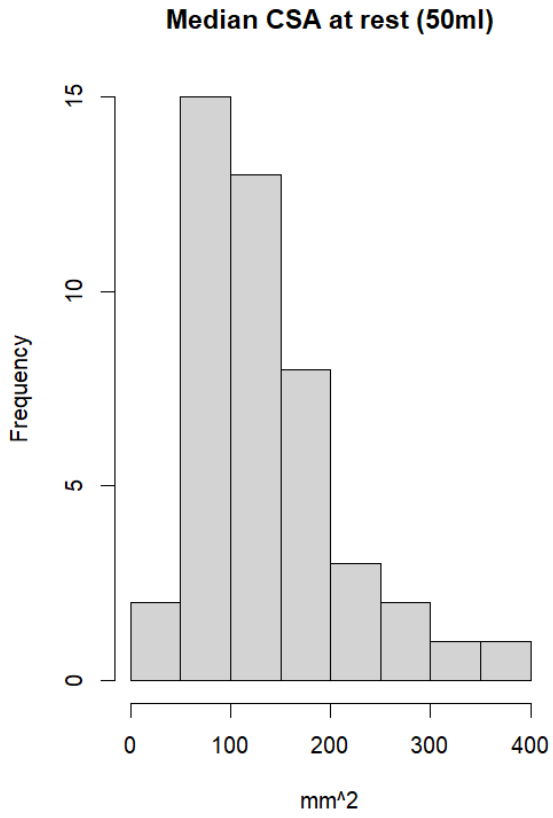
	Sum of Squares	df	Mean Square	F	p-value
Between Groups	0.428	2	0.214	0.941	0.398
Residuals	9.560	42	0.228		
Total	9.989	44			

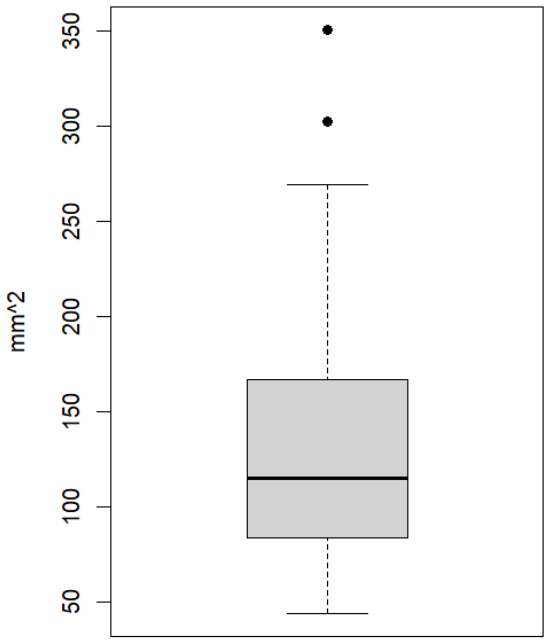
Distensibility index at rest (50 ml distension volume)

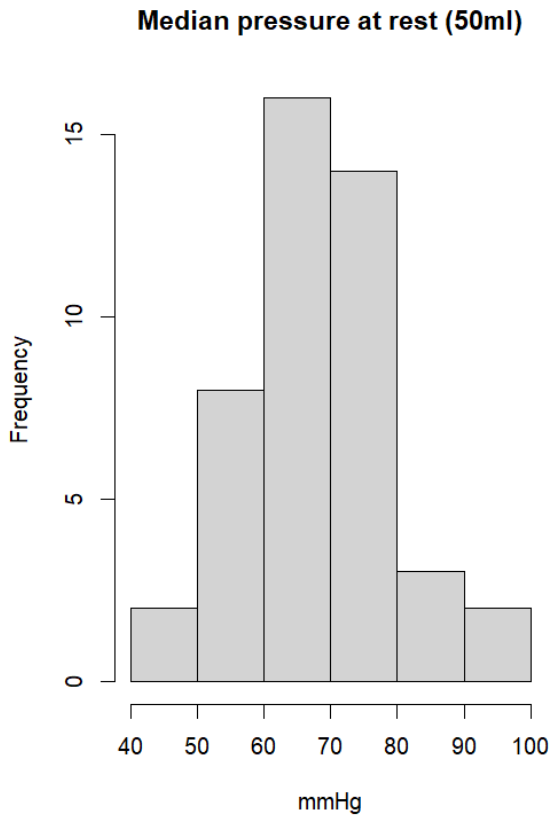
	n	Mean	sd	Median	se	95% CI
Duration	45	15.23	5.24	14.1	0.78	13.7-16.8
CSA	45	134.98	68.82	115.01	10.26	114.3-155.7
Pressure	45	67.67	10.76	67.86	1.6	64.4-70.9

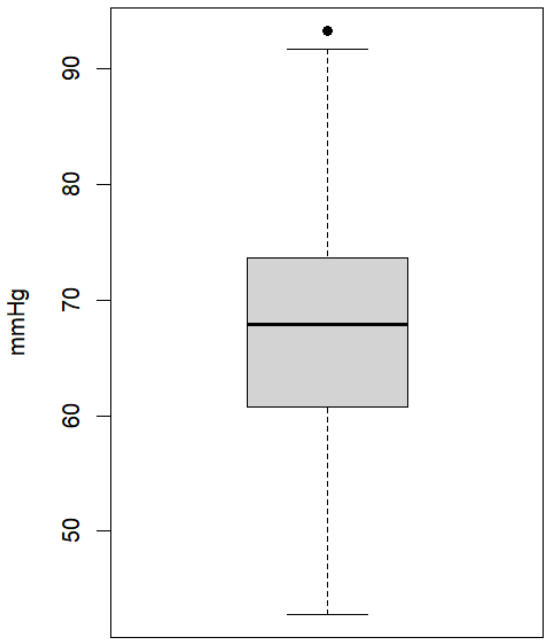
DI	45	2.14	1.42	1.94	0.21	1.71-2.57

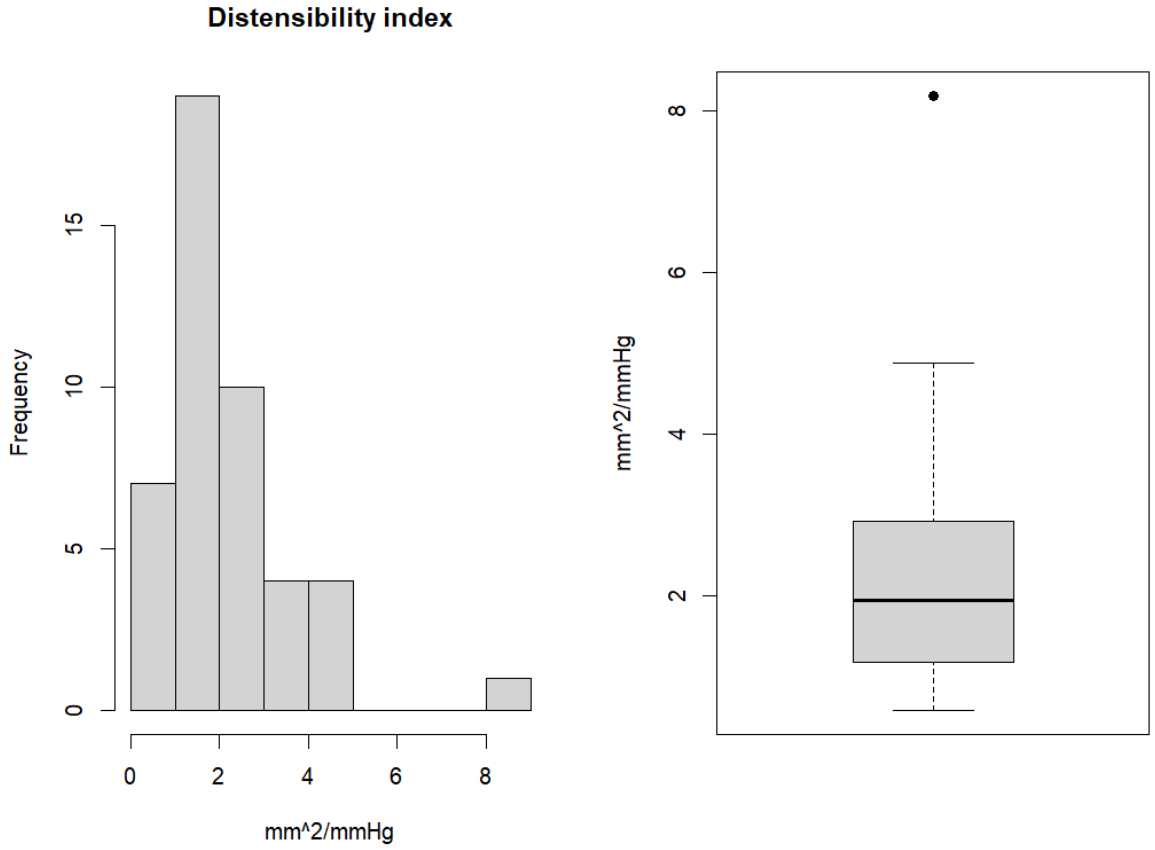



Median CSA at rest (50ml)
Frequency
0
5
10
15
0
100
200
300
400
mm^2


mm^2
50
100
150
200
250
300
350


Median pressure at rest (50ml)
Frequency
0
5
10
15
40
50
60
70
80
90
100
mmHg


mmHg
50
60
70
80
90



Compare grouped differences for the variables at rest:

Duration of resting period

		Total	Nulliparous	Parous	Male
N		45	19	16	10
Mean		15.2	15.4	16.2	13.4
Std. Deviation		5.2	6.9	3.3	4.0
Std. Error		0.78	1.58	0.82	1.26
95% Confidence Interval for Mean	Lower Bound	13.7	12.1	14.4	10.5
	Upper Bound	16.8	18.8	17.9	16.2
Minimum		7.7	8.2	11.7	7.7
Maximum		31.7	31.7	23.3	22.1

ANOVA table

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	50.11	2	25.06	0.910	0.410
Residuals	1156.77	42	27.54		
Total	1206.88	44			

Cross-sectional area at 50ml distension

		Total	Nulliparous	Parous	Male
N		45	19	16	10
Mean		135.0	118.5	158.9	128.0
Std. Deviation		68.8	59.3	78.5	64.9
Std. Error		10.3	13.6	19.6	20.5
95% Confidence Interval for Mean	Lower Bound	114.3	89.9	117.1	81.6
	Upper Bound	155.7	147.1	200.7	174.4
Minimum		44.2	60.3	83.8	44.2
Maximum		350.4	269.0	350.4	228.1

ANOVA table

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	14787.82	2	7393.91	1.604	0.213
Residuals	193594.55	42	4609.39		
Total	208382.36	44			

Pressure at 50 ml distension

		Total	Nulliparous	Parous	Male
N		45	19	16	10
Mean		67.7	69.0	65.4	68.7
Std. Deviation		10.8	10.6	11.6	10.2
Std. Error		1.6	2.4	2.9	3.2
95% Confidence Interval for Mean	Lower Bound	64.4	64.0	59.2	61.4

	Upper Bound	70.9	74.1	71.6	76.0
Minimum		42.8	47.0	42.8	50.2
Maximum		93.3	93.3	91.6	82.7

ANOVA table

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	129.14	2	64.57	0.547	0.583
Residuals	4962.43	42	118.15		
Total	5091.57	44			

Distensibility index at 50 ml distension

		Total	Nulliparous	Parous	Male
N		45	19	16	10
Mean		2.1	1.8	2.6	2.0
Std. Deviation		1.4	1.2	1.8	1.1
Std. Error		0.2	0.3	0.4	0.4
95% Confidence Interval for Mean	Lower Bound	1.7	1.3	1.7	1.1
	Upper Bound	2.6	2.4	3.6	2.8
Minimum		0.6	0.8	1.0	0.6
Maximum		8.2	4.9	8.2	3.8

ANOVA table

	Sum of Squares	df	Mean Square	F	p-value
Between Groups	5.81	2	2.90	1.461	0.244
Residuals	83.51	42	1.99		
Total	89.32	44			

Normal values

0-50ml distension parameters

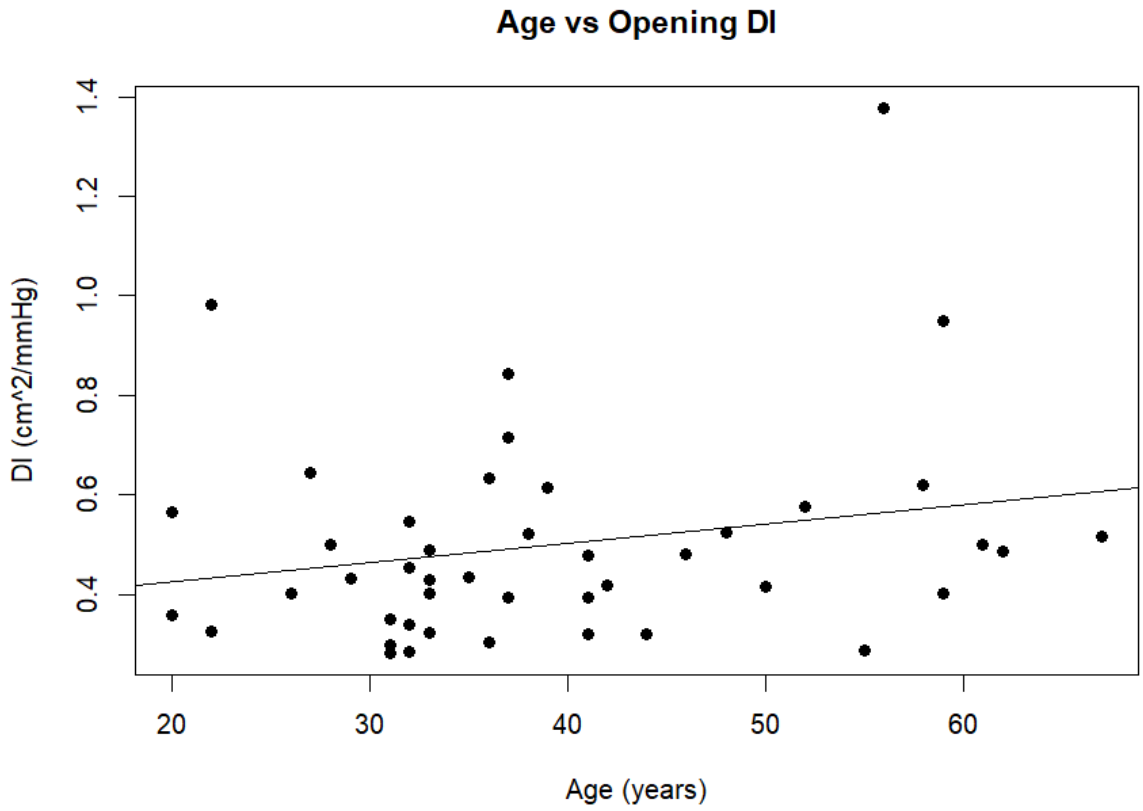
	Median	5th percentile	95th percentile
Opening pressure (mmHg)	47.0	22.3	75.1
Opening volume (ml)	38.0	29.6	44.7
Distensibility index (cm^2/mmHg)	0.43	0.28	0.97
Compliance (ml/mmHg)	0.96	0.67	2.30
Distension rate after opening (ml/mmHg)	0.43	0.36	0.66

50ml resting parameters

	Median	5th percentile	95th percentile
CSA (cm^2)	115.0	51.1	292.1
Pressure (mmHg)	67.9	48.0	89.0
Distensibility index (cm^2/mmHg)	1.94	0.76	4.75

Association between age and distensibility

Age vs Opening DI



H0= No linear slope

H1= Linear slope

P-value: 0.15

Interpretation: No statistically significant linear association between opening DI and age (total variation explained: R^2= 4.9%.

Equation of the regression line:

Opening DI=0.35 + 0.004 *age

As age increases by 10 years, mean opening DI increases by an estimated 0.04 units, 95% CI (-0.01-0.09).

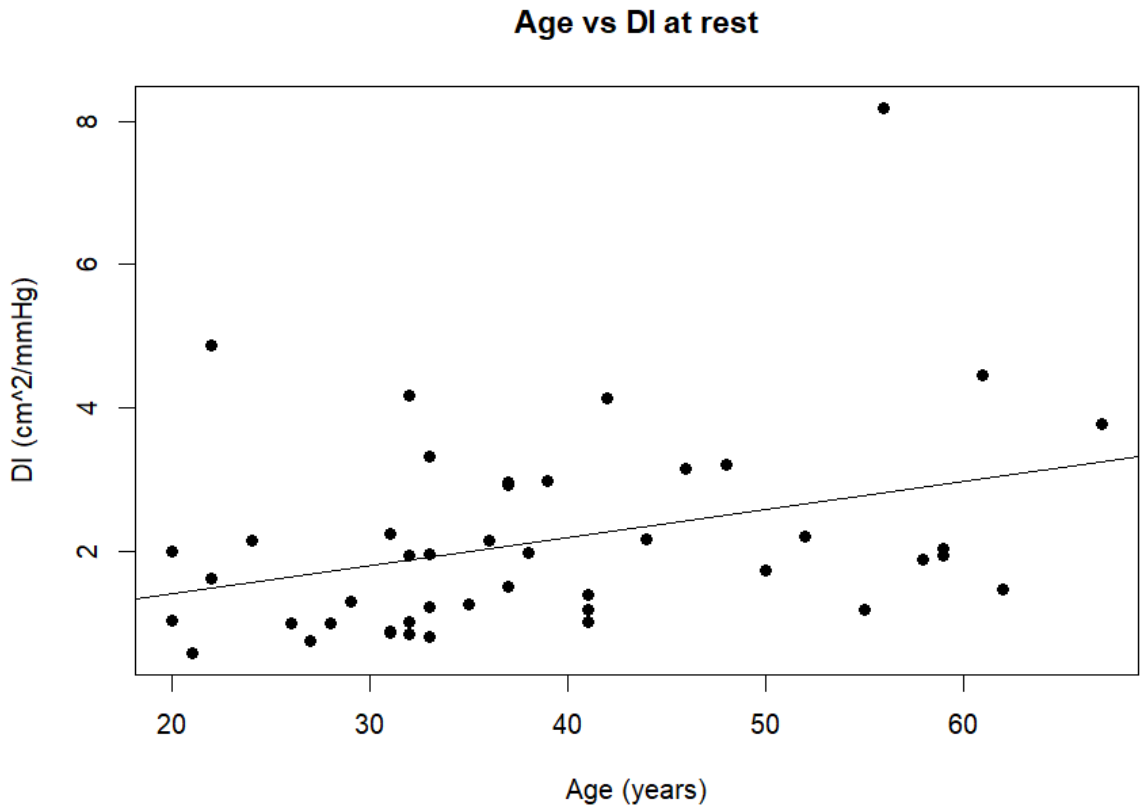
	df	Sum of Squares	Mean Square	F	p-value
Between Groups	1	0.095	0.095	2.16	0.1489

Residuals	42	1.85	0.044		

R-squared: 0.04895

95% CI: -0.0014 to 0.0091

Age vs Opening DI



H0= No linear slope

H1= Linear slope

P-value: 0.02

Interpretation: 2

Equation of the regression line:

DI at rest= 0.64 + 0.039 *age

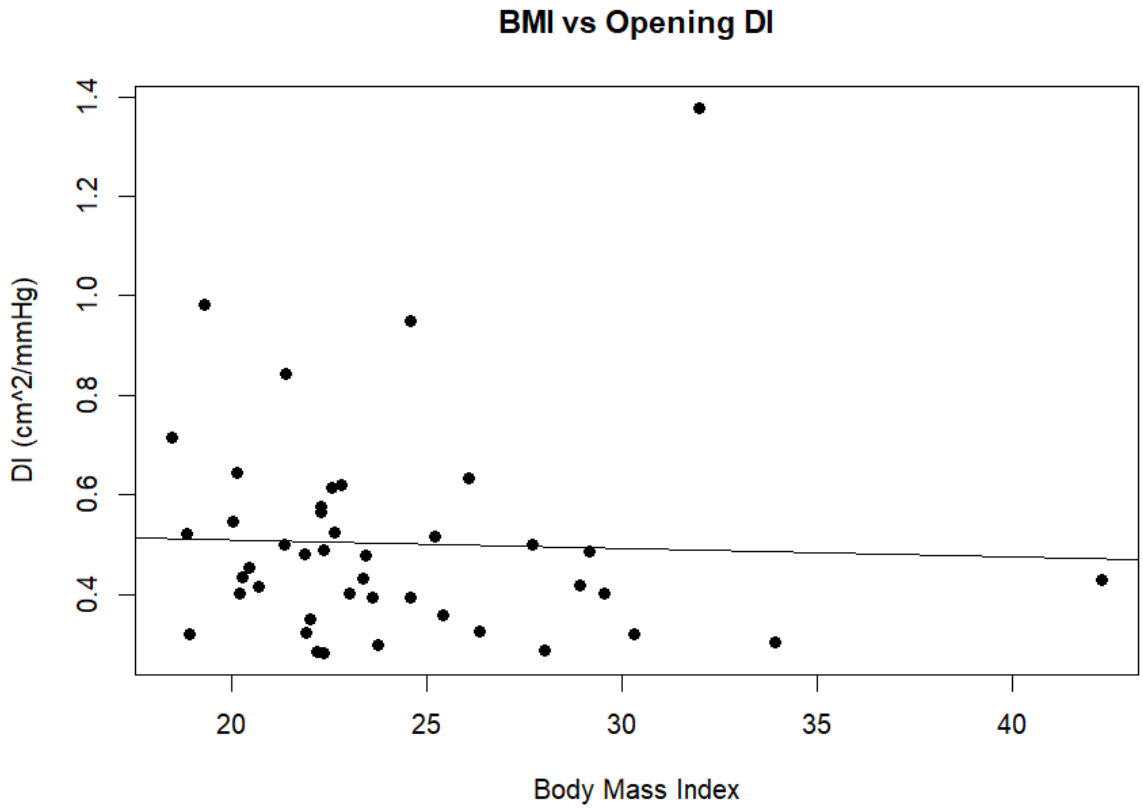
As age increases by 10 years, mean resting DI increases by an estimated 0.4 units, 95% CI (0.06-0.7).

	df	Sum of Squares	Mean Square	F	p-value
Between Groups	1	10.57	10.57	5.7709	0.02068
Residuals	43	78.754	1.8315		

R-squared: 0.1183

95% CI: 0.0063 to 0.0719

BMI vs Opening DI



H0= No linear slope

H1= Linear slope

P-value: 0.8226

Interpretation: No statistically significant linear association between opening DI and BMI . (total variation explained by model: 1%.

Equation of the regression line:

DI at rest= 0.54 - 0.0016 *age

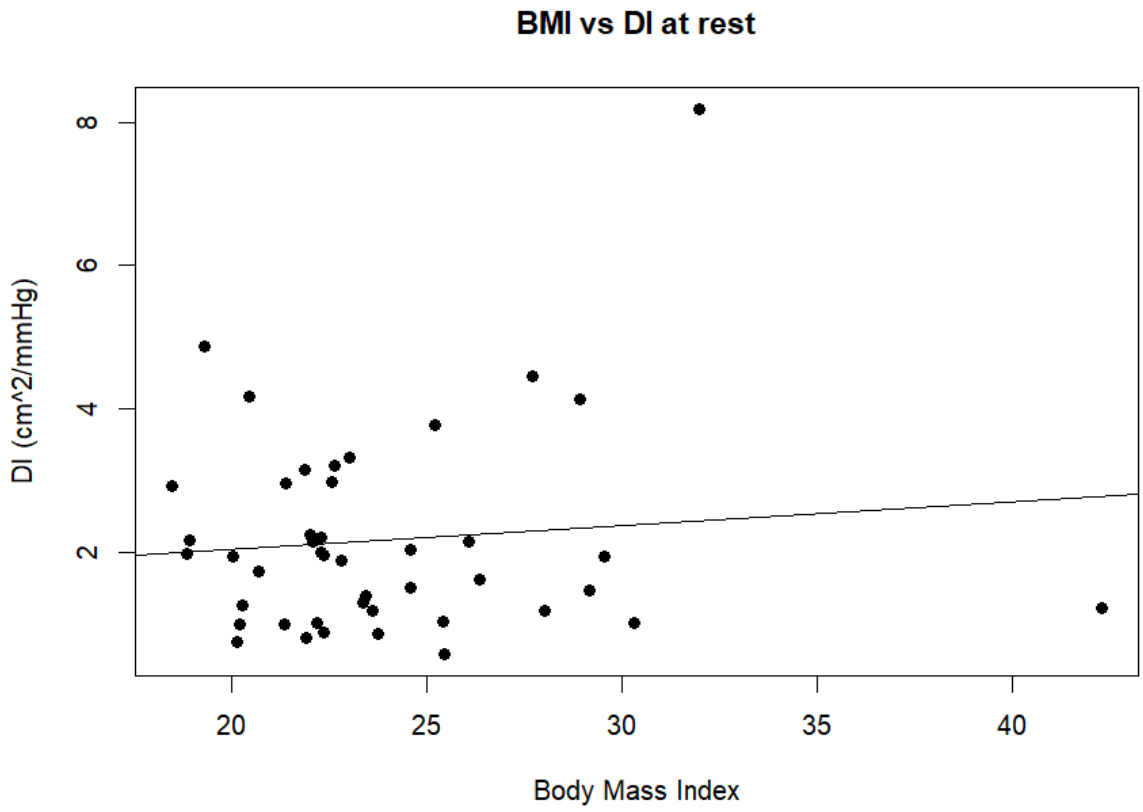
As BMI increases by 1 unit, mean opening DI decreases by an estimated 0.002 units, 95% CI (-0.02-0.01).

	df	Sum of Squares	Mean Square	F	p-value
Between Groups	1	0.00238	0.0028	0.0509	0.8226
Residuals	41	1.916	0.047		

R-squared: 0.00124

95% CI: -0.016 to 0.013

BMI vs DI at rest



H0= No linear slope

H1= Linear slope

P-value: 0.518

Interpretation: No statistically significant linear association between DI at rest and BMI. (total variation explained by model: 10%.

Equation of the regression line:

DI at rest= 1.38 – 0.033 *age

As BMI increases by 1 unit, mean opening DI decreases by an estimated 0.002 units, 95% CI (-0.02-0.01).

	df	Sum of Squares	Mean Square	F	p-value
Between Groups	1	0.877	0.877	0.4249	0.518
Residuals	42	86.703	2.064		

R-squared: 0.01002

95% CI: -0.0692 to 0.135

Mean age and BMI

	Total	Males	Females	Nulliparous	Parous
n	46	11	35	19	16
Age (sd)	38.5 (12.4)	38.2 (16.3)	38.5 (11.2)	35.3 (11.8)	42.4 (9.3)
Body Mass Index (sd)	24.1 (4.5)	24.8 (3.8)	23.9 (4.8)	23.7 (5.5)	24.0 (3.8)

Appendix 12

Chapter 8 Summary tables for squeeze distensibility assessment

Squeeze pressure

	Total	Males	Female	Nulliparous	Parous
n	41	8	33	17	16
mean	102	125	96	98*	94*
SD	22.3	29.6	15.9	16.8	15.1
95% CI	95 - 109	101 - 150	90 - 102	90 - 107	85 - 102
median	97	126	95	97	92
5th	71	81	70	68	71
95th	141	179	131	130	132

Squeeze DI

	Total	Males	Female	Nulliparous	Parous
n	41	8	33	17	16
mean	0.8	0.3	0.9	0.7	1.1*
SD	0.61	0.15	0.61	0.49	0.70
95% CI	0.6 – 1.0	0.1 - 0.4	0.7 - 1.1	0.5 – 1.0	0.7 - 1.4
median	0.6	0.2	0.7	0.6	1.1
5th	0.2	0.2	0.2	0.2	0.2
95th	2.2	0.6	2.3	1.8	2.4

*p<0.05 vs males

Appendix 13

Chapter 8 Summary tables for cough distensibility assessment

Cough pressure

	Total	Males	Female	Nulliparous	Parous
n	31	8	23	13	10
mean	103	130	93	94*	93*
SD	28.5	35.6	18.7	22.2	14.0
95% CI	92 - 113	100 - 159	85 - 102	68 - 146	72 - 114
median	95	111	90	92	88
5th	70	93	69	68	72
95th	173	201	140	146	114

Cough DI

	Total	Males	Female	Nulliparous	Parous
n	31	8	23	13	10
mean	1.0	0.2	1.3	1.0*	1.6*
SD	0.84	0.06	0.82	0.64	0.94
95% CI	0.7 - 1.3	0.2 - 0.3	0.9 - 1.6	0.6 - 1.4	0.9 - 2.3
median	0.8	0.2	1.2	0.8	1.5
5th	0.1	0.1	0.2	0.1	0.3
95th	2.8	0.3	2.9	2.2	3.0

*p<0.05 vs males