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Biomechanical Measures of Function of the Ano-rectal Region of the Human Body

by

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A dissertation submitted to the University of Dublin for the degree of
Doctor of Philosophy

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March 2015

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Declaration

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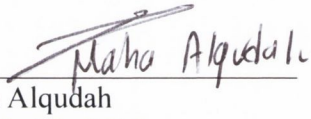

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Dedication

I would like to dedicate this thesis to my husband Esmat for his unfailing love and ever present support.

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Summery

In this research, the Functional Lumen Imaging Probe (FLIP), a novel measurement tool, was adapted to measure the biomechanical properties and morphological changes of the ano-rectal region during distension and provocative manoeuvres. Two customs made FLIP probes (prototype 1& prototype 2) and original EndoFLIP® (prototype 3) were developed and tested in bench top studies and in pilot studies. Different aspects were considered when the FLIP probe was developed to measure the distensibility along the anal canal: the accuracy of the CSA and pressure measurements along the anal canal; the length of the probe should be capable of measuring the morphological changes along the anal canal; and the size of the probe should be narrow enough to not elicit relaxation of the IAS and accordingly causing a continues movement inward and outward of the probe during the pilot studies. EndoFLIP ® (prototype 3) originally designed to measure OGJ compliance, with a length of 10cm and maximal diameter of 2.5cm, was safely positioned and distended in the anal canal of patients with FI without continuous movement along the anal canal and at the same time was capable of plotting the hour glass shape of the anal canal at different volumes. Hence this design was selected for the ano-rectal region evaluation.

Twenty-one healthy volunteers 36.5±2.5 years (mean ± SEM) were studied using the EndoFLIP® probe (FLIP prototype 3). The test was well tolerated from all volunteers. A new protocol for studying ano-rectal function involved anal canal distension and subsequent morphological evaluation was developed from the interpretation of selected results. The physiological interpretation of that experiment was hindered by the absence of any validation components of this technology with other systems. Pilot study with Endoflip® under video-fluoroscopy confirmed the probe position in the anal canal and introduced three outcome measures of the anal canal distensibility including narrow zone length, narrow zone average CSA and bag pressure. Another EndoFLIP® pilot study with Endoanal Ultrasound estimated the muscular structure surrounding the anal canal at different distension volumes.

Nineteen faecal incontinence (FI) patients 58.3± 2.8 years (mean ± SEM years) with dysfunctional anal sphincter were studied. The test was well tolerated from all patients. The data from the healthy volunteers and FI patients were investigated using distensibility test. At each step volume, the outcome measures were evaluated. During 40ml ramp distensions, long and wide narrow zone was observed in the healthy volunteers at low volumes indicating that the upper and lower parts of the anal canal were the least distensible. At high volume, this narrow zone shortened and tightened in the middle of the anal canal indicating that the middle part of the anal canal was the least distensible. In FI patients, a significant short and tight narrow zone was observed at low volumes indicating that the

middle part was the least distensible. This narrow zone became more distensible and opened widely at high volume.

During squeezing test significant elongation was observed in the narrow zone of healthy volunteers, this elongation trapped fluid in the middle of the narrow zone and increased the value of the average CSA at low volumes. However, only at high value a significant reduction in the CSA was observed when the middle part was the least distensible and the fluid was pressed away. No significant morphological changes were observed in FI volunteers suggesting a weakening in the contractile properties of the EAS and PRM. Quantitative measures of squeezing durations were also obtained at different volumes with squeezing time significantly higher in healthy volunteers.

Contour plot of the sixteen CSA was used to present the cough and straining data from both groups. During coughing all healthy volunteers managed to keep the bag in the anal canal, however, the eject bag were proportional to the distended volumes in FI group. During straining in healthy volunteers, the bag was pushed outside the anal canal gradually over a period of time which was significantly longer than in FI.

In order to initiate the diagnostic accuracy process, EndoFLIP® measures resting and squeezing pressure and average CSA were compared to ano-rectal manometry resting and squeezing pressure. Findings from this study indicate that there is a single significant negative correlation between EndoFLIP® resting CSA at 40ml and ano-rectal manometry resting pressure. This work contributes original quantitative information that can describe the biomechanical properties and the morphological changes along the anal canal during distension and provocative manoeuvres. This new measurements can be used to improve our understanding of continence mechanism.

List of Tables

Page

Table 1.1: Etiology of faecal incontinence..... 16

Table 2.1: The voltage measured at each electrode (E) during the calibration of FLIP prototype 1..... 51

Table 2.2: Change in anal canal Cross-Sectional Area and FLIP bag Pressure During 10ml,20ml, 30ml, 40ml and 50ml ramp distensions (N=4) 62

Table 2.3: Change in anal canal Cross-Sectional Area and FLIP bag Pressure during rest, squeeze, cough and strain manoeuvres at 30ml and 40ml step volumes (N=4)..... 64

Table 2.4: The average changes in PMAXi (mmHg) and PMINi(mmHg) during rectoanal inhibitory reflex (RAIR). Average CSA measured at the min bag pressure (P MIN) higher than the CSA measured during resting when the rectum balloon was filled with 30ml, 40ml, 50ml and 60ml air. The measurements were observed at two step volumes of 30ml and 40ml inside the FLIP bag (N=4)..... 66

Table 2.5: Changes in anal canal CSA and FLIP bag pressure during rectal sensation test (N=4)..... 68

Table 3.1: CSA measurements of EndoFLIP® inflated with diluted ionic contrast agent in the accuracy test..... 77

Table 4.1: FI patients background information..... 109

Table 4.2: Comparing the pressure, average cross-sectional area (CSA), and length between the FI and controls during ramp distension (0-40) ml at rate of 40ml/min. Medians and interquartile ranges; * Significant differences between volumes, Friedman test $p<0.001$; ** Significant differences between disease and control, Mann-Whitney test..... 110

Table 4.3: Medians and interquartile ranges from length, pressure and CSA from the Squeeze test were compared with the rest measurements at 20ml, 30ml and 40ml..... 114

Table 4.4: Bag position during cough test for FI volunteers at 20ml, 30ml and 40ml bag volume..... 114

Table 4.5: Medians and interquartile ranges from length, pressure and CSA from the coughing test were compared with the rest measurements at 20ml, 30ml and 40ml..... 115

Table 4.6: Medians and interquartile ranges of pressures and duration for healthy and FI volunteers during straining at 40ml volume 116

Table 4.7: Medians and interquartile ranges of pressures for healthy and FI volunteers during resting and straining tests at 40ml volume..... 116

List of Figures

Page

Figure 1.1: Axial view of the pelvic floor. *(Photograph kindly provided by Professor FBV Keane)*..... 4

Figure 1.2: Anatomy of the Ano-rectum. *(Photograph kindly provided by Professor FBV Keane)*..... 6

Figure 1.3: Innervation of the pelvic floor. *(Photograph kindly provided by Professor FBV Keane)* 8

Figure 1.4: The anorectal angle at rest (A), during squeezing (B) and during defecation(C). *(Reprinted with kind permission from Prof Bharucha)*..... 12

Figure 1.5: Recording of anorectal pressure of FI female patient from water perfusion four port catheters during: (a) resting, (b) squeezing and cough (c) 25

Figure 1.6: Normal RAIR due to distension of the ano-rectal manometry balloon. RB (rectal balloon), IS (internal sphincter and ES (external sphincter)..... 26

Figure 1.7: High-definition manometry and pressure topography during rest and voluntary squeeze. In the healthy subject (*left*), normal resting and normal increase in sphincter pressure is seen whereas in the incontinent subject the sphincter is weak during squeeze. *(Reprinted with permission from Prof Bharucha)* 28

Figure 1.8: Simple manometry catheter shown in position in the anal sphincters when it is toned. A: The toned or competent sphincter causing the sensor to be occluded by the sphincter; B: The pressure is indicative of the state of the chamber created by the opening of the lumen. *(Modified from McMahon et al, 2009)* 29

Figure 1.9: dynamic MRI (A, B, C) in one patient. Images were acquired at rest (*A*), during squeeze (B), and defecation (C). MR and evacuation defactographic images demonstrate abnormal descent of the ano-rectal junction during defecation to 5.5 cm and 9.4 cm below the pubococcygeal line respectively. *(Reprinted with kind permission from Prof Bharucha)*..... 33

Figure 1.10: Sagittal dynamic MRI images of normal puborectalis relaxation (left panel, subject 1) and puborectalis contraction (black arrow, right panel, subject 2) during rectal evacuation. (*Reprinted with permission from Prof Bharucha*) 34

Figure 1.11: Endoanal ultrasound (EAUS) and MRIs of anal sphincters. (A) The IAS is higher signal intensity than the EAS on MRI. The IAS is thin (thin white arrows). The EAS tear is between the thick white arrows. (B) On MRI, the intact internal anal sphincter (small white arrows) is of higher signal intensity than the external anal sphincter and demonstrates the same defect, between large black arrowheads. The EAS is located between the large white arrowheads. (C) Axial endoanal MRI at a more caudal level demonstrates similar abnormalities in the IAS between the large black arrowheads and EAS between the small 35

black arrowheads. (*D*) Axial endoanal MRI demonstrating normal IAS (short white arrows), longitudinal muscle (long white arrow), and EAS (black arrowheads). *(Reprinted with kind permission from Prof Bharucha)* .....

Figure 1.12: Sketch of an impedance planimetry probe with one set of excitation (E) and two detecting electrodes (S), for measurement of cross sectional area in the middle of the bag.... 39

Figure 1.13: GMC-IP1000 impedance planimeter is a multipurpose box that can be used with all impedance planimetric probe types and most pressure transducers 40

Figure 1.14: Schematic representation of the dynamic manometry probe for measurement of pressure and cross sectional area in the anal canal. B=bag, C=probe, D=detecting electrode, O=infusion channel. CSA is estimated from measurement of electrical impedance of the fluid inside the bag 41

Figure 1.15: The FLIP is using impedance planimetry technology to measure 8 CSAs along the geometry of the narrowing region of a bodily sphincter (modified from scientific paper.... 41

Figure 1.16: Once the bag is filled with a conductive solution, 16 intra-luminal cross-sectional areas (CSA) are measured within the central part of the bag by the impedance measuring electrodes, whereas the pressure transducers provide the corresponding intra-bag pressure. The volume of conductive solution injected from the syringe to the bag is controlled via the touch screen on the recording unit. The screen displays the calculated CSAs as a cylinder of varying diameter in real time along with the corresponding bag pressure. There is also the option of a split-screen display, which can display a snapshot from previous distension simultaneously with the current acquisition. (*Photograph courtesy of Crospon Ltd., Galway, Ireland.*)..... 44

Figure 2.1: FLIP (prototype 1) consists of 8 electrodes. The distance between the middle of adjacent double electrodes is 6mm. Hence, the length of measurements equal to the distance from the first electrode till the eighth electrode (5*7+1*8=43mm). The maximum measured diameter is equal to the maximum diameter of the non-compliant bag which is 30mm)..... 48

Figure 2.2: The FLIP bag dimensions 49

Figure 2.3: study protocol..... 50

Figure 2.4: FLIP probe (prototype 1) built to be positioned safely in the anal canal cavity The probe was constructed of a three lumen polyethylene catheter with an outer diameter of 1.6mm..... 51

Figure 2.5: Calibration curve showing the CSAs represented by voltages at eight sensing electrode pairs in the FLIP. The horizontal lines represent the variation of the voltage measured over calibration cylinders 52

Figure 2.6: a schematic draw of the FLIP positioned in the anal canal 52

Figure 2.7: Results of the 50ml bag distension from the 8-electrode FLIP. (A) Pilot one and (B) pilot two. Top of each image is towards the rectum and the bottom is more external. Volume increased in steps of 10ml until a volume of 50ml. Diameters are given in the 8 boxes on the right hand side of the FLIP diagrams and bag pressures in mmHg and volume in millilitre are given at the bottom of each diagram 53

Figure 2.8: Result from the squeezing manoeuvre at 30ml, 40ml and 50ml bag volume in the anal canal. Top of each image is towards the rectum and the bottom is more external. Diameters are given in the 8 boxes on the right hand side of the FLIP diagrams and bag pressures in mmHg are given at the bottom of each diagram..... 54

Figure 2.9: Schematic draws of the designed FLIP which was used in experiment 2. It consisted of; a stretchable balloon (rectum balloon) was used to distend the rectum during the rectoanal inhibitory reflex (RAIR) and the rectal sensation tests. The second bag is a non-compliant bag located in the anal canal contains 16 sensing electrode pairs capable of measuring the geometry of the anal canal at different manoeuvres tests. The pressures inside the Flip bag and the rectum balloon were measured 57

Figure 2.10: This image represents the equipment set up for experiment-2. It consist of an EndoFLIP® system comprising recording unit, GMC-IP1000 impedance planimeter, syringe pump , pressurised cuff and the custom made FLIP (prototype 2)..... 58

Figure 2.11: A schematic draw of the FLIP prototype 2 in position 59

Figure 2.12: (A) FLIP (prototype 2) design for anorectal evaluation. (B)The rectal balloon was filled with 250ml of air and the FLIP bag was filled with 50ml of conductive saline 61

Figure 2.13: A graph represents the relation between the measured and actual measurements during accuracy test, the fitting equation was 1.1 * displayed diameter+2.5..... 61

Figure 2.14: A graph represents the relation between the pressure and volume of the FLIP bag during ramp distension. A dramatic increase in pressure occurred at 50ml volume 62

Figure 2.15: Geometric profile of the anal canal during ramp distension from four healthy volunteers. A narrow zone is formed along the anal canal during the ramp distension. A wide zone formed near top as more volume is distended in the bag. Top of each image is towards the rectum and the bottom is more external. The volume in each image represents the volume in the bag 63

Figure 2.16: The pressure-CSA relation at the narrowest point in the anal canal for four healthy controls (HC) 64

Figure 2.17: A series of rectal distensions were performed to measure the RAIR. The PMAX as well as the PMIN were measured at each rectal distension. This figure represents the RAIR test for two volunteers at 30ml step volume in the FLIP bag. PMAX1 and PMIN1 were calculated when the balloon rectum was inflated with 30ml air; PMAX2 and PMIN2 were calculated when the balloon rectum was inflated with 40ml air; PMAX3 and PMIN3 were calculated when the balloon rectum was inflated with 50ml air; PMAX4 and PMIN4 were calculated when the balloon rectum was inflated with 60ml air 65

Figure 2.18: This figure represents the recto-anal inhibitory test at 30ml FLIP bag volume. 1. During resting, the rectum balloon was empty; 2. When the rectum balloon was inflated with 60ml of air, both FLIP bag pressure and rectal balloon pressure reached their maximum value; 3. After less than one second, the anal canal relaxed and the FLIP bag pressure reduced to its minimum value 66

Figure 2.19: This figure represents the rectal sensation test for one subject at 30ml step volume in the FLIP bag. At (A) the changing in the FLIP bag pressure and the minimum CSA during slow rectal balloon distension is plotted. At (B) a still images of the anal canal 67

from the EndoFLIP® screen during the rectal sensation test. 1. During resting, the rectum balloon was empty; 2. First sensation occurred at 80ml air inflated in the rectal balloon; 3. Strong sensation occurred at 110ml air inflated in the rectal balloon; 4. Maximum tolerable volume was 240ml air. (The sudden decrease in minimum CSA at 100ml of air in the rectum balloon is a technical error) .....

Figure 2.20: A still images from EndoFLIP screen. Diameters are given in the sixteen boxes on the right hand side of the FLIP diagrams and bag pressures in mmHg and volume in mL are given at the bottom of each diagram 72

Figure 3.1 EndoFLIP® probes were immersed in each cup of the two sets of radiant dilutions..... 75

Figure 3.2: An illustration showed the FLIP bag inflated in a phantom and placed in a patient phantom. It is a Perspex material with similar density to water that is routinely used to simulate a patient in an x-ray beam during testing. We will most likely have 10cm of Water on top of the probe (towards the tube side of the x-ray machine), and 5cm below (towards the detector side of the x-ray machine) to simulate the probe within a patient body 75

Figure 3.3: An X-ray image of the diluted (a) non-ionic and (b) ionic contrast agents. The highlighted circle represent the FLIP bag immersed in (1:12) diluted ionic contrast agent. 76

Figure 3.4: An X-ray image of EndoFLIP® bag inflated with 1:12 diluted contrast agent inside a phantom and placed in a patient phantom 77

Figure 3.5: (a) Illustration showing the EndoFLIP® in position along the anal canal in the anorectal region. EndoFLIP® distal end was positioned toward the rectum and the EndoFLIP® proximal end was positioned outside the anal verge (black arrows). (b) Diagram for volunteer protocol starting with 3 ramp distensions(0-40ml) at 40ml/min. (c) Then three further step distensions at 20, 30 and 40ml, at each step volume the volunteers were asked to perform two provocative manoeuvres (squeeze, cough, strain) 80

Figure 3.6: An X-ray image of EndoFLIP® in the anal canal of male volunteer during different manoeuvres (rest, squeeze, cough and strain). The blue line represents the anal verge. The number of the electrode rings that were seen below the anal verge was not the same in all manoeuvres. The sixteen ring electrodes are highlighted by the white dots and were not always clearly visible during the provocative manoeuvres 82

Figure 3.7: An X-ray image of EndoFLIP® in the anal canal of female volunteer during rest on the left and squeezing on the right. The blue line represents the anal verge. The sixteen ring electrodes are highlighted in yellow. The probe was angulated inside the anal canal as an effect of the anatomical structure of the region as highlighted by the thin black arrow. The angle was accentuated during the squeezing which shifts the probe toward the rectum 83

Figure 3.8: An X-ray image of the EndoFLIP® bag when inserted in the anal canal. The bag was distended by 40ml of prepared radiant conductive solution. The figure clearly shows the sixteen ring electrodes inside the probe. The distal end of the bag was located in the rectum as the probe was curved by 77.4◦ 83

Figure 3.9: Geometric profile of the anal canal during ramp distension of the healthy male subject. The top of each profile is directed toward the distal end of EndoFLIP®. Diameters are given in the sixteen boxes on the right hand side of the FLIP diagrams and bag pressures in mmHg at the bottom of each diagram. The longitudinal white line highlights the narrow zone length at each volume. The orange rings represent the average estimated diameters 84

along the narrow zone. A distinct narrowing towards the centre of the colour profile was observed at higher volumes. EndoFLIP® distal end was positioned toward the upper part of the anal canal and the EndoFLIP® proximal end was positioned toward the lower end of the anal canal .....

Figure 3.10: Geometric profile of the anal canal during ramp distension of the FI female subject. The top of each profile is directed toward the EndoFLIP® distal end. Diameters are given in the sixteen boxes on the right hand side of the FLIP diagrams and bag pressures in mmHg at the bottom of each diagram.The longitudinal white line highlighted the narrow zone length at each volume. The orange rings represent the average estimated diameters along the narrow zone. A distinct opening in the narrow zone was observed at higher volumes. EndoFLIP® distal end was positioned toward the upper part of the anal canal and the EndoFLIP® proximal end was positioned toward the lower end of the anal canal 85

Figure 3.11: Still images from the male healthy volunteer during resting and squeezing at three step volumes 20ml, 30ml and 40ml. Diameters are given in the sixteen boxes on the right hand side of the colour profile and bag pressures in mmHg at the bottom of each diagram. The longitudinal white line highlighted the narrow zone length at each volume. The orange rings represent the average estimated diameters along the narrow zone. The narrow zone was tightly closed during both resting and squeezing manoeuvres. Both narrow zone length and bag pressure increased noticeably during squeezing at each step volume. EndoFLIP® distal end was positioned toward the upper part of the anal canal and the EndoFLIP® proximal end was positioned toward the lower end of the anal canal 86

Figure 3.12: Still images from the female FI volunteer during resting and squeezing at three step volumes 20ml, 30ml and 40ml. Diameters are given in the sixteen boxes on the right hand side of the colour profile and bag pressures in mmHg at the bottom of each diagram. The longitudinal white line highlighted the narrow zone length at each volume. The orange rings represent the average estimated diameters along the narrow zone. The estimated diameter of the narrow zone increased significantly at 40ml volume. Narrow zone length increased during squeezing only at 20ml step volume. The bag pressure increased during squeezing at all step volumes. EndoFLIP® distal end was positioned toward the upper part of the anal canal and the EndoFLIP® proximal end was positioned toward the lower end of the anal canal 87

Figure 3.13: Squeezing pressure at three step distensions 20ml, 30ml and 40ml. t1, t2, t3 represents the squeezing duration time at 20ml, 30ml and 40ml respectively..... 88

Figure 3.14: The contour plot for the coughing manoeuvre for the healthy male subject and the FI female subject. The FLIP bag was still in position during coughing in the healthy volunteer and was ejected in the FI volunteer 89

Figure 3.15: The contour plot for the straining manoeuvre for the healthy male subject and the FI female subject. The FLIP bag was moving gradually toward outside the anal verge in 3seconds in the healthy volunteer and was ejected in less than 1 second in the FI volunteer.... 90

Figure 3.16: The ultrasound probe was assembled by filling the small rigid balloon (60mm) on the end of the ultrasound probe with degassed water then covering the end of the probe with a latex balloon. The evaluation of the anal sphincter was done using special radial rotary transducers (15mm) spinning 360° around a fixed shaft 92

Figure 3.17: A comparison between the anal canal (upper (A), middle (B) and lower (C) levels as measured by the ultrasound probe (i) and the anal canal profile as measured by the EndoFLIP® (ii). The EAUS probe was used to scan the muscular structure around the probe in the upper part (A) of the anal canal (40mm in depth from the anus). Then the probe was 93

withdrawn down approximately 5-10 mm toward the middle of the anal canal (B). Then the probe was withdrawn down again approximately 5-10 mm toward the lower end of the anal canal.....

Figure 3.18: A representation of images for female patient (p001). On the left (EndoFLIP®), anal canal profiles at 10ml, 20ml, and 30ml and 40ml ramp distensions. On the right (2.EAUS) A, B and C represented the upper, middle and lower portions of the anal canal as measured by the Endo-anal Ultrasound. On the right the muscular images measured by the ultrasound probe at three levels. The muscular image consisted of a striated muscle sling (white shaded) and a thick circle of smooth muscle (black shaded) around the probe. The red arrows indicated the striated muscles sling ends and the yellow circle highlighted the smooth muscle structure. The small dotted red circles at B&C represented a muscular defect in the striated muscles 94

Figure 3.19: A representation of images for female patient (p002). On the left (EndoFLIP®), anal canal profiles at 10ml, 20ml, and 30ml and 40ml ramp distensions. On the right (2.EAUS) A, B and C represented the upper, middle and lower portions of the anal canal as measured by the Endo-anal Ultrasound. On the right the muscular images measured by the ultrasound probe at three levels. The muscular image consisted of a striated muscle sling (white shaded) and a thick circle of smooth muscle (black shaded) around the probe. The red arrows indicated the striated muscles sling ends and the yellow circle highlighted the smooth muscle structure..... 95

Figure 4.1: Probe positioning in five volunteers. The red arrow and the white arrow pointed to the distal border and the proximal border of the anal canal profile respectively. The EndoFLIP® was retracted in the anal canal till thee CSA measurements were seen outside the distal border. The number of CSA measurements seen outside the proximal border varied according to the narrow zone length; it can contain 3-CSA measurements as in volunteers (b&d) or 4-CSA measurements as in volunteers (a&e) 99

Figure 4.2 Still images of geometric changes in the anorectal region during distension for four volunteers. The top of each image (the upper anal canal) is towards the rectum and the bottom (lower anal canal) is outside the anal verge .The x axis represents the distended profile diameter. The y-axis on the left side represents the position of sixteen sensing electrodes across the EndoFLIP® bag with electrode 1 located toward the anal verge. The y-axis on the right side represents the changing in diameter in millimetres compared with the change in colour in the anal profiles as appeared on the EndoFLIP® display. Three main regions shown with the annotations A, B & C were formed during volume distension in the anal canal. A & B illustrate narrow regions formed at low volumes in the upper and lower segments and C illustrates a distinct narrowing towards the centre (middle segment) of the colour profile at higher volumes..... 100

Figure 4.3: Distensibility plot of the distal border (pressure plotted against the cross-sectional area of the upper segment) for ramp distensions at 40ml/min for the male and female healthy controls. The distensibility index was 0.086 for females and 0.077 for males. The opening pressure for females was higher than for males 101

Figure 4.4: : (a) The changes in the anal profile during squeezing at 20ml in the EndoFLIP® bag between at rest (blue outline) and squeeze (red line) for one health control. This image was used to determine the anal canal borders. The changes at both borders are represented by δ prox (proximal border toward anal verge) and δ distal (distal border towards rectum) which are as a result of changes in the CSA values at the relevant detectors. This figure suggests that the anal canal is narrower at rest than during squeezing and the distended narrow zone is longer during squeezing than at rest. (b) CSA for δ prox and δ distal and bag pressure for the same volunteer as shown in (a). (c) CSA measurements along the anal canal that lie between 102

the δ prox and δ distal CSA measurements for the same volunteer. Figure 4(b) and figure 4(c) use the same x-axis time scale and represent identical points in time .....

Figure 4.5: The changes in the anal profile during coughing at 20ml in the FLIP bag between at rest (blue outline) and cough (red line) for one healthy control. This image was used to determine the anal canal borders. The changes at both borders are represented by δ prox (proximal border toward anal verge) and δ distal (distal border towards rectum) which are shown as changes in the CSA values at the relevant detectors. This figure suggests that the distended narrow zone was longer during coughing than at rest. (b) CSA measurements for δ prox and δ distal and bag pressure for the same volunteer as shown in (a)..... 103

Figure 4.6: Average pressure plotted against volume during squeezing and coughing (n=20). Anal canal pressure during coughing was higher than during squeezing at all three step volumes. Also the squeezing and coughing pressure at 20ml was significantly lower than the pressure measured at 30 and 40ml 103

Figure 4.7: The changes in the anal profile during straining at 30ml in the FLIP bag in (A) one male and (B) one female healthy subject. The straining manoeuvre started with a sharp increase in the bag pressure at time T1 and then a change in the morphology of the anal canal occurred at T2. The straining time was defined as the time between T1 and T2 104

Figure 4.8: This figure illustrated the EndoFLIP® bag pressure as well as the proximal and distal CSAs of anal canal narrow zone for one subject during straining manoeuvre. The bag pressure at rest (blue line) increased to 65mmHg when the straining started. After 3 seconds the proximal CSA (red line) increased and the distal CSA (green line) decreased. There was a time (T) between the T1 (the time when the pressure reaches its max value) and T2 (the time when the anal profile change). Straining time= T2 – T1 105

Figure 4.9: Changes in length between control (N=20) and disease (N=19) 111

Figure 4.10: Changes in pressure between control (N=20) and disease (N=19) 112

Figure 4.11: Changes in cross sectional area between control (N=20) and disease (N=19) 112

Figure 4.12: Distensibility for control (N=20) VS disease (N=19) at each volume 113

Table of Contents

List of Tables vi
List of Figures vii
Table of Contents xiv
Chapter One: Introduction and Literature Review 1
1.1 Introduction 1
1.2 Structure of the thesis 2
1.3 Aims, Research Questions and Hypothesis 3
1.3.1 Aim 3
1.3.2 Research questions 3
1.3.3 Hypothesis 3
1.4 Anatomy 4
1.4.1 Pelvic floor 4
1.4.2 Rectum 5
1.4.3 Anal canal 5
1.4.3.1 External anal sphincter 7
1.4.3.2 Internal anal sphincter 8
1.4.4 Male versus female anatomy of the anal sphincter complex 9
1.5 The mechanism of preserving continence 9
1.5.1 Rectum Function 9
1.5.2 Anal sphincter function 10
1.5.3 Ano-rectal angle 12
1.6 Mechanism of defecation 13
1.7 Theories of anal continence 14
1.7.1 High Pressure zone 14
1.7.2 Resistance to opening 14
1.8 Faecal incontinence 15
1.8.1 Prevalence of FI 15

1.8.2 Causes of FI 16
1.8.2.1 Childbirth (Obstetric Trauma) 16
1.8.2.2 Surgery 17
1.8.2.3 Dysfunctional of the internal sphincter 17
1.8.2.4 Impaired rectal sensation and compliance 17
1.8.2.5 Aging 17
1.8.2 Idiopathic Faecal Incontinence (IFI) 18
1.9 Management of faecal incontinence (FI) 19
1.9.1 Bowel habit modification 19
1.9.2 Pharmacological Approach 20
1.9.3 Biofeedback therapy 20
1.9.4 Surgical approaches 20
1.9.5 Sacral nerve stimulation 20
1.9.6 Conclusion 21
1.10 Clinical evaluation of the faecal incontinence 21
1.10.1 Digital rectal examination (finger index) 21
1.10.2 Ano-rectal manometry 22
1.10.2.1 Ano-rectal manometry systems 22
1.10.2.2 The technique for measuring anal canal pressures 23
1.10.2.3 Parameters measured with ano-rectal manometry probe 24
1.10.2.4 Optional measured parameters 26
1.10.3 Developing anal pressure techniques: High-resolution manometry (HRM) 27
1.10.3.1 Limitation of the manometry system 28
1.10.4 Electromyography of the pelvic floor 30
1.10.4.1 Single-fibre EMG 30
1.10.4.2 Concentric-Needle electrode 30
1.10.4.3 Surface EMG 30
1.10.5 Endoanal ultrasound (EAUS) 31
1.10.6 Evacuation proctography 32
1.10.7 Pelvic Magnetic resonance Imaging (MRI) 33
1.10.7.1 Static and Dynamic MRI 33
1.10.7.2 Endoluminal MRI 34
1.10.8 Conclusion 35
1.11 Developing a new approach to functional testing in the ano-rectum region 36

1.11.1 Background 36
1.11.2 Balloon distension technique (Barostat) 37
1.11.3 Impedance planimetry technique 37
1.11.3.1 Distensibility aspects 37
1.11.3.2 The principle of the impedance planimetry 38
1.11.3.3 Impedance planimetry probe (dynamic anal manometry) 40
1.11.3.4 Errors and Limitations 41
1.11.4 Functional lumen imaging probe (FLIP) technique 41
1.11.4.1 FLIP for Evaluation of the Oesophago-Gastric Junction (OGJ) 43
1.11.5 EndoFLIP® system 43
1.11.5.1 Clinical Utility of EndoFLIP® 44
1.12 Conclusion 45
Chapter Two: Developing a Functional Lumen Imaging Probe (FLIP) For Evaluation of the Anal Sphincters 47
2.1 Introduction 47
2.2 Experiment 1: Designing a FLIP probe for evaluating the anal sphincters (FLIP prototype 1): in vitro & in vivo pilot study 47
2.2.1 The objectives 47
2.2.2 Materials and methods 47
2.2.2.1 FLIP prototype1 Design 47
2.2.2.2 Equipment 49
2.2.2.3 Calibration of the FLIP 49
2.2.2.4 Subjects 50
2.2.2.5 Study protocol 50
2. 2.2.6 Data recording 50
2.2.3 Results 51
2.2.4 Discussion 55
2.3 Experiment 2: Designing a FLIP probe for evaluating the anal sphincters (FLIP prototype 2): in vitro & in vivo pilot study 56
2.3.1 The objectives 56
2.3.2 Material and methods 56
2.3.2.1 FLIP (prototype2) design 56
2.3.2.2 Accuracy test of the FLIP 57
2.3.2.3 Equipment 57
2.3.2.4 Subjects 58

2.3.2.5 Study protocol 58
2.3.2.6 Sensory assessment 60
2.3.2.7 Data analysis 60
2.3.3 Results 60
2.3.3.1 Probe construction 60
2.3.3.2 Ramp distension 62
2.3.3.3 Rest, squeeze, cough, strain 64
2.3.3.4 Recto-anal inhibitory reflex (RAIR) test 65
2.3.3.5 Rectal volume sensory threshold test 67
2.3.4 Discussion 68
2.4 Experiment 3: Original EndoFLIP ® (FLIP prototype 3): A pilot study 71
2.4.1 The objectives 71
2.4.2 Materials and methods 71
2.4.2.1 Subjects 71
2.4.2.2 Equipment 71
2.4.2.3 Study protocol 71
2.4.2.4 Data analysis 72
2.4.3 Results 72
2.4.4Discussion 72
2.5 Conclusion 73
Chapter Three: Pilot Studies to Validate Study Protocol and to Define Outcome Measures 74
3.1 Introduction 74
3.2 Design and Preparation of a Radio-opaque EndoFLIP probe for Validation of Probe Position 74
3.2.1 Objectives 74
3.2.2 Material and methods 74
3.2.2.1 Equipment 74
3.2.2.2 Preparing a radiant conductive solution 74
3.2.2.3 The accuracy of EndoFLIP® CSA measurements 76
3.2.3 Results 76
3.2.4 Discussion 77
3.3 Pilot study1: Validation of the probe position by Video-fluoroscopy 78
3.3.1 Objectives 78
3.3.2 Materials and methods 78
3.3.2.1 Subjects 78

3.3.2.2 Distensibility measurements 78
3.3.2.3 Study Protocol 79
3.3.2.4 Probe Positioning 81
3.3.3 Results 81
3.3.3.1 Probe positioning via video-fluoroscopy 81
3.3.3.2 EndoFLIP® outcome measurements 84
3.3.4 Discussion 90
3.4 Pilot study 2: Endoanal Ultrasound validation tool 92
3.4.1 Objectives 92
3.4.2 Materials and methods 92
3.4.2.1Subjects 92
3.4.2.2 Equipment 92
3.4.2.3 Study protocol 93
3.4.3 Results: 94
3.4.4 Discussion 95
3.5 Conclusion 96
Chapter Four: Clinical Applications of the Functional Lumen Imaging Probe in the Ano-Rectal Region 97
4.1 Introduction 97
4.2 Study One: Identification of Component Muscle Function in the Ano-rectal Region of Healthy Controls using EndoFLIP® Distension 97
4.2.1 The objectives 97
4.2.2 Materials and methods 98
4.2.2.1Subjects 98
4.2.2.2 Equipment 98
4.2.2.3 Study protocol 98
4.2.2.4 Statistical analysis. 98
4.2.3 Results 98
4.2.3.1 Ramp Distensions 99
4.2.3.2 Squeeze Manoeuvres 101
4.2.3.3 Cough Manoeuvres 102
4.2.3.4 Straining Manoeuvres 104
4.2.4 Discussion 105
4.2.5Limitations of the current study 107

4.2.6 Conclusion 107
4.3 Study two: Evaluation of the continence mechanism using EndoFLIP®: Clinical outcome from healthy control and FI groups 107
4.3.1 Objectives 107
4.3.2Material and methods 108
4.3.2.1Subjects 108
4.3.2.2 Equipment 108
4.3.2.3 Study protocol 108
4.3.2.4 Data analysis 108
4.3.2.5 Statistical analysis 110
4.3.3 Results 110
4.3.3.1 Distension data set 110
4.3.3.2 Provocative manoeuvres 113
4.3.4 Discussion 117
4.3.4.1 Distension 117
4.3.4.2 Squeezing 118
4.3.4.3 Coughing 119
4.3.4.4 Correlation of narrow zone CSA and bag pressure measured by EndoFLIP® at rest and during squeezing with pressure derived from ano-rectal manometry at the same manoeuvres .. 119
4.3.4.5 Straining data 120
4.3.5Limitations of the current study 121
4.3.6 Conclusion 121
Chapter 5: General Discussion and Future Work. 122
5.1 Discussion 122
5.2 Future work 124
5.2.1 Methodological Issues 124
5.2.2 Biofeedback 124
5.2.3 Defecatory Position 124
References 125
Appendices 139
Appendix 1: Letters of ethical approval 139
Appendix 2: Patient information leaflet 142
Appendix 3: Protocol: A study of the use of the FLIP probe in the assessment of ano-rectal function in normal volunteers 144
Appendix 4: Radiant Solutions 148

Appendix 5: Video-fluoroscopy parameters 149
Appendix 6: Healthy contour plot cough data 152
Appendix 7: Non-healthy contour plot cough data 153
Publications and Presentations 154

Chapter One: Introduction and Literature Review

1.1 Introduction

Faecal incontinence (FI) is characterized by involuntary loss of rectal contents through the anal canal. It is a complex, challenging and multifaceted clinical problem (1). Between 7.1% and 9.5% of the adult population under age 70 and 15.3% of the population over age 70 suffer from FI (2). However, 12% of adult women are known to have FI (3). Although a significant proportion of the population suffer from FI, very little attention has been paid to it. The reason for this is attributable to social embarrassment, which prevent sufferers from speaking about it.

The mechanisms of defecation and continence, regulated by voluntary and reflex actions, are complex and depend on the interactions of several components. The ano-rectal region consists of two sphincters. The internal anal sphincter (IAS) which is composed of smooth muscle extends more than 1cm above the external anal sphincter (EAS). The EAS is composed of striated muscle and under conscious control. Function in this area is also affected by the pelvic floor muscles which maintain the ano-rectal angle (4). The IAS is persistently tonically contracted and participates in the act of defecation by reflex relaxation in response to rectal distension known as the recto-anal inhibitory reflex (RAIR) (5). The EAS with the puborectalis muscles (PRM) are mainly responsible for the squeezing pressure as a response to voluntary effort, or induced by increased intra-abdominal pressure during coughing and sneezing (6, 7). Erratic function of any of these components can result in FI.

In recent years, the increasing interest in FI and greater awareness of the available treatment modalities has increased the demand for specialize investigating tools (8). Investigations for ano-rectal region assessment fall broadly into two categories: physiological and radiological. Physiological tests include ano-rectal manometry, electromyography; while the radiological tests include evacuation proctography, endoanal ultrasound and magnetic resonance imaging of the ano-rectal region.

Although findings from any of these investigations may associate with incontinence, they are inadequate measures to determine incidents and severity of incontinence or response to therapy. For instance, there is no clinical significance for either normal or abnormal manometric values as abnormal values may not have clinical symptoms and vice versa. Patients with clinical problems may exhibit normal values which highlights the limitations of measuring incontinence with manometry (9, 10). Similarly, in endoanal ultrasound, the presence of sphincter defects does not mean the diagnostic with faecal incontinence. Despite the importance of endoanal ultrasonography in diagnosing pelvic floor dysfunction, this demonstrates the poor measurement of incontinence using this technique (11).

This thesis is based on the theory that if FI assessment is developed to become more sensitive, specific and reliable then clinical practice would be improved. The availability of an objective and reliable diagnostic tool that is looking at the concept of distending ano-rectal regions will be a better measure of its performance. This is may be expedient since movement of stools within the anal canal during defecation and faecal incontinence are dynamic processes (12). Furthermore, identifying the biomechanical properties of the muscle structure at different locations in the anal canal would benefit healthcare setting involved.

1.2 Structure of the thesis

This thesis is divided into four chapters. In the first chapter, the background of the research is introduced, beginning with an explanation of the anatomical structure and physiological function of the anal canal and the nature FI. Limitations to current FI evaluations are presented and the Functional Lumen Imaging Probe (FLIP), a novel method to evaluate anatomical lumens, is introduced.

Second chapter addresses general methodological design and pilot studies. Three FLIP prototypes are developed; two of them are tested in bench top study. These prototypes are positioned and distended in the anal canal in pilot studies. Based on these finding the original EndoFLIP® probe designed for oesopho-gastric unction (OGJ) evaluation is selected for measuring the ano-rectal region.

In the third chapter, two pilot studies using EndoFLIP® with two imaging techniques, video-fluoroscopy and Endoanal Ultrasound are carried out. Study protocol for evaluating ano-rectal region is subsequently established and new outcome measures are established. The new outcome measures are: narrow zone length, narrow zone average CSA and bag pressure.

Fourth chapter reports the methodology and results from two separate research studies. Anal canal distensibility and morphological changes is evaluated in a healthy non-elderly subject group in the first study. The clinical limitation of this study is investigated and resolved in the chapter three. The second study reports the narrow zone length, narrow zone average CSA and bag pressure for healthy and FI groups during ramp distension and different provocative manoeuvres.

1.3 Aims, Research Questions and Hypothesis

1.3.1 Aim

The overall aim of this study was to use the functional lumen imaging probe in the ano-rectal region of the intestinal tract to determine if aspects to the components of incontinence could be measured by probe distension as a method in the biomechanical evaluation and diagnosis of ano-rectal dysfunction.

1.3.2 Research questions

In order to achieve the research aim, the research questions are the following:

1. Can the FLIP probe be used to provide the biomechanical measurement of the continence components during anal sphincter distension?
2. What are the quantitative measurements of the anal sphincter distensibility and anal canal morphology in adult healthy volunteers that can be measured by FLIP during distension and the anal provocative manoeuvres?
3. How are new measurements of anal sphincter distensibility and anal canal morphology using FLIP during distension and provocative manoeuvres validated?
4. Can the anal sphincter distensibility and anal canal morphology be evaluated in a population with known anal sphincter dysfunction using FLIP at rest and during provocative manoeuvres?

1.3.3 Hypothesis

The experimental hypothesis is that the a functional lumen imaging probe (FLIP) can be designed and tested in clinical studies to monitor CSAs and bag pressure during distension in the ano-rectal region and that these measurements can be used as a practical test to describe ano-rectal function and define objective differences in healthy continent subjects and faecal incontinent patients.

1.4 Anatomy

Of functional importance for anal continence and defecation are the rectum, levator ani, puborectalis muscle, external anal sphincter, internal anal sphincter, anal cushions and their innervations. Factors considered important in normal anal continence and defecation are central to a discussion of pelvic floor physiology. The researcher will discuss the present theories of anal continence and mechanism of defecation

1.4.1 Pelvic floor

The striated levator ani or pelvic diaphragm is subdivided into four muscles according to their attachments, namely pubo-coccygeus, ileo-coccygeus and coccygeus. It is not easy to distinguish between the boundaries of different parts of muscle as they share many similar functions. These muscles receive innervations from a branch of the fourth sacral nerve which is one of the five pairs of spinal nerves (13). See Figure 1.1.

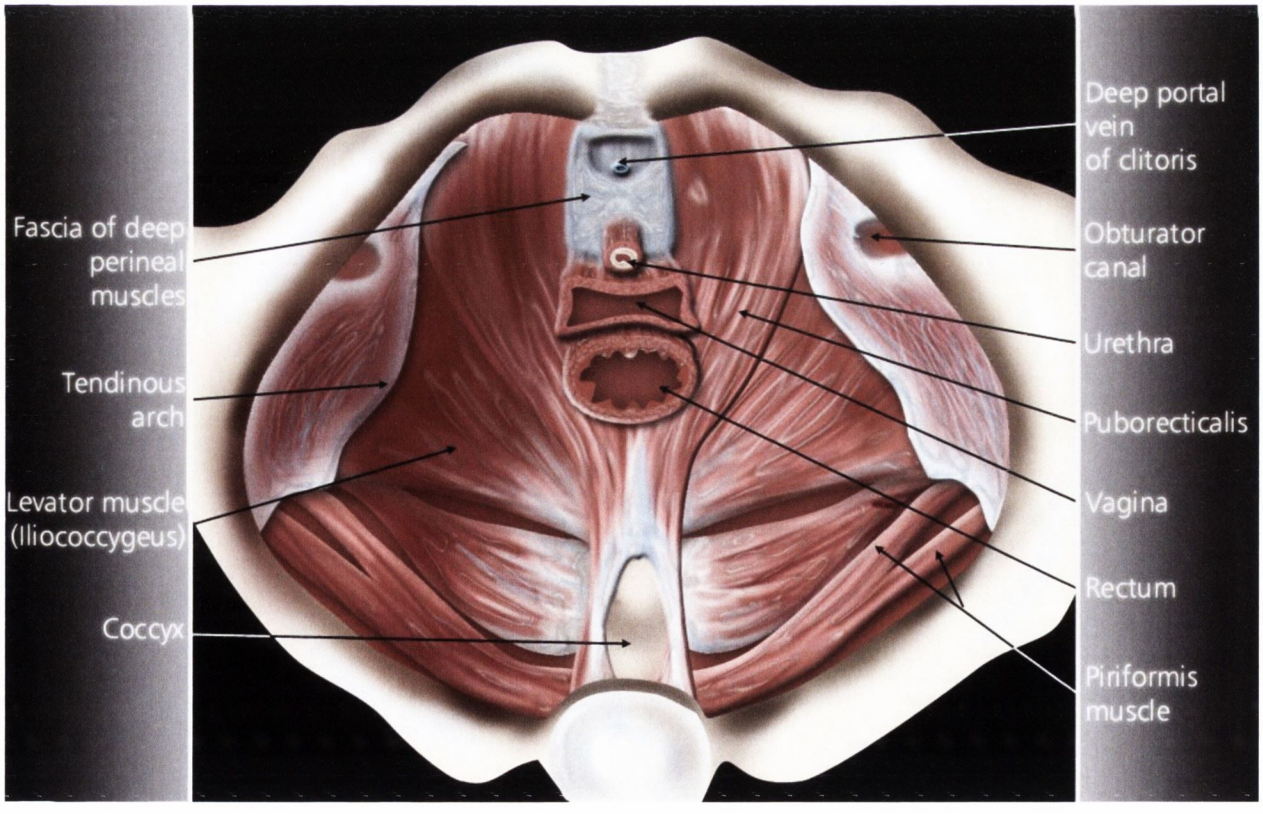

Figure 1.1: Axial view of the pelvic floor *(photography kindly provided by Professor FBV Keane).*

The most medial fibres of the pubo-coccygeus form a sling around the rectum and are named the puberectalis muscle (PRM). The PRM passes directly backward from the back of the pubis with its inner surface in intimate contact with the lateral walls of the vagina or prostate and the ano-rectal junction. The two legs of the puborectalis muscle meet posterior to the ano-rectal junction to form a sling which angulates the ano-rectal junction to an angle of 92° (mean) during rest and 147° (mean) during straining (14).

Shafik and others suggest that the puborectalis corresponds to a component of the external sphincter (see section 1.4.3.1) and not to the levator ani. The puborectalis muscle functions anatomically with the external sphincter muscle and fuses with the external sphincter as it forms a loop around the ano-rectal junction. Based on developmental evidence; the puborectalis muscle appears distinct from the majority of the levator ani. Similar to the external sphincter the puborectalis receives innervations from the fourth sacral nerve (15).

1.4.2 Rectum

The rectum is 15-20 cm long, and extends from the recto-sigmoid junction at the level of third sacral vertebra to the anal orifice which is the opening where stool exits the body through the anal sphincter (16). The rectum wall contains two single layers of smooth muscle; longitudinal and circular. Ganglion cells are distributed throughout the rectum in three separate plexuses: the myenteric (Auerbach's), the deep submucosa, and the superficial submucous plexuses. The rectum is innervated by both sympathetic and parasympathetic nerves (17).

1.4.3 Anal canal

Anal canal appears as a cylindrical structure that extends from the attachment of the levator ani muscle to the rectum (to the lower edge of the external anal sphincter) (18). Knowledge of the exact anatomy of the anal sphincter complex is essential for management of pathology in this area. Three muscle layers surround the anal canal sub-epithelium. The innermost layer is the internal anal sphincter. The longitudinal muscle lies laterally to the internal anal sphincter. The external anal sphincter lies laterally to the longitudinal muscle and may be closely applied to it (19).

There are two definitions of the boundaries of the anal canal. The first one is the anatomic anal canal, it is approximately 2.2cm in length (range 1.4-3.8 cm) and extends from the dentate line (a line which marks the end of the rectum and the beginning of the anal canal) to the anal verge (anal orifice). The second definition is the longer surgical or physiological anal canal as determined by digital examination of the subject in the conscious state. This is approximately 4.2 cm in length (range 3.0-5.0 cm) and extends from the ano-rectal ring or upper margin of the puborectalis muscle in the contractile state to the orifice (20, 21). See Figure 1.2.

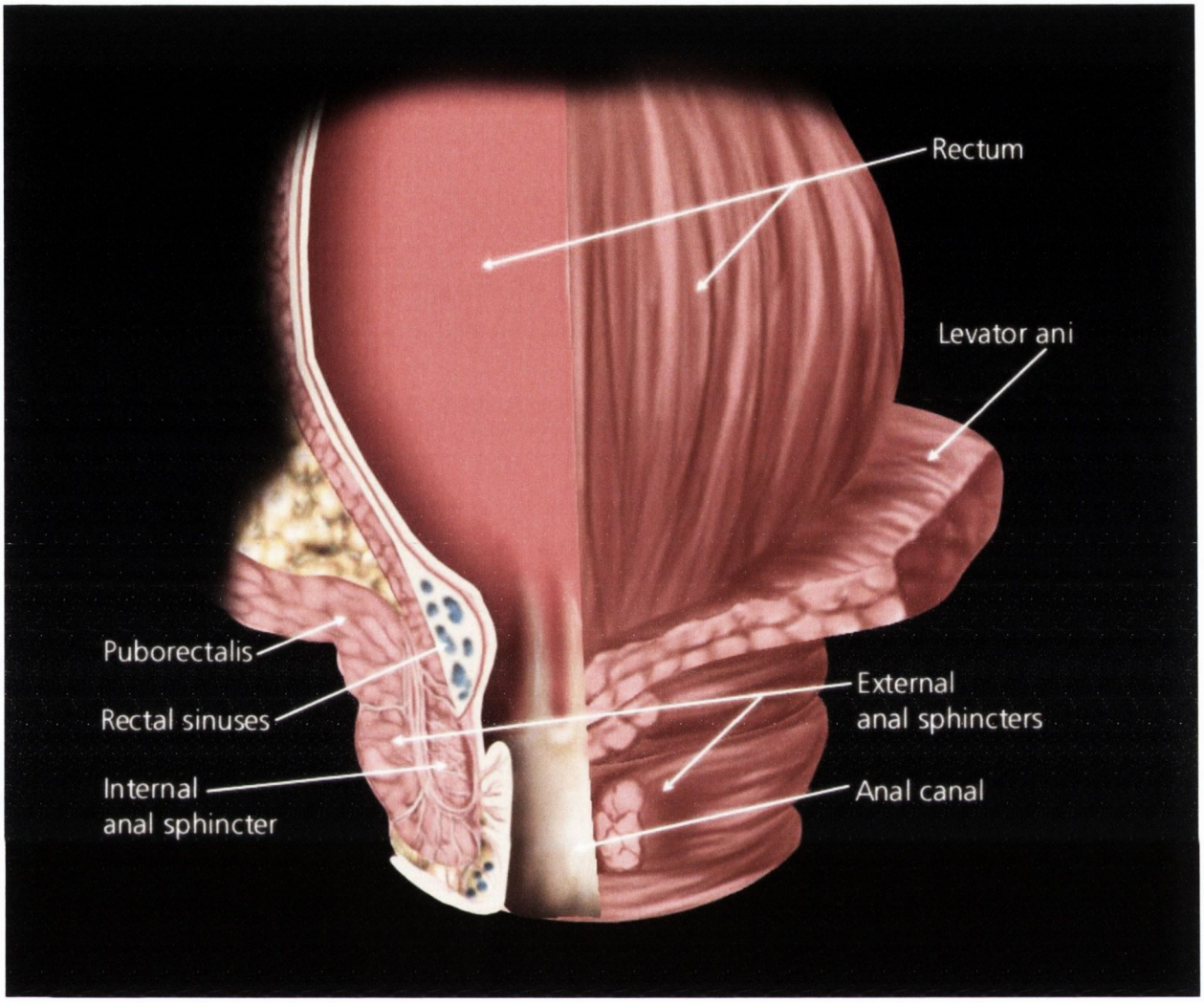

Figure 1.2: Anatomy of the ano-rectum (Photography kindly provided by professor FBV Keane)

The anal canal contains two concentric muscular tubes called the sphincters. The outer tube is the external anal sphincter (EAS) which is composed of voluntary innervated striated voluntary muscle. The innermost tube is automatically innervated smooth muscle, the internal anal sphincter (IAS). The two sphincters are separated by a conjoint longitudinal layer. The sphincters encircle the anal canal just distal to the ano-rectal angle formed by the muscular tone of the loop of PRM pulling the rectum anteriorly (22).

The muscle layers along the anal canal are different. The upper parts comprise the IAS, the longitudinal muscle layer and the PRM, which acts as a sling around, instead of completely surrounding the anal canal. The PRM blends into the EAS in the middle part of the canal, forming a complete ring anteriorly and the IAS is thickest in the middle portion of the anal canal. The subcutaneous EAS, lying below the termination of the IAS, defines the lower part of the anal canal (23) .

Between the internal anal sphincter, the external anal sphincter and the puborectalis muscle, there is a slit like space that is called the inter-sphincteric space which contains the longitudinal muscle layer. It is obvious that the inter-sphincteric space has to be filled up by some material in order to close the anal canal hermetically (24).

1.4.3.1 External anal sphincter

The external anal sphincter (EAS) is a voluntary striated muscle elongated around the anal canal. Its fibres are circumferentially oriented and very small, separated by connective tissue.

Early studies believed that the EAS represented a homogenous single muscle (25, 26). Milligan and Morgan (1934) proposed that the EAS mechanism was a triple loop system consisting of deep, superficial and subcutaneous loops. These loops were separated by planes of tissues and each received individual innervations, had a unique orientation and attachment to surrounding tissue (27, 28).

Shafik (1975) suggested that the EAS consists of muscle bundles arranged along the anal canal in a series of U-shaped loops which are distinguishable into three main 'loops': the top, intermediate and base. This division depends on the attachment, direction and innervations of each loop. The deep or upper loop, the muscle bundles of the deep external sphincter and puborectalis are fused together and they could not be differentiated either morphologically or on histologic examination; both were made up of striated muscle bundles which are innervated by a rectal nerve which is a branch of the pudendal nerve. Both muscles form a single U-shaped loop which slings the upper anal canal to the symphysis pubis. The contraction of this loop pulls the ano-rectal junction up and forward. The superficial or intermediate loop is innervated by a branch of the fourth sacral nerve. It encircles the mid portion of the anal canal and inserts into a small triangular bone at the base of the spine, known as coccyx. No concentric circular muscle bundles of the intermediate loop could be identified. The muscle thus form a U-shaped loop. The contraction of this loop pulls the mid portion of the anal canal backward. The subcutaneous or lower loop is the smallest loop and forms the lower portion of the external anal sphincter. The muscle bundles are attaché anteriorly to the perianal skin in and close to the midline. The muscle thus forms a third U-shaped sling. The lowermost and medial bundles of the base loop are circular and form a complete ring around the anal verge. It is innervated by the inferior rectal nerve. The contraction of the subcutaneous loop cause the distal portion of the anal canal to be pulled forward (29). See Figure 1.3.

The outer thickness of the EAS is 2.5mm (range 1-4mm), the inner is 16mm (range 10-20mm) and lateral is 4.0mm (range 2-5mm) (30).

1.4.3.2 Internal anal sphincter

The internal anal sphincter (IAS) is a thickened downward continuation of circular smooth muscle of the rectum starting at the ano-rectal junction with an anterior length of 2cm and a posterior length of 4 cm, and ending to the most proximal portion of the external sphincter. The average thickness is 2.5mm (range 2-4mm) (31, 32). As the IAS is a continuation of the circular fibres of the rectum, hence it shares the same sympathetic (L5) and parasympathetic nerves (S2-S4) innervations. Sympathetic innervation is responsible for sustained tonic contraction, whereas the parasympathetic mediated activity causes relaxation of the internal sphincter (33), see Figure 1.3.

The internal anal sphincter can be identified by the characteristic arrangement of the muscle bundles in cross section. The rectal smooth muscle, in cross section, is aggregated into longitudinal bundles. At the level of the pelvic floor, circular fibres of the upper internal sphincter are arranged in oval bundles, thus the junction between the internal sphincter and the rectal smooth muscle can be readily distinguished (34).

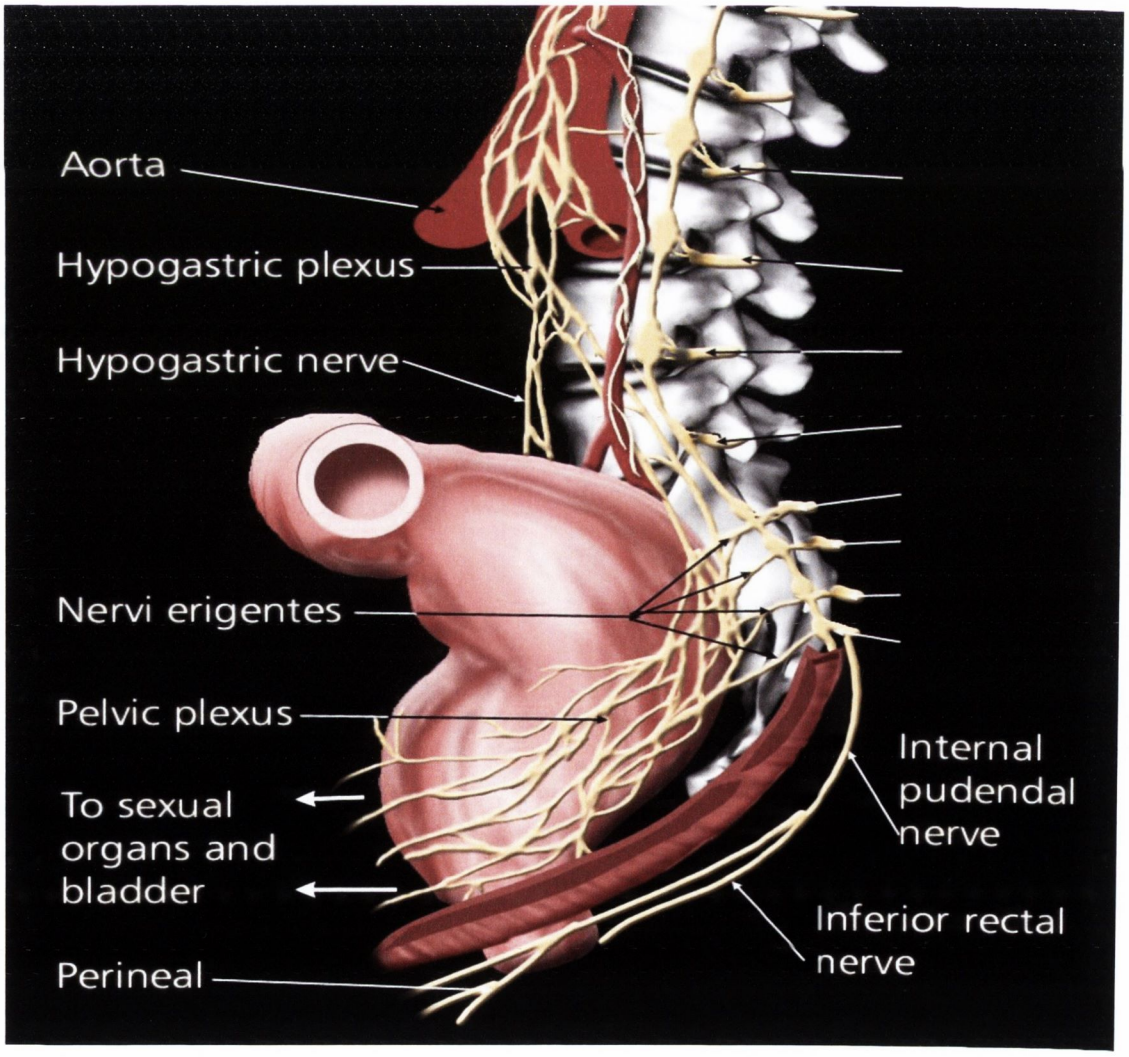

Figure 1.3: innervations of the pelvic floor *(Photography kindly provided by Professor FBV Keane).*

1.4.4 Male versus female anatomy of the anal sphincter complex

Most studies do not mention differences in structure between the male and female anal sphincter complex and usually male anatomy is represented. Generally, the anal canal sphincters are thicker and longer in men. There is no difference in morphology of the IAS between the male and female but there are differences between the sexes of the EAS (35).

Two parameters determine the structure of the anal sphincters; sphincter thickness and sphincter length. The external sphincter is thicker in males (8.6±1mm), compared to females (7.7±1.1mm), (36). The mean maximum thickness of the internal sphincter (1.8±1mm vs. 1.9±0.6mm) and the longitudinal muscles (2.5±0.6mm vs. 2.9±0.5mm) in females and males are not significantly different. The inner thickness of the external striated sphincter is much higher (24.7±4.6mm) than the outer one (6.6±1.7mm), while the inner and outer thickness of the internal smooth muscle sphincter is nearly equal (9.0±1.7mm, 9.6±1.7mm respectively) (37).

The length of the anal sphincter is varied also between males and females; the IAS is longer in males than in females (25.6±6.4 vs. 19.8±4.0mm), but the mean length of the internal sphincter length as a percentage of total anal canal length is approximately the same (78.4% vs. 78.7%). The EAS is also shorter in females compared with males (15.4±2.8mm vs. 42.6±5.4) and forms a less percentage of total anal canal length (100% vs. 62.9 %) (38, 39).

Oh and Kark addressed the differences in EAS length between male and female, concluding that in male, the inner and outer length of the EAS was approximately the same , whereas in the female the outer portion condensed to a narrow bundle of muscle less than half the inner length (40).

1.5 The mechanism of preserving continence

Physiology of the ano-rectal region is a complex matter. The currently available techniques only allow the study of the mechanisms of anal continence. From a review of the work of several investigators, it can be realized that maintenance of anal continence depends on a highly integrated series of complicated events on which there is no uniform agreement. Following is a brief discussion of factors that had been considered important in the overall maintenance of continence.

1.5.1 Rectum Function

The main function of the rectum is to act operates as a reservoir for only a short period of time to allow voluntary defecation at the appropriate time (41).

The change in volume per unit of pressure changes is called compliance. From a mechanical point of view, the adaptive compliance of the rectum along with the rectal capacity is an important factor for effective reservoir function (42). The compliance of the rectal reservoir refers to the ability of the rectum to distend. Despite compliance is considered as a significant factor of the accommodation process, yet it is very difficult to measure it. During filling of a normally functioning rectum, an increasing volume of stool is associated with passive distension of the rectum that allows rectal pressure to remain low (43). When the maximum tolerable volume is reached, the rectal pressure will rise eventually to overcome the pressure generated by the anal sphincters. The end result of this series of event is the evacuation of the rectum (44). Rectal distensibility is approximately 16 ml per cmH_2O in normal individuals; urgency develops with distension of approximately 200 ml, whereas maximum tolerable rectal distension capacity is approximately 400ml. The threshold at which distension, urgency, and maximum tolerable capacity is varied. A surprisingly small volume, about 10ml, is all that has to be introduced into the rectum to initiate relaxation. The rectum accommodates passively in response to increases in volume. However, accommodation of the rectum helps to slow the delivery rate of stool. Anal continence can be threatened breached when the distensibility of the rectum is reduced even if the sensitivity and motility of the anal canal are normal (45, 46).

Rectal motility is periodic and occurs as either contractions or as sequences of rhythmic rectal pressure waves. The duration of these sequences can vary from one to several minutes. Rectal waves are of higher amplitude in the rectum than in the sigmoid (the sigmoid is the distal part of the colon). This reverse gradient provides a pressure barrier resisting the progression of stool. Therefore, the most important function of the rectum is delaying the passage of intestinal contents (47).

1.5.2 Anal sphincter function

The main function of the sphincter is e a tight seal. There are different function of the sphincters muscles in GI tract; either forming a valve-like function in one-directional food transport or to provide a tight-seal of the anal canal at rest and to control contraction and relaxation (48). The ano-rectal mechanism for preserving continence is complicated. It consists of two sphincters, one IAS composed of smooth muscle, the other EAS of striated muscle under conscious control, the PRM and nervous reflexes which control the sphincters. The lumen of the anal canal in the normal resting state is occluded by the PRM , the resting tone of the IAS and EAS (49) together with the anal cushions (50).

The function of the IAS and EAS in maintaining continence was examined by obtaining a continuous recording of the pressure in the anal canal before and after the EAS had been entirely paralyzed by bilateral pudendal block. The amount of relaxation was bigger after the block which leads to the pressure falling to the lower level. The activity of the internal sphincter contribute of the 85% of anal pressure at rest but only 40% during the pressure fall following substantial rectal distension (51).

The IAS is a smooth muscle in a state of continuous maximal contraction or tone that represents a natural barrier to the involuntary loss of stool from the rectum. The striated sphincter tonic activity EAS plays relatively little part in the resting tone 25%-30% (52). Another study with increase in anal canal probe size there was an increase in resting pressure; the major increase related to the passive components of the anal canal tissue (53).

The mean anal canal resting pressure in healthy adult volunteers is 87±7 cmH_2O. The IAS has two functions: it is persistently tonically contracted for ensuring the anal canal is closed at rest and it initiates the act of defecation by reflex dilation in response to rectal distension (this reflex is called the recto anal inhibitory reflex) (54, 55).

The EAS is under voluntarily control and has a normal state of elastic tension or partial contraction in resting, known as the tone of the muscle. The pressure of the EAS is the greatest during the waking hours in the upright position and significantly reduced during the sleep. During constant tonic activity at rest the EAS generates approximately 30% of the basal resting anal pressure. It also undergoes a contraction reflex. Because of rapid fatigue, the pressures generated by the EAS mechanism alone cannot be responsible for sustained continence (56). However, the relative contribution of the IAS and EAS to maintain resting and squeezing pressure is difficult to determine because of muscle overlap.

Because of the large degree of anatomical and functional overlap between the smooth muscle of the IAS and the voluntary muscle of the EAS, the relationship between contraction of the EAS and the IAS is poorly understood, although both muscles relax during defecation, they contract in a complementary manner during attempts to maintain continence (57). Phillips and Edwards claimed that it is impossible to demonstrate two separate zones of contraction of the sphincters using manometry (58). Furthermore, they demonstrated the difficulty in recording the function of both muscles simultaneously in human subjects. Schuster et al. attempted to record from two muscles by sitting two balloons in the anal canal; the inner balloon recording from the bulk of the EAS, this technique affords only a partial separation of the actions of the voluntary and involuntary muscles of the anal sphincter (59).

These results do not agree with those of Duthie and Watts who compared pressures in conscious subjects and in anaesthetized subjects in whom the EAS had been paralyzed. Although the resting pressure was reduced in the latter group, the level to which the pressure fell on rectal distension was the same in both groups (60). This type of experiments reveals the difficulty of pressure measurements interpretation in terms of separating the activity of the anal sphincters.

The EAS with the PRM is mainly responsible for the squeezing pressure. The squeeze response may be voluntary, or induced by increased intra-abdominal pressure (for example when sneezing or coughing), or by simply moving a finger across the anal canal lining. This contraction of the EAS

mechanism (squeeze) coincides with a sharp elevation in pressures throughout the anal canal. The EAS and PRM function as one unit bring about voluntary sphincter contraction, and normally double the resting pressure of the anal sphincter during voluntary contraction(61). During squeeze, the anal canal lengthens as the EAS contracts and the PRM shortens and elevates the anal canal. After 4 minutes of sustained voluntary contraction of the EAS mechanism, the anal canal pressure returns to resting levels as the muscle is fatigued by the maximum voluntary effort (62).

1.5.3 Ano-rectal angle

It is recognized from our knowledge of the dynamics of flow that is not necessary to completely seal a tube to prevent flow of a solid or semi-solid if an acute angle is incorporated in the system. Using this principle, an in vitro study demonstrated that the inclusion of angulations in a tube reduces the occlusion pressure required to hold back solids and semi-solids but is of no benefit for fluids or gas (63).

Tonic contraction of the PRM pulls the junction of the rectum and the anal canal forward, forming the ano-rectal angle. The ano-rectal angle represents an important factor in the continence mechanism. At rest, the ano-rectal angle is approximately 80° and 90° degrees (28). During squeeze or the increase of the intra-abdominal pressure, the tonic activity increases in the PRM and the ano-rectal angle become more accentuated (decreased to 60◦) causing the anal canal to be in closer position. Likewise, voluntary contraction of the PRM will assure that the anal canal remains closed. The PRM relaxed at the start of the rectal contraction to permit straightening (increased to 140◦) the ano-rectal angle, thus preparing the anal canal for receiving the rectal contents. The PRM and the EAS function work as a unit to interrupt the commencement of defecation (64). See figure 1.4.

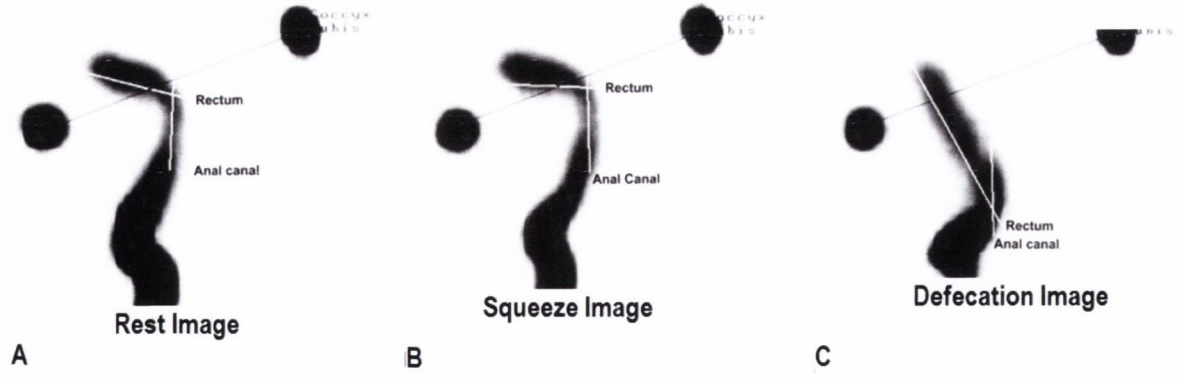

Figure 1.4: The ano-rectal angle at rest (A), during squeezing (B) and during defecation(C). *(Reprinted with kind permission from Prof Bharucha)*

Ano-rectal angle plays an important role in anal incontinence during manoeuvres such as coughing, lifting or straining(65). The relevance of the PRM to continence is illustrated by the high degree of continence in children with congenital absence of IAS and EAS (66).

1.6 Mechanism of defecation

Defecation is initiated by the propagation of the intra-luminal contents to the rectum. Stool is often transferred into the rectum by colonic high amplitude propagated contractions, which often occur after awakening or meals.

The individual senses of rectal fullness mediated by puborectalis and levator ani stretch receptors, without distension of the rectum, there is no call to stool. Integral to the dynamic nature of anal canal activity is intermittent, transient relaxation of the IAS, which allows descent of distal rectal contents into the upper anal canal, endowing a subconscious or conscious perception of their physical nature (67). The sampling reflex is IAS relaxation and ano-rectal angle straightening induced by rectal distension. It occurs approximately seven times per hour in healthy control subjects, but less frequently in patients with FI (54). This reflex can be reproduced under laboratory conditions, where rectal distension causes reflex relaxation of the IAS (in this case known as the recto-anal inhibitory reflex RAIR) as well as a significant increase in voluntary EAS activity (61).

The rectal contents would reach into the distal end to contact the sensitive receptors facilitated by the decrease in rectal pressure. These receptors can assess the state of the material either solid, gas, or liquid(66).

Consequently, reflex contraction of the EAS muscle is seen when the rectum is distended by small volumes, this reflex is known as the recto-anal contractile reflex (68). Based on EMG recording of the EAS, the contraction of EAS was not related to the volume of distension but to the speed of distension. Thus, the EAS contraction can maintain the anal continence during the time of faecal sampling and meanwhile, allows time for impulses to reach conscious awareness (69). After assuming a posture convenient for defecation, the subject strains by contracting the abdominal muscles. This is associated with a relaxation of the EAS. It has been suggested that the levator plate and the longitudinal muscle of the anus contract simultaneously during evacuation. The resultant force is causing the anorectal angle to open. The contraction of the longitudinal muscles of the anus is flattening the anal vascular cushions which also shorten the length of the anal canal. These factors which occur at the same time reducing the anal canal pressure less than the rectal pressure leading o pressure gradient from the rectum toward the external area (70). At the end of evacuation, the tonic activity returns to the IAS, EAS and PRM, restoring the ano-rectal angle and anal canal resting pressure.

1.7 Theories of anal continence

Anal continence has been characterized by the ability of controlling defecation, sensing the rectal contents quality and maintaining full control continuously. Continence is maintained by the coordinated function of the pelvic floor, rectum and anal sphincters. So far, no generally accepted theory of continence has emerged. The reason for this is probably that there are many factors responsible for a perfect mechanism of continence and defecation (71).

1.7.1 High Pressure zone

The most commonly explained reason for anal continence is the high pressure zone (resting pressure) in the anal canal. The high pressure zone (average 25-120mmHg) at rest can provide an effective barrier against pressure in the rectum (5-20mm Hg) (72). This high pressure zone increases the resistance to the passage of enteric contents from the rectum through the anal canal. (72). Both anatomical and physiological tests demonstrate the length of high pressure zone to be at least 4 cm. Many studies suggested that this high pressure zone is found in the mid anal canal, due to the action of anal sphincteric muscles (71). It was also suggested that low pressure denotes incompetent sphincter, and, on the other hand, high resting sphincter pressure denotes a competent sphincter (73).

Because of the difficulties of interpretation, the literature abounds with unexplained results and some doubtful conclusions about the anal canal pressure measurements. Most researchers have agreed on the existence of a high pressure zone or pressure barrier in the anal canal. However, they disagree about its importance in maintaining anal continence and on the part played by the IAS and EAS in its production (74).

1.7.2 Resistance to opening

Some studies have been looking at the concept of distension sphincter region as a better measure of its performance. They assume that the measurement of the sphincter resistance to distension is physiologically more significant than measurement of simple resting pressure (high pressure zone). Hence, sphincters that control defecation act in response to the presence of stools distending the anal canal. Harris and Pope found that the pressure profile obtained as the manometry tube was withdrawn through the anal canal from the rectum differs considerably from that obtained as the tube was inserted into the rectum from the exterior (75). The different in pressure recorded when the probe was withdrawn from the rectum and reinserted in the anus indicated that the competence of the sphincter in the anal canal does not depend too much on the capability of the muscle to squeeze around the anal canal but on their ability to resist the opening of the anal canal as stated by Harris and Pope (76).

1.8 Faecal incontinence

Faecal incontinence (FI) is the involuntary loss of stool or soiling at a socially inappropriate time or place. According to Rome II criteria, FI was defined as 'recurrent uncontrolled passage of faecal material for at least one month, in an individual with a developmental age of at least 4 years' (77). Loss of bowel control can be devastating. It is a complex and challenging problem of multi-factorial reasons and rarely attributable to a single factor (78).

It is physically and psychologically disabling symptoms that results in progressive isolation and loss of an individual's potential (79). Despite the considerable advances that have been made during the past decades, the understanding of the evaluation of FI remains limited. It is underreported, under-recognized and a poorly understood problem (80). People who have FI may feel ashamed, embarrassed, or humiliated. It can lead an afflicted person to resist leaving home or the comfort of a nearby toilet. Because FI is not life threatening, the implications for quality of life need to be considered when dealing with these patients(81).

1.8.1 Prevalence of FI

The exact prevalence of faecal incontinence is unknown, chiefly because of embarrassment or lack of awareness(82), it is often a hidden problem(83). The difference in reported prevalence rates may be due to the variation in defining the FI or the method of data collection (84, 85). In the same vein, only a minority of patients with FI report their symptoms to their physicians, so the data on the prevalence of FI in general population is misleading (86).

Geibel et al, reported that the incontinence of solid stool was 4.8% of general population (87). In the Australian community, Kalantar et al reported that 7% of physically independent adults over the age of 65 have daily or weekly episodes (88). Pretlove et al and Davis et al found that this disorder is present in all age groups and in both genders, varying from 1.5% in children to more than 50% in nursing home residents (89-91). Johnson et al, estimated that up to 13.7% of all people suffer from FI and that its prevalence increases with age (80). Although the condition is widely accepted as a problem in the elderly (92); it is now becoming apparent that much younger age groups are frequently affected (93). Bharucha et al found that more than 1 of 10 adult women in the general population have FI; almost 1 in 5 of these people have moderate to severe FI (94). Whitehead et al reported that the prevalence of FI in non-institutionalized US adults is 8.3% with the prevalence in women of 8.9 % similar to men at 7.7%. This prevalence increased up to 15.3% in participants aged 70 years and older (95).

Symptoms of FI can be categorized into four grades. Grade I implies full continence, Grade II incontinence to flatus (gas), Grade III incontinence to liquid stools and Grade IV incontinence to solid

stool (96). More importantly, Parks detailed questionnaire should be undertaken to assess the degree and frequency of FI and its effects on quality of life (97).

1.8.2 Causes of FI

The main cause of FI is still poorly understood. Progressive loss of anal sphincter function happens with no specific time from the start of the symptoms (98). At the beginning, losing the control of flatus starts, followed by losing the faecal material and then incontinence to solid stool. Finally, The urge to stool is lost (99). Two types of history with prior perineal intervention are typical in colorectal practice. Patients may have a history of prolonged or difficult childbirth with vaginal delivery or they may have a history of surgical procedures such as anal fissure repair and haemorrhoidectomy (100, 101). See table 1.1.

Incontinence is either passive where the leakage of faeces happens without the patient being aware, or motor, where the patient is aware of the call to defecate but is unable to control the passage of faeces. Some studies relate the passive faecal incontinence to internal sphincter dysfunction and the urge faecal incontinence to external sphincter dysfunction (102).

Table1.1: Etiology of faecal incontinence.

Etiology of faecal incontinence (103)	
Anal sphincter weakness	Injury: obstetric trauma, related to surgical procedures (e.g., haemorrhoidectomy, internal sphincterotomy ,fistulotomy, Ano-rectal infection) Non-traumatic: scleroderma, internal sphincter thinning of unknown cause.
Neuropathy	Stretch injury, obstetric trauma, and diabetes mellitus.
Anatomical disturbances of pelvic floor	Fistula, rectal prolapse, descending perineum syndrome.
Inflammatory conditions	Crohn's disease, ulcerative colitis, radiation, proctitis.
Central nervous system disease	Dementia, stroke, brain tumours, spinal cord lesions, multiple system atrophy
Diarrhoea	Irritable bowel syndrome, post-cholecystectomy diarrhoea

1.8.2.1 Childbirth (Obstetric Trauma)

The most common cause for symptoms of FI in healthy women is obstetric trauma (89). Obstetric trauma is the rupture of the external and/or internal anal sphincter during traumatic vaginal delivery, especially if the forceps were required (104). FI following vaginal delivery can be part of a double pathology; involving both direct sphincter division and nerve injury (105). The passage of the foetal head through the pelvis during child birth obviously causes marked stretching of the tissues around the vagina and the anal canal. Tissue and nerve damage is caused by either direct pressure or over-

stretching(106). Ultimately, after an interval of many years secondary muscle wasting occurs as a result of anal nerve injury (107). It has been shown that 35 per cent of women delivering vaginally for the first time develop anal sphincter defects and that 13 percent of the same group have problems with the control of their bowel function (108). When the women have been followed through two vaginal deliveries, it was found that although the risk of sphincter injury is greatest after the first vaginal delivery, a second delivery was found to place the women at high risk of worsening any bowel symptoms already present (109).

There is no evidence that pregnancy itself can change the appearance or function of the anal sphincter. Rather it is during the process of labour and delivery itself that damage may occur. This is supported by the fact that women delivered by elective caesarean section report no change in their bowel habit (110).

1.8.2.2 Surgery

The second common cause of FI is the Sphincter damage after anal surgery related to obstetric trauma. Also, FI can be the cause or the effect of direct anal sphincter trauma following anorectal surgery (anal fistula surgery or haemorrhoidectomy) (111).

1.8.2.3 Dysfunctional of the internal sphincter

Both measured resting anal pressure and electrical activity in the IAS was reduced in FI patients. Low resting pressures are found in incontinent patients of all ages. The possible causes for this include physical disruption of the sympathetic innervation of the sphincter or structural changes of smooth muscle (112). The relaxation of the IAS as a response to rectal distension occurs more frequently and last longer in FI compared to healthy controls (113).

1.8.2.4 Impaired rectal sensation and compliance

In this case, FI may occur in the presence of normal pelvic floor and sphincter function. An increased volume of liquid stool coupled with rapid colonic transit time may fill up the rectum, rectal adaptive compliance is overwhelmed by rapid filling or by overloading total capacity (114). This type of FI is often associated with faecal impaction as a result of diminished rectal sensation. In this case the impacted faecal bolus causing reflex relaxation of the IAS and EAS sphincter, resulting in incontinence. Faecal impaction is predominantly seen in elderly people(115, 116). An overall decrease in total rectal volume may result from infectious diarrhoea, inflammatory bowel disease and radiation enteritis (117).

1.8.2.5 Aging

A high correlation between age and anal sphincter dysfunction has recently been demonstrated. With increasing age, the maximum resting and maximum squeeze pressures of anal sphincters decreases. These abnormalities may be found after the ages of 50 (118, 119). Age- related variations included a

significant decrease in the thickness of the EAS in men. Significant decrease in the thickness of the PRM and increase in the thickness of the IAS were noted in both sexes (39).

The reduction in pressures that occurs with aging is greater in women than in men, and greater for squeezing pressure than resting pressure; the maximum squeeze in elderly women is 6.3% of that in elderly men, whereas the maximum squeeze pressure in younger women is 75% of that in younger men (120-123).On other hand, other studies have reported that age are independently associated with FI (124-126).

1.8.2 Idiopathic Faecal Incontinence (IFI)

Faecal incontinence (FI) occurs as a result of several conditions as discussed previously. Patients, who are suffering from incontinence without any known underlying disorder have idiopathic FI (IFI). Around 80% of FI cases are considered to be idiopathic (127). Usually these patients have lower sustained intra-anal squeeze pressures than normal control subjects and abnormal resting pressure, and have lost the ability to maintain the normal ano-rectal angle, or both (128). Most patients with IFI are incontinent to liquid stool (129).

There are several gaps in our understanding of the pathophysiology of IFI, even though there is an increasing array of tools that can diagnose ano-rectal structure and function. While different studies have generally appraised one or two mechanisms of incontinence, the relationship between symptoms and dysfunction of the ano-rectal region in FI is unclear (77).

Around 65% of IFI cases are associated with weakness of the anal sphincters(130). In addition, 67% of patients had one or more functional gastrointestinal disorders including diminished rectal capacity or sensation (131). These factors alone or in combination with age increase the possibility of IFI dysfunction(132). Histological studies of the pelvic floor and the external sphincter have shown that IFI may be due to direct stretch injury (133)or recurrent traumatic of the pudendal nerve (134, 135).

Most IFI patients are women. The most commonly factor that imply IFI in women is anal sphincter injury. However most of the women who suffered anal sphincter injury during vagina delivery do not develop FI until several decades after traumatic delivery, consequently, other risk factors and not sphincter injury contribute to the pathophysiology of IFI (136).

Bannister et al developed a new combined multiport manometric and electromyographic technique to partly identify the individual function of the IAS and EAS. Two distinct manometric patterns were observed. The first one has normal resting pressure but low squeezing pressure and the second one showed the opposite reaction to rectal distension. The researcher concluded that an abnormally weak IAS would allow the liquid faeces to enter the anal canal. This results in EAS having insufficient time

to respond to the entry of faeces into the rectum and to become desensitised by continuous exposure to rectal contents after a period of time. It was also found that the impairment of both IAS and EAS muscles lead to severe incontinence because of the reciprocal relationship in the contractile activities of the EAS and IAS (69).

Another study which used combined test of Anorectal manometry, electromyography, and sensation to determine the mechanism of IFI reported that most of the IFI patients had the impairment in EAS. It was also observed that all incontinence patients who had normal sphincter pressure suffered impaired rectal sensitivity (137)

Bharucha et al demonstrated that the structural and functional disturbance in IFI women is not only impairment of the anal sphincter but also in rectal capacity. It was observed that 25% of FI patients have a significant reduction in rectal capacity associated with rectal urgency and increased perception of rectal balloon distension. The inter-individual variation are related to predicative factors like age, BMI , symptoms, obstetric history and anal sphincter appearance (131).

Most of published studies provide some understanding of the pathogenesis of IFI in women. However, there are very few studies and therefore less is known regarding IFI in men. Mitrani et al compared men with women and found that women with IFI more often demonstrate abnormalities of anal sphincteric motor function. This is related to sex difference in muscle mass and child birth experience (138).

1.9 Management of faecal incontinence (FI)

All treatment of the anal canal should always be directed at the cause. For most people the management of faecal incontinence often requires a combination of treatments including attention to medications, regular toileting, and sometimes use of gentle laxatives. When non-invasive treatments have failed, minimally invasive treatments can be considered (139, 140). The leakage caused by weak internal anal sphincter can be improved by injecting a silicon biomaterial. Surgery should only be carried out if patients suffer major incontinence which is not responding effectively to conservative treatment (141). However, the surgery is not able to restore the sphincter to its full function. Obstetric causes can lead to major disruption of the sphincter which can be treated by overlap repair of the sphincter. Unfortunately, this treatment is satisfactory only for short time(142).

1.9.1 Bowel habit modification

Modifying irregular bowel habits can provide simple interventions to try to improve FI symptoms. Dietary modification should be used as first line therapy, and it can also be used as an adjunct to surgical treatment (143). The addition of supplementary fibre and bowel habit training are useful for most patients. Reduce food substances that contain caffeine which may worsen the symptoms of

incontinence by predisposing to diarrhoea or urgency. Sometimes, they are the only treatment necessary for patients with mild FI (144).

1.9.2 Pharmacological Approach

Opiate derivatives such as loperamide or diphenoxylate with atropine may reduce FI associated with diarrhoea. Also, making the stool firmer and slowing the transit can improve stool control in FI patients who does not suffering from diarrhoea. For patients who leak a small volume of stool up to several hours after defecation, a small volume phosphate enema or a warm tap water enema is useful to clean out the rectum and eliminate retained stool and mucous (145).

1.9.3 Biofeedback therapy

Biofeedback is a form of physical therapy and muscle retraining offered to patients with FI. Patients who suffered mild to moderate FI and did not respond to medical treatment are the best candidate for biofeedback. The concept of this technique depends on a trial and error learning process which involves the use of an auditory or visual representation to monitor anal canal pressure changes in order to provide biofeedback which can tell the patient if their performance was appropriate. Manometry equipment (section 1.10.2) is often utilized for biofeedback (146). The goal is to improve the contraction of EAS in response to the relaxation of the IAS as a response to rectal distension by stimulating the RAIR using a rectal balloon. In addition, these manoeuvres can increase the strength of EAS contraction and/or the duration of sustained contractions (147). Interpretation of reports on biofeedback is difficult because of the lack of uniform reporting concerning the exact method and duration of biofeedback. Several uncontrolled studies suggest continence improved in approximately 60% of patients (148, 149). Not all studies reveal such strong results for biofeedback, a recent study by Lee et al shows a fair improvement that continued for at least a year (150).

1.9.4 Surgical approaches

Sphincteroplasty (overlap repair) has long been the standard of care of the management of FI related to sphincter injury(151). Overlapping sphincter repair for simple defects of the EAS muscle was introduced by Park and McPartin in 1970 (152). Anterior overlapping is most commonly conducted to repair an obstetric injury (153). The curvilinear incision placed over the perineal body during this technique and the healthy muscles of the sphincters is dissected to the scarred sphincter. The scar is transacted and an overlap repair is performed to ensure an intact ring of muscle. Despite the reported improvement in the symptoms of FI (154) (155), only 50% of initial success was maintained after five years of the surgery(142)..

1.9.5 Sacral nerve stimulation

Sacral nerve stimulation is a treatment of moderate to severe faecal incontinence which is associated with structurally intact anal sphincters but weak anal sphincter muscles or in patients with disruption of the internal anal sphincter in isolation (156). Sacral nerve stimulation involved the implanted of

surgically electrical devices that can stimulate the sacral nerve root which is responsible for innervating the rectum and anal canal with the aim of treating FI. If symptoms improve objectively, a permanent electrode is placed with a simulator implanted in the anterior abdominal wall or buttocks (157). It can provide short and medium term results indicate improvements in FI symptoms without any direct intervention on the anal sphincter complex. Pain at the surgical site is the most commonly reported complaint (158).

1.9.6 Conclusion

Each of the interventions described have been logically designed to manage impaired anal sphincters and are being employed in clinical practice. However, their evidence base is limited to preliminary studies and little evidence. Consequently, there is much clinical uncertainty regarding candidacy for the interventions, the optimal protocol to follow and the efficacy of these interventions. Unfortunately, patients with faecal incontinence are undergoing intensive rehabilitation which is unbeneficial or invasive surgery with potential adverse events while others are being deprived of beneficial care. Until the diagnostic evaluation of anal sphincters dysfunction is developed further in an objective and accurate manner, uncertainty regarding indications for and benefits of these treatments will persist. In the following chapter, current methods to evaluate the faecal incontinence are reviewed.

1.10 Clinical evaluation of the faecal incontinence

Clinical assessment of the function of the continence mechanism can provide dynamic information about the integrated function of each component. To evaluate patients with FI, a detailed clinical assessment with physiological and imaging tests of the ano-rectum is considered (159, 160). These information should provide data related to the etiological factors, the severity of the problem and its impact on the quality of the life (159, 160).

Currently, several complementary tests are available for evaluating ano-rectal function. The most commonly performed tests are: the digital rectal examination, ano-rectal manometry, electromyography and endoanal ultrasonography (161). The challenge is to conduct ano-rectal function testing in combination with the trials of treatments especially that FI is a multi-factorial disorder(137).

1.10.1 Digital rectal examination (finger index)

The digital rectal examination is the first test performed to diagnose FI patients, which can give an approximate indication about the degree of incontinence. In this test, a lubricant index finger is inserted slowly to the anal. From laboratory experience, the gastroenterologist can evaluate the sphincter competence by asking the patient to hold tightly around the finger. The resting sphincter

tone and the length of the anal canal can be also estimated. Unlike the examination of other organs, the clinician needs to exercise considerable sensitivity and allay any fears the patient might have when performing it (162). The digital examination is an approximation and is prone to inter-observer differences, which is influenced by many factors including the size of the examiner finger, the technique and the cooperation of the patient.

1.10.2 Ano-rectal manometry

Ano-rectal Manometry is commonly used for recording of luminal pressure in the anal canal. It is not a single test but rather a series of measurements to assess anal sphincter function, rectal sensation, recto-anal inhibitory reflexes (RAIR), and rectal compliance. It is considered as the most informative frequently utilized procedure in the evaluation of the anal sphincter function. It is one of the basic tests of ano-rectal function and it is widely used as a first line of investigation for incontinence (163, 164). Ano-rectal manometry is used to establish if there is any weakness of the pelvic floor sphincter muscle by measuring the luminal pressure and reflexes of anal sphincters. Also, it can be used to assess the length of the high pressure zone (165).

Currently, several types of ano-rectal manometry probes are available. Each system has distinct advantages and disadvantages; the most commonly used systems are reviewed here.

1.10.2.1 Ano-rectal manometry systems

The main difference between systems is the technology used in the probes. The two types of probe systems are described below.

1.10.2.1.1 Solid state probe (micro-transducer technique)

The strain-gauge technique is the simplest way of measuring the luminal pressure. It consists of pressure sensors that are mounted directly on the probe and placed in the anal canal so the measurements are recorded on position where the probe is left. Strain gauges consist of pressure-sensitive diaphragms with semiconductor strain gauges that are mounted on its inner surface (165). Microtransducer can be incorporated directly into the probe to eliminate the need for perfusion systems. Although this technique is considered as the most accurate system for performing manometry, it has limited use because of the high cost and fragility of the system (166).

1.10.2.1.2 Perfused low-compliance manometric probe (perfusion technique)

The perfusion technique is widely used although it is more prone to sources of error than the strain-gauge technique. The probes are single- or multi-lumen with up to 12 channels and side holes. The most important advantage of perfused probes is that the multiple channels can be set on a single probe. Also, the side holes on the channels can be displayed in either a radial or a stepwise fashion (10). Most probes have a diameter from 0.4 to 1.0 mm. Each channel is perfused with water at a low

perfusion rate (3ml/min or less). High feeding pressure in the fluid reservoir and high resistance capillaries were used to maintain the low rate of the water. The fluid flows from the transducer chamber via the channels in the probe and into the anal canal. The measured pressure represents the resistance to the flow of water through the side hole of the perfused tube produced by the approximation of the wall of the anal canal to the probe. The pressure is transmitted via the fluid in the channel to the transducer. The analogue signal from the transducer is amplified and visualised on computer monitors after analogue-to-digital conversion(166, 167).

It has been reported that fluid from water perfusion system leakage may result in sphincter contractions (168). In order to avoid this leakage and to obtain more reliable recordings, a balloon or a sleeve may cover the perfused side hole. Hence, it is called the microballoon ano-rectal manometry probe. The sleeve extended along the whole sphincter so that any contraction will increased the resistance to the flow of the water. This provides multi-directional rather than unidirectional pressure measurement. Air or water may be used with micro balloon systems; some study had shown good correlation between air-and water-filled systems (169), whereas the others had shown the systems to be identical (170). Unfortunately, sleeve and non-sleeve catheters are unable to distinguish between external and internal anal sphincter activity or assess the highest pressure point in the anal canal. In addition, perfusion systems are dependent on the compliance of the system and rate of perfusion as well as the size of the probe (171) and limited by the number of perfusion side holes (50, 172).

When the system is perfused at a constant flow rate ($F=\Delta V/\Delta t$) and the distal opening is suddenly occluded, a rapid pressure increase is observed. The ratio of the flow rate and the rate of pressure increase ($\Delta P/\Delta t$) is also equal to the compliance of the transducer-catheter assembly. The catheter compliance (C_{cath}) defined as the system's volume change for a given pressure increase, depends on the length, diameter, and the material of the pressure transmitting tube, the type of transducer used, and the presence of minute leaks (173).

$$C_{cath} = \Delta V/\Delta P = (\Delta V/\Delta t) / (\Delta P/\Delta t) = F / (\Delta P/\Delta t),$$

So the rate of pressure increase depends on the rate of perfusion:

$$\Delta P/\Delta t = F/C_{cath}$$

The technique of manometric measurements varies; the catheter can be left at one position (stationary technique) or can be manually moved at constant intervals (continuous pull through technique).

1.10.2.2 The technique for measuring anal canal pressures

Two techniques are currently in common use;

1.10.2.2.1 Station pulls through technique

With the stationary pull through technique, resting and squeeze pressure measurements are recorded as the transducer is stationary at 1cm intervals along the canal from the rectum to the anal verge (174).

1.10.2.2.2 Continuous pull through technique

The continuous pull through technique can assess anal canal pressures on a cross sectional bases at each recording station from anal verge, allowing examination of pressures which can provide a more longitudinal profile, which is important because of the considerable variation in pressures along the axis of the anal sphincter. The catheter tip is initially positioned 6cm from the anal verge into the rectum and then gradually and progressively withdrawn at intervals of 1cm (175).

1.10.2.3 Parameters measured with ano-rectal manometry probe

Typically recorded data include resting pressure, maximum squeeze pressure and squeeze duration, anal canal pressure in response to cough, anal canal pressure in response to defecatory manoeuvres, the recto anal inhibitory reflex (RAIR) elicited by balloon distension of the rectum, rectal compliance and sensory thresholds in response to balloon distension. The pressure recordings are given in millimetre of mercury (mmHg) (176).

1.10.2.3.1 Resting anal pressure

The resting pressure is defined as the difference between the baseline pressure (atmospheric pressure) and the maximum anal sphincter pressure at rest (176) .Tonic contraction of the IAS is suggested to contribute up to 85 per cent of the resting anal pressure and it is independent on the size of the sphincter (26). Resting pressure is not constant; it is dependent on whether the individual alert or sleep, and on his or her position. Hence resting anal pressures in the normal individual in the left lateral position is between 50 and 95 mmHg and increases by 25 percent when the individual is ambulant; moving, walking, etc (177). Pressure recording of the anal canal using side hole manometry and station pull through technique show that the highest pressure during rest is located in the part of the anal canal that is surrounded by the EAS (178). See Figure 1.5 (a).

1.10.2.3.2 Maximal squeeze pressure and squeeze duration

The maximum squeeze pressure is defined as the difference between the baseline pressure (atmospheric pressure) and the highest pressure recorded within the anal canal during squeeze as a result of the voluntary contraction of the EAS (176). This is usually 50% to 100% greater than resting anal pressure in normal individuals. The duration of squeezing between the onsets increases in anal sphincter pressure till the return to the resting pressure is also measured (176). The squeezing pressure for males was found higher than for females, however, no difference was found in resting pressure for both of them (179) . Some studies suggested that the use of an inflated balloon catheter can increase the maximal squeeze pressure in the anal canal. An explanation for this is that trying to retain

a balloon in the rectum is more accurately replicates the normal physiology of continence than the verbal command to squeeze (180). See Figure 1.5 (b).

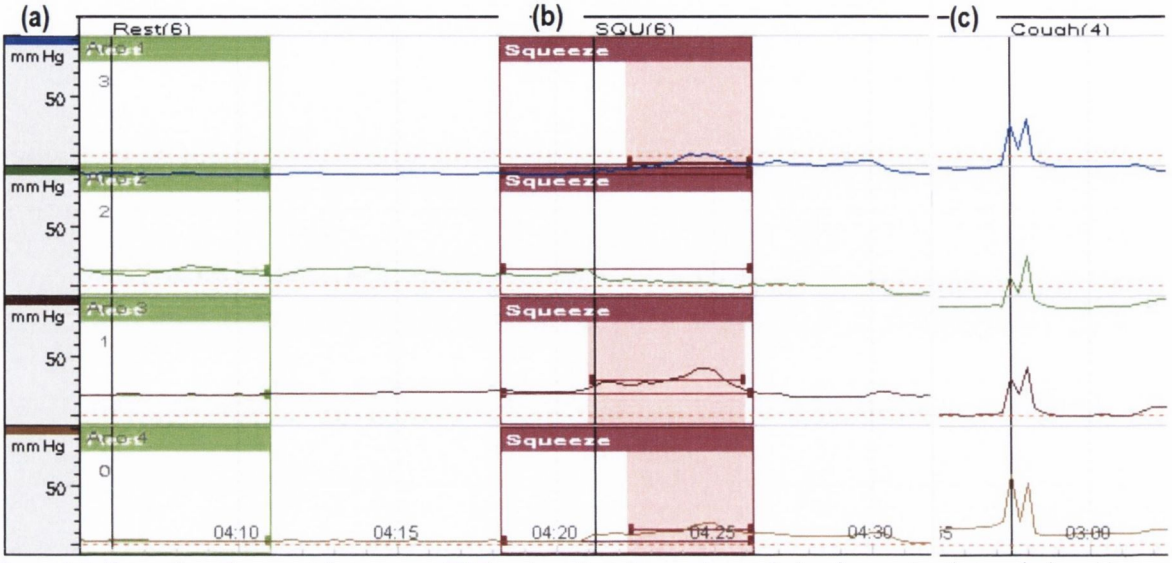

Figure 1.5: Recording of ano-rectal pressure of FI female patient from water perfusion four port catheters during: (a) resting, (b) squeezing and cough (c)

1.10.2.3.3 Anal canal pressure in response to cough

Normally, an intra-abdominal pressure increment induces a reflex contraction of the external anal sphincter. This reflex is performed by asking the patient to cough or to blow up a balloon, or simply to cough (176). This test is particularly useful for estimating the anatomical level of neuronal injury in a patient with FI (181). See Figure 1.5 (c).

1.10.2.3.4 Anal canal pressure in response to defecatory manoeuvres (Straining)

During this test, the patient is asked to push (strain) as if trying to defecate. The external sphincter should relax during this manoeuvre and the anal relaxation can be measured by pressure recordings along the anal canal(164). Manometric evaluation of the anal canal relaxation requires multiple, closely spaced recording ports along the anal canal, because during straining the anal canal shortens and these morphological changes may displace the probe. If both intra-rectal and anal pressure recordings are combined, it is important to locate the anal manometry probe very close <5mm to the rectal balloon, otherwise a short no relaxing segment of the anus may be missed (182).

1.10.2.3.5 The recto anal inhibitory reflex (RAIR) elicited by balloon distension of the rectum

Anal responses to rectal distension are the lowest balloon volume required to elicit relaxation of the internal anal sphincter and to cause sustained relaxation of this sphincter during the intermittent balloon distensions (183). RAIR evaluation is performed by the progressive increases in volumes of air in the rectum and recording anal canal pressure responses. During this test, the rectal balloon is inflated manually at maximum rate of 10ml of air or body temperature water. The inflated air or water

should be completely withdrawn within 3-5s of inflation (184). Most ano-rectal laboratories report RAIR as either being present or absent and state the volume of air required eliciting the reflex. The difficulties in reproducing RAIR may necessitate more investigation. Occasionally rectal distension with different volumes resulted in transient and large pressure increases of the entire anal canal. It appears likely that the latter changes are caused by contraction of the deeper bundles of the EAS, which possibly override IAS relaxation (57, 185). See Figure 1.6.

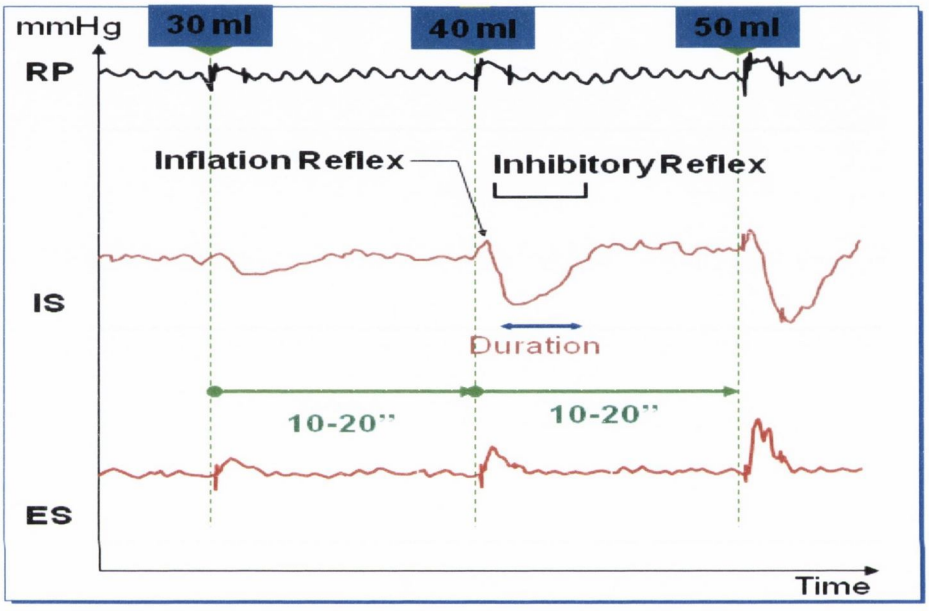

Figure 1.6: Normal RAIR due to distension of the ano-rectal manometry balloon. RB (rectal balloon), IS (internal sphincter and ES (external sphincter)

1.10.2.3.6 Rectal compliance and sensory thresholds in response to balloon distension

Rectal compliance and sensation testing accompanies manometric evaluation and using the same equipment and helps in providing information about sensory defect and extensibility of the rectum. To conduct the procedure, air will be injected into the rectum balloon which is positioned in the rectum. The point of first sensation, the point of urge to defecate and the maximal tolerable volume are the measurements which are recorded during sensory. Normal rectal compliance would adapt by expanding with the injecting of air to maintain the pressure relatively steady (183). Patient's response to distension is noted by marking each distension: 0=no sensation, 1=first sensation, 2=constant sensation/urge, 3=maximum tolerable sensation (165).

1.10.2.4 Optional measured parameters

1.10.2.4.1 High pressure zone length

The high pressure zone length is the distance in centimetre from the start of the pressure increase from the upper anal canal to the point at which pressure fell to zero leaving the lower anal canal during a rapid pull-through retraction of the manometry probe in the anal canal (183). Using rapid pull through, the ano-rectal manometry probe is connected to a puller which is automatically withdraws

the probe at a constant speed of 0.5-1.0cm/s. As speed of catheter withdrawal was constant and known, the length of the high pressure zone of the anal canal could be accurately measured (186).

1.10.2.4.2 Vector volume analysis

The pressure profile of the sphincter can also be studied using a specific manometry probe with six to eight radially oriented channels; so called 'vector volume' probe. These measurements provide radial and longitudinal pressure profile from each vector diagrams; when plotted together can generate three dimensional picture of the pressure profile of the anal canal, known as vector volume. Possible deficiencies and asymmetries in the anal canal can be estimated (187).

1.10.3 Developing anal pressure techniques: High-resolution manometry (HRM)

HRM is solid-state probe of 4.2mm outer diameter with 36 or 256 circumferential sensors spaced 1-cm intervals (188). This device uses a novel pressure transduction technology that allows each sensor to detect pressure over a length of 2.5 mm and in each of 12 radially dispersed sectors. The average sector pressures allow the detection of the circumferential pressure at each detector (189). The accuracy of pressure measurements in HRM is much higher than the 4 sensors water perfused manometry system which can miss important findings(190). See Figure 1.7.

HRM provides greater physiological resolution and minimize movement artefact. Also, HRM capable of generating a three dimensional topographical plots of the intra-luminal pressure relative to time and location (191). This would help in detecting the pressure changes over a longer length of the rectum and anal canal with detection of abnormalities and increasing accuracy (192). To date, few reports suggested that HRM may enhances our understanding of anal pressure profiles, normal physiology, puborectalis function, and sphincter defects, and may increase diagnostic yield (193, 194).

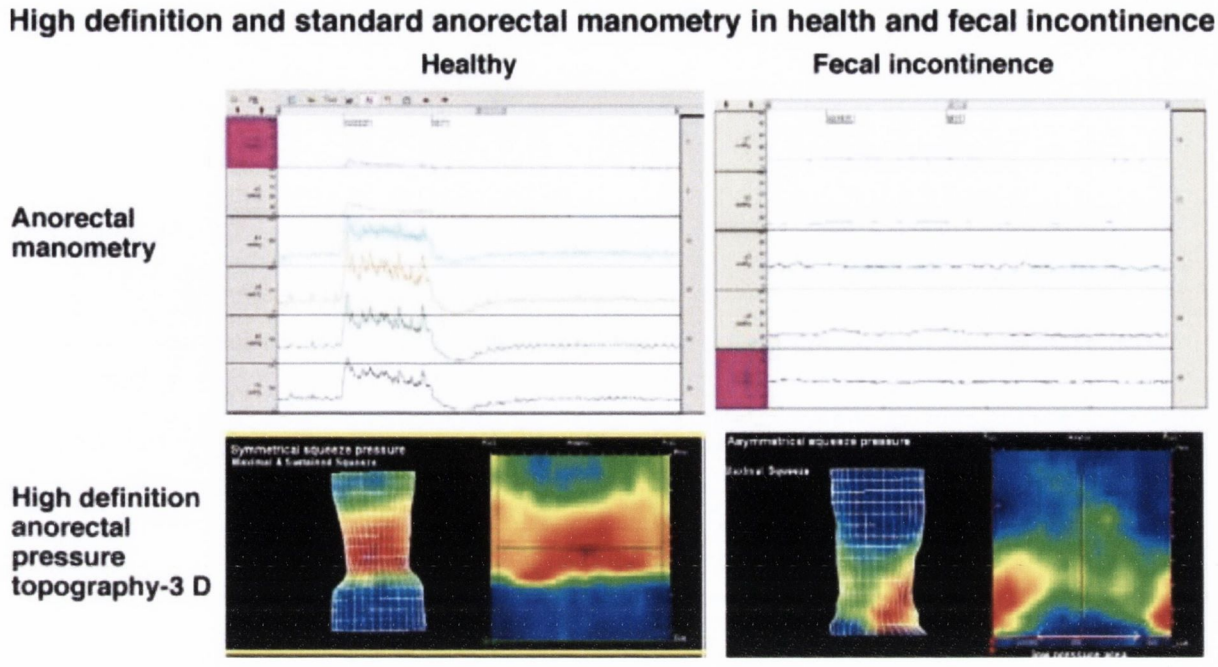

Figure 1.7: High-definition manometry and pressure topography during rest and voluntary squeeze. In the healthy subject (*left*), normal resting and normal increase in sphincter pressure is seen whereas in the incontinent subject the sphincter is weak during squeeze. *(Reprinted with permission from Prof Bharucha)*

1.10.3.1 Limitation of the manometry system

Manometry with a balloon placed in the rectum has been considered as the gold standard for measuring ano-rectum function for many years. Anal canal pressure measurements are the commonest means of assessment of sphincter function (49, 166, 176, 181, 195-198). However, the literature abounds with confusing, unexplained results and some doubtful conclusions about sphincter activity. There can be considerable overlap between the pressure profiles of normal and incontinence patients (51); patients with normal resting pressures and reduced squeeze pressures may be incontinent because of failure of the EAS to contribute to continence patients at critical time (74). A significant number of continent patients have impaired pressure profiles and some incontinence patients generate normal pressure profiles. Manometric studies of the anal canal do not always predict the patient's clinical status. In general, a lack of uniformity in materials and methods used has reduced the value of inter-unit comparisons of results (199, 200). Hence, the clinical significance of normal and abnormal manometric values remains unclear, subjects with values outside the normal range may not have clinical symptoms and vice versa. Patients with clinical problems may exhibit normal values (9, 10). In general, the association between conventional manometric findings and symptoms is often poor and the pathological basis of symptoms remains unclear in a substantial number of patients (201). Moreover; the measured value depends on the method used for the manometric assessment as well as its influence by the abdominal pressure (202).

Harris and Pope found that the pressure profile obtained as the tube was withdrawn through the anal canal from the rectum differs considerably from that obtained as the tube was inserted from the exterior (75). Differential pressure recorded by withdrawing a probe from the rectum and reinserting it via the anus led Harris and Pope to propose that the competence of the sphincters in the anal canal depends not so much on the ability of the muscles to squeeze around the anal canal but rather on their ability to resist the opening up of the anal canal (76).

Furthermore, the relaxation could not be captured by manometry, the manometry can measure the function of toned sphincter, and so it can exert a squeeze or force on the sensor. However, if the sphincter is relaxed, the pressure measured is more indicative of the state of the chamber created by the lumen opening up between the rectum and the anal canal. See figure 1.8. Therefore; simple manometric recording of the sphincter pressure will not be able to assess intrinsic abnormalities of distensibility (203).

Figure 1.7 indicates how this concept of measuring pressure may be limiting. When a normal sphincter is toned or competent, it exerts a squeeze or force on the sensor causing it to be occluded by the sphincter. Hence, the pressure measured provides a sensible reading of the force in the tightened sphincter. However, if the sphincter is in relaxation state, it is represented by no or low pressure which is more created by the opening of the lumen (203).

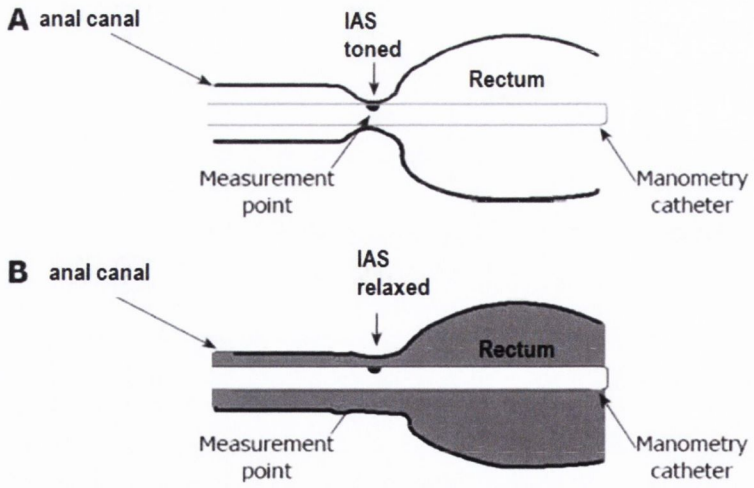

Figure 1.8: Simple manometry catheter shown in position in the anal sphincters when it is toned. A: The toned or competent sphincter causing the sensor to be occluded by the sphincter; B: The pressure is indicative of the state of the chamber created by the opening of the lumen. *(Modified from McMahon et al, 2009)*

1.10.4 Electromyography of the pelvic floor

Electromyography (EMG) provides a sensitive measure of denervation (fibrillation potentials) and can usually identify myopathic damage, neurogenic damage or mix injury. It can assess the neuromuscular activity of the anal canal as well as the neurological damage of the area of interest by recording the motor unit potential generated by the PRM and the EAS at rest, during voluntary contraction, during evacuation and in response to different reflexes (204). Abnormal EMG activity, such as fibrillation potentials and high-frequency spontaneous discharges provide evidence of denervation. It occurs in patients with faecal incontinence following pudendal nerve injury (191). The EAS is examined on each side with 1 or 2 needle insertions. The PRM is examined by inserting a needle in the middle line between the anus and the tip of the coccyx, passing the needle through the EAS and into the deeper PRM. Insertion activity at rest, motor unit potential amplitude and duration and following mild to moderate voluntary muscle contraction are assessed. Motor unit potential is a reliable measurement of the neurogenic lesion (175). The test can be carried out with single fibre, concentric needle techniques or surface EMG.

1.10.4.1 Single-fibre EMG

The single-fibre EMG allows quantitative studies of denervation and subsequent re-innervation of individual motor units by the measurement of 'Fibre Density'. Fibre density is an index of the mean number of muscle fibres supplied by one motor unit within the uptake area of the electrodes. This technique can be performed to map the muscles for patients who have EAS injury to confirm the damage of its innervation. Increased fibre density suggested by the increase of the number of spike potentials detected. Incontinence patients usually show smaller and prolonged motor potentials (205).

1.10.4.2 Concentric-Needle electrode

A concentric-Needle electrode is inserted lateral to the anal canal either in the PRM or the EAS, so the electrical activity is recorded (206). This investigation may be used to map EAS defects that caused by obstetric or surgical procedures. Although the concentric needle EMG has long been used in the assessment of traumatic sphincter, the technique is difficult to quantify in routine investigations of these muscles. Endoanal ultrasonography has now largely replaced EMG in the assessment of sphincter anatomy (207).

1.10.4.3 Surface EMG

Surface EMG can provide useful information about sphincter function recorded within the anal canal. Although conducting the procedure is not comfortable to the patients, it has less of a risk of infection than needle EMG. Surface EMG appears to have a definite role in the evaluation of sphincter function and in biofeedback training as it can provide visual or audible signal during the training (208).

1.10.5 Endoanal ultrasound (EAUS)

Visualization of the sphincter and detection of sphincter lesions is playing a crucial role in diagnosis of FI Patients who may benefit from surgical therapy. EAUS is an imaging technique used to visualize the sphincter anatomy. It reliably identifies both external PRM and internal anal sphincters (209). EAUS is used to examine the anal sphincters; a discontinuity indicates a tear. It provides an assessment of the thickness and structural integrity of the EAS and IAS and can detect muscle tissue scarring, defects and other local pathology (210). A defect is defined as a "discontinuity of the muscle ring". Scarring is defined as a "hypo-intense deformation of the normal pattern of the muscle layer due to replacement of muscle cells by fibrous tissue" (211, 212). It is of value in exploring the cause of anal incontinence because it can show up clinically undetected sphincteric defect or scars. It can elucidate the background to anal incontinence notably after vaginal delivery (213).

This examination does not require any preparation or sedation and it is normally tolerated by the patients. The cylindrical nature of the anal structures favours the 360◦ axial view at right angles to the lumen obtained with a mechanically rotary probe of 360◦. It is introduced into the rectum, aligned in standard orientation with the anterior end uppermost with a focal length of 1 to 4 cm, and then slowly withdrawn sown the anal canal. When Images are obtained at upper, middle and lower anal canal, some image asymmetry may be induced especially if the patient is laying in a left lateral position. Accordingly, it is preferable to examine the patient in prone position (214).

The PRM and transverse perineal demarcate the upper part of the anal canal. The former blends into the EAS in the middle part of the canal, forming a complete ring anteriorly. The IAS is thickest in the middle part of the anal canal and is of uniformly low reflectivity, contrasting sharply against the EAS the longitudinal muscle laterally. The subcutaneous EAS, lying below the termination of the IAS, defines the lower part of the anal canal (215).

EAUS has shown 85% of patients with incontinence of traumatic origin have EAS defects and 40% of these patients also have disruption of the IAS; an IAS thickness of less than 2 mm or more than 4 mm is considered abnormal in adults(216). Such defects are often associated with a weak PRM that can be relatively inert on dynamic testing. It is currently the diagnostic imaging of choice for providing information about the integrity of the IAS and EAS and detecting sphincteric defects and has the advantage of avoiding radiation (217).

A major impact of the EAUS has been to image tears of the sphincters not apparent on clinical examination: so called occult defects. These were documented in around 35% of first time vaginal deliveries. Major defects are relatively easy to visualise but minor defects of the sphincter can be quite subtle especially EAS defects. Differentiation of the various muscle layers is poor, where the majority

of the obstetric defects arise and the distinction between defects of the sphincter and the support structures requires careful analysis (218, 219).

Although, diagnostic procedures such as EAUS and EMG have been found to be reliable measures for the detection of sphincter defects, they contribute little to the evaluation of the functional significance of the lesions detected (220). EMG can still detect functional abnormalities and even absence in incontinence patients in whom normal anal sphincter is found on EAUS (221), so these two techniques are complementary and not mutually exclusive (191). On other hand, some investigators prefer EAUS for the assessment of sphincter morphology, because the latter is widely available, less expensive and less painful than needle EMG (222).

1.10.6 Evacuation proctography

Evacuation proctography (EP) is a simple radiologic technique that provides a dynamic study of the rectal emptying and the complex process of defecation. Its main indication is difficult or infrequent rectal evacuation. Other indications are rectal prolapse, anal incontinence and pelvic pain. The defactography can provide information about the function of the PRM, efficiency of emptying length of the anal sphincter and movement of the ano-rectal angle (223).

This technique involves the filling of the rectum with 100 to 200 ml of barium past with a consistency similar to stool. Some investigators fill contrast barium until a strong urge to evacuate is provoked. During the study the patient should lie in left lateral position on the fluoroscopy table. The barium paste is injected with a syringe into the rectum and the injection is continued during withdrawal of the syringe, to mark the anal canal and verge. After that, the table is positioned into upright position; a commode is placed on the footrest. Also, the patient must be seated during imaging related to the physiological position importance. Recording the image of the rectal evacuation by spot image or video-fluoroscopy must be quick or continuous. Despite the spot image is preferable because of the best spatial image, the capability to capture the process if the evacuation is rapid is minimal (219).

EP comprises three stages: the pre-evacuation, evacuation and post-evacuation. Before evacuation or rest; the ano-rectal junction should be at the pubococcygeal line and the anal canal should be closed without leakage, see figure 1.9. The ano-rectal angle should be approximately 90◦. Some investigators advocate the acquisition of views during a squeeze manoeuvre to evaluate the strength of voluntary pelvic floor muscle, during cough to stress the continence mechanism and during straining to assess pelvic floor descent. The Anorectal angle increased by approximately 20◦ and anal canal shortened during the initiation of evacuation. It take about 4.5 seconds to open to its maximum diameter of 1.5cm (224). After the evacuation process finishes, the anal canal closes and the anorectal angle and junction return to their original positions (225).

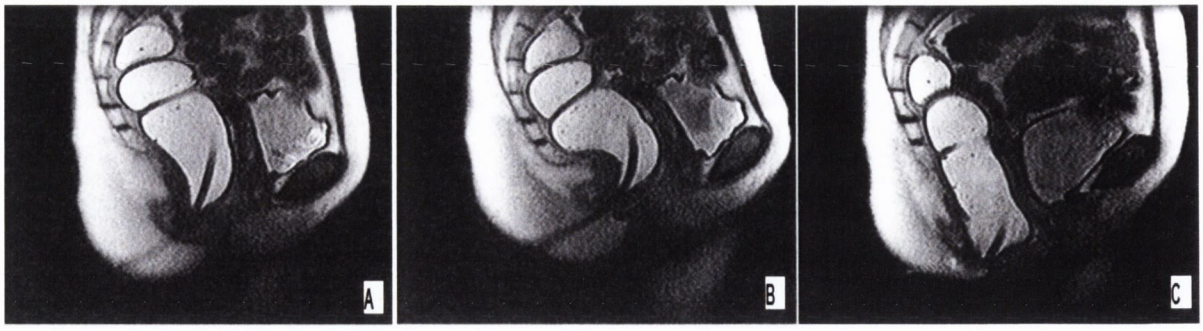

Figure 1.9: dynamic MRI (A, B, C) in one patient. Images were acquired at rest (*A*), during squeeze (B), and defecation (C). MR and evacuation defactographic images demonstrate abnormal descent of the ano-rectal junction during defecation to 5.5 cm and 9.4 cm below the pubococcygeal line respectively. *(Reprinted with kind permission from Prof Bharucha)*

Five parameters describing normal evacuation should be defined; (i)the increase in ano-rectal angulation,(ii) obliteration of the impression of the puborectal muscle, (iii)wide opening of the anal canal, (vi)total evacuation of contrast medium, and (v)normal pelvic floor resistance (226). Although EP is now well established, its use is still confined to specialist centres and its role remains controversial despite several years of clinical use. The test interpretation is lacking standards for incontinence patients as 50% of normal subjects will show some abnormality (189).

1.10.7 Pelvic Magnetic resonance Imaging (MRI)

1.10.7.1 Static and Dynamic MRI

Although EP is rapid and easy to perform, the modifications needed to image other organs are time consuming and invasive. Also, the musculature of the pelvic floor is not easily to be visualized, and irradiation of younger patients is an essential factor. To overcome these limitations, MR imaging has been applied to pelvic floor dynamics with excellent outcome, although the experimental nature of this procedure must be emphasized (214).

The basic examination requires no patient preparation. A soft catheter is inserted in the vagina and rectum to facilitate identification. MRI of the pelvic floor is performed on the patient in a supine position. Static MRI with a body coil gives a global view of the pelvic organs. T1-weighted sequences in coronal and sagittal planes visualise the morphology of the pelvic organs. T2-weighted sequences obtained with an endo-vaginal coil can provide a description of the anatomy of the three pelvic compartments in the three planes of space and the thickness of levator ani muscle. Images may be conducted in sagittal, coronal and transverse plane. Static images can also be obtained (219).

Dynamic MRI of the pelvic floor requires no patient preparation. The patient is placed in a supine position in the magnet and fast spin-echo images of the pelvis are taken at rest. The sagittal images best reveal the relationships between the pelvic organs and pelvic floor. Further images are taken

during maximal pelvic strain, as practised by the patient before the MRI evaluation. Imaging is then repeated while the patient performs a maximal straining effort (227). Some authors fill both bladder and the rectum and encourage the patient to evacuate within the magnet (225). See Figure 1.10.

There is considerable discrepancy between the results of dynamic MRI when compared with proctography. This is probably a consequence of the necessary supine position, which can be overcome by using vertical open MRI system (228). Major advantages of MRI are its non-invasiveness; no radiation exposure, no injection of contrast medium.

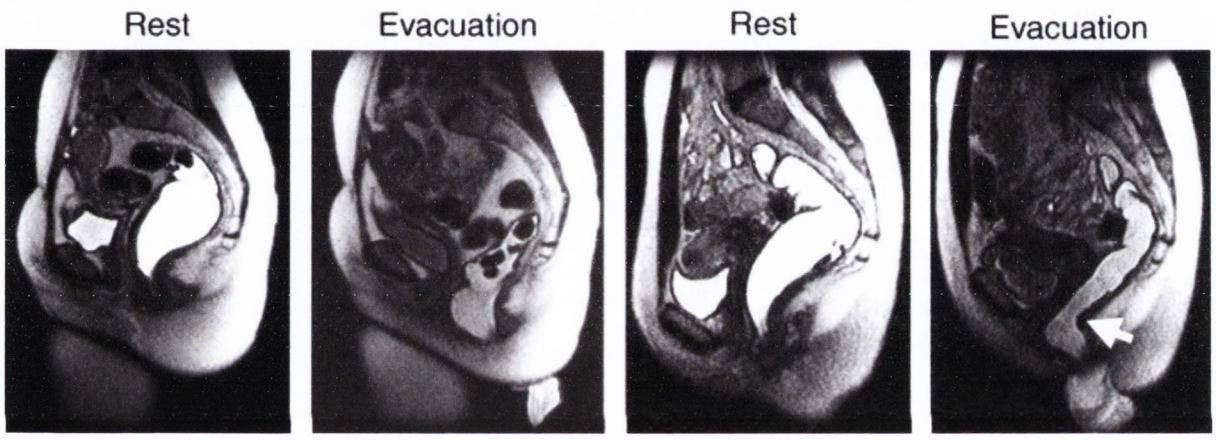

Figure 1.10: Sagittal dynamic MRI images of normal puborectalis relaxation (left panel, subject 1) and puborectalis contraction (black arrow, right panel, subject 2) during rectal evacuation. *(Reprinted with permission from Prof Bharucha)*

1.10.7.2 Endoluminal MRI

Endoluminal MRI provides a detailed anatomical picture of the anal sphincter. This technique provides high resolution images of the normal anatomy and pathologic condition of both EAS and IAS. It can be used to investigate FI and may be of considerable diagnostic value (229).

The examination is normally done with the patients in supine position after placing a cylindrical rigid receiver coil in the anal canal. Such an examination is well tolerated by most patients and easy to perform (212). Rigid endoanal coils are mostly chosen related to its optimal image. A T2-weighted sequence is usually used. MRI is performed to obtain sagittal planes and transverse planes oriented at a right angle to the anal canal. The most relevant plane is the transverse plane but sagittal planes provide information on disease spread. The smooth muscle of the internal sphincter is fairly hyper-intense whereas the striated muscle of the external sphincter and the levator ani muscle are hypo-intense (230).

Endoluminal MRI is superior to the endoanal sonography because of its multi-planner capability and the higher inherent contrast resolution of the former, especially in detecting external sphincter (23)

Rapid MRI sequences can visualize he anatomy of both the IAS and the EAS. MRI is better than US in identifying EAS and visualizing PRM as it can assess pelvic floor motion in real time without radiation exposure (231). See figure 1.11.

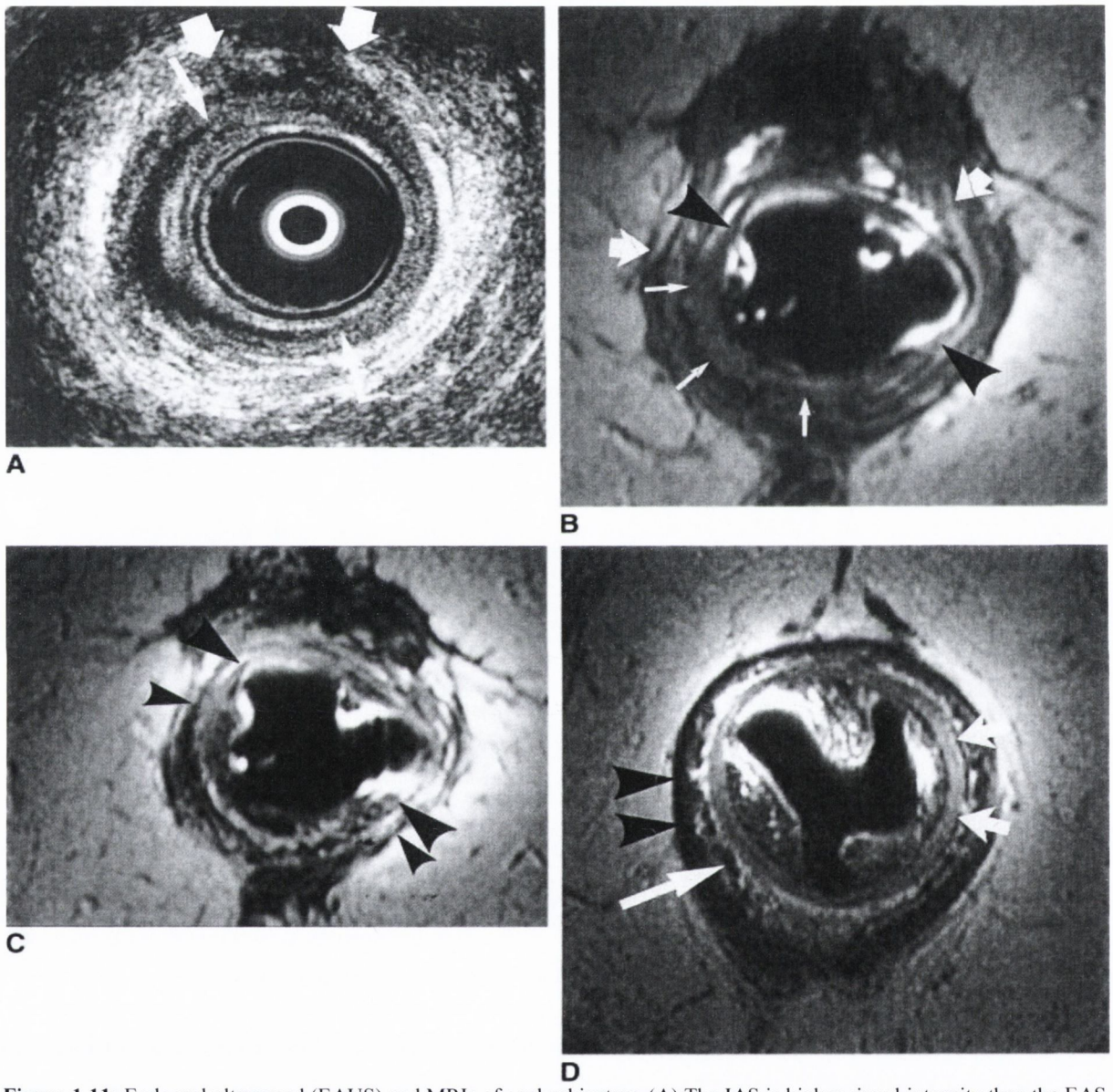

Figure 1.11: Endoanal ultrasound (EAUS) and MRIs of anal sphincters. (A) The IAS is higher signal intensity than the EAS on MRI. The IAS is thin (thin white arrows). The EAS tear is between the thick white arrows. (B) On MRI, the intact internal anal sphincter (small white arrows) is of higher signal intensity than the external anal sphincter and demonstrates the same defect, between large black arrowheads. The EAS is located between the large white arrowheads. (C) Axial endoanal MRI at a more caudal level demonstrates similar abnormalities in the IAS between the large black arrowheads and EAS between the small black arrowheads. (*D*) Axial endoanal MRI demonstrating normal IAS (short white arrows), longitudinal muscle (long white arrow), and EAS (black arrowheads). *(Reprinted with kind permission from Prof Bharucha)*

1.10.8 Conclusion

As outlined above, several methods exist for evaluation of anal sphincter. There are many tests for imaging the pelvic floor but no standards have been established for when they should be prescribed. MRI and EAUS are commonly used for description of the morphology. EAUS images are necessary to document the severity of weakness and identify abnormality in sphincter morphology. EP provides

a dynamic study of the rectal emptying and the complex process of defecation. Dynamic MRI imaging with barium exposure is particularly useful for identifying the musculature of the pelvic floor. EMG should be considered for incontinence patients who underlying disease associated a neurological sphincter weakness unexplained by morphology as visualised by EAUS (103). Ano-rectal manometry is generally used to measure the length of the high pressure zone in the anal canal and to determine the anal resting and squeeze pressures.

The aims of pelvic floor diagnostic tools are to prevent persistence of an uncorrected defect and recurrence of a badly corrected defect, and to avoid the complications that would arise on attempting to correct a non-existent disorder (219). However, these techniques cannot provide us with reliable or validated quantitative data. As a result, diagnostic testing is deemed a qualitative exercise and clinicians are advised to interpret current data cautiously (11). Until diagnostic evaluation is developed further, progress in our understanding and treatment of faecal incontinence will be hampered. Researchers, therefore, agree that future work in the area of faecal incontinence and ano-rectal dysfunction will likely centre on more accurate and physiologic diagnostic techniques. It is probably that this will ultimately improve intervention practices.

The next section will introduce a novel evaluation technique; the Functional Lumen Imaging Probe (FLIP) which is designed to quantitatively evaluate anatomical lumens.

1.11 Developing a new approach to functional testing in the ano-rectum region

1.11.1 Background

As described earlier, the ano-rectum is physiologically a highly complex segment of the bowel. The mechanical features are modified by the sensory and reflex factors and integrated immediately to initiate normal defecation within a few seconds and to maintain continence within a fraction of a second. These highly complex natures of the region may be responsible for the lack of knowledge of how the anal sphincters work. The difficulties of interpretation exist despite the availability of an enormous pool of research data where many different factors have been considered in an un-integrated approach (232).

The standard ano-rectal manometry techniques described in section 1.10.2 are valuable because they are easy to perform and supply the investigator with a simple expression of the state of the almost closed anal sphincter in terms of resting and squeezing pressures. Since the sphincters act in response to the presence of stools distending the anal canal as well as the fact that anal canal is subject to

dimensional changes, the measurement of resting pressures is a poor way of describing the continence mechanism as the biomechanical and morphological properties changes in the ano-rectal region (233).

1.11.2 Balloon distension technique (Barostat)

Most balloon distensions in the gastrointestinal tract are done with simultaneous measurements of the balloon pressure and volume using barostat methods (234). The balloon can be distended with fluid or air filled balloons (235). The technique of distending the anal sphincters to measure its distensibility was proposed by Katz et al. They suggested that resting anal sphincter resists distension until a resting yield pressure is reached, after which further distension of sphincter produces no further pressure arise. The squeezing pressure is reached by voluntary contraction after recording balloon had been distended to the volume needed to reach the resting yield pressure. Using this technique, they were able to differentiate patients with incontinence as the result of abnormalities of the voluntary component of anal sphincter from those patients with incontinence of other cause (236). However, this technique rely on the pressure being control which is not always the situation as control is not always possible related to increasing or reducing volume through a narrow catheters (237).

The response to anal distension was found to be similar to that seen in rectal distension. That is confirmed by a study of Bouchoucha et al. indicating that the sphincters act both as a barrier of pressure at rest and then as a filter related to the deformation rate to allow or to impede stool passage. The anal sphincter acts as a low-pass filter (238).

Unfortunately, biomechanical parameters of the anal canal such as wall tension and distensibility that defined using barostat technique are over estimated, especially that the biomechanical properties of the smooth muscles and striated muscles in the anal canal are different (10).

1.11.3 Impedance planimetry technique

Harris *et al.* introduced a method using impedance measurement based on the field gradient for assessment of CSA in biological tubes (239). Over years, a significant number of studies were conducted in order to improve this system (240). Subsequently, Gregersen *et al.* introduced an improved Impedance Planimetry system with the ability to measure the Cross-Sectional Area (CSA) in the gastrointestinal tract; it contains a simple sine wave generator and current amplifier with low supply current. The system which automatically offset the adjustment and has a large linear range of measurement can provide a tool for the measurement of CSA in the gastrointestinal tract (241).

1.11.3.1 Distensibility aspects

Theoretically, distensibility is the measure of tube compliance and is represented as the ratio between the average pressures to the average CSA at defined distended volume. The flow rate through a tube is

inversely related to its narrowest CSA and therefore a low distensibility tube provides a stronger continence barrier function (232).

The distensibility data available are based mainly on balloon distension technique. The most important aspects relate to the experimental design, the probe design, the method of measurement, the geometry and mechanical properties of the balloons, and the assumptions for the analysis, i.e. the analysis depends on the mode of filling (continuous or stepwise). In general, the boundary of a geometrically measurable parameter must be in complete contact with the measured sphincter, i.e. the whole circumference and surface area of the balloon must be in contact with the tissue (242). Also it is important to ensure that the applied pressure is properly transmitted to the tissue. Besides, it is essential that measurements are obtained at one location. Thus, cross sectional area could be measured at the desired location. Biomechanical analysis is controlled by geometrical factors. Most of the equations used in biomechanics are derived under certain geometrical assumptions (243).

1.11.3.2 The principle of the impedance planimetry

Impedance planimetry is a technique for measurement of the luminal CSA in hollow organs, specifically cylindrical tubes. This novel technique subsequently is offering the possibility of characterising biomechanical properties of the gut wall (10). Impedance planimetry utilises a simple electrical principle based on Ohm's Law for estimation of active and passive biomechanical wall properties of the intact. This method provides an accurate single measure of CSA under changeable conditions together with bag pressure in animal and human segmental studies. The advantage of this method is that two variables are measured at the same time as opposed to most other methods (241, 244).

Consider a linear array of four electrodes mounted on a catheter. When a constant alternating current I is induced in an electrical conductor by the two outer (excitation) electrodes, the potential difference V between the two inner (detection) electrodes, according to Ohm's Law, is $V = I Z$, Z represent the electrical impedance of the saline. See Figure 1.12.

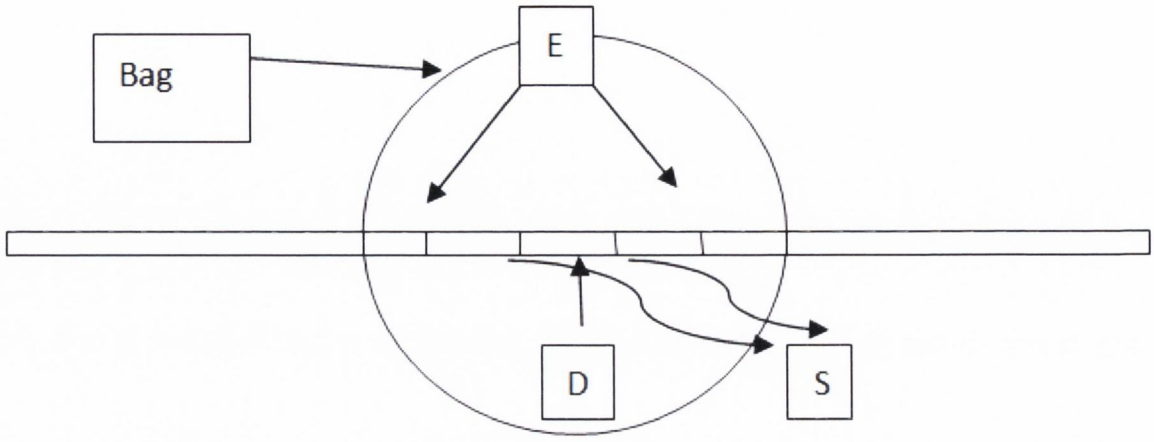

Figure 1.12: Sketch of an impedance planimetry probe with one set of excitation (E) and two detecting electrodes (S), for measurement of cross sectional area in the middle of the bag.

The impedance is a function of the distance (D) between S electrodes; the two excitation electrodes are sufficiently far away from the detection electrodes in order to create a uniform electrical field in the area surrounding the two detection electrodes, the conductivity c of the liquid in the bag and the cross sectional area (CSA) of the bag between the S electrodes.

Thus, the potential difference can be expressed as:

$V = I\ D\ c^{-1}\ CSA^{-1}$

Where I, D, and c are constant. Thus, the voltage crossing the electrodes is proportional to the inverse of the CSA,

$V = k\ CSA^{-1}$. Where k is a calibration constant.

Thus, voltage measurement was made at one single sensing electrode pair (S) and the voltage measured was proportional to the impedance between the sensing electrode pair. This impedance change was inversely proportional to the CSA change at the sensing electrode pair in the bag, i.e. the impedance decreased as the bag filled with saline. Using the assumption that the sphincter has a circular CSA, the CSA measurement can then be used to obtain the diameter at the location of the electrode pair (S) (245-247).

The electrical field created by the current from the excitation electrodes is confined to the conducting fluid inside a bag which embraces the electrodes on the probe. In order to control the pressure or volume inside the bag, a channel connects the bag with a fluid container which can be raised in such a way as to increase the bag's pressure (10).

Impedance planimetry was deemed both useful and reliable in measuring oesophagus competence(248), measuring the distensibility and biomechanical wall properties in the rectum (249-255)as well as measuring anal CSA and pressure during anal distension (256-259)

1.11.3.3 Impedance planimetry probe (dynamic anal manometry)

The impedance planimetry probe consists of four electrodes; the two outer electrodes using excitation are placed with an inter-electrode distance of 1-5cm, and they are connected to an Impedance Planimeter which gives a constant alternating current of 30-100μA at 10 k Hz. The measured impedance depends on the frequency of the current; it was found in previous work that 10 kHz is the most optimal frequency (240). See Figure 1.13. The current is then delivered to the two excitation electrodes, which generate an electrical field between them. The potential difference between the two detection electrodes is amplified using a high signal to ratio amplifier, filtered, analogue-to-digital converted and sent to the computer (260). Two inner ring electrodes for the purpose of detection are placed between the excitation electrodes with an inter electrodes distance of 1-2mm, all of which are connected to an impedance planimetry system. The detection electrodes should be small high-impedance electrodes (241).

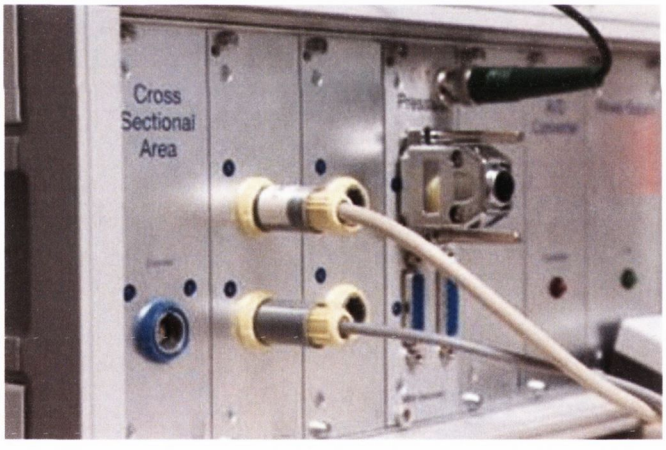

Figure 1.13: GMC-IP1000 impedance planimeter is a multipurpose box that can be used with all impedance planimetry probe types and most pressure transducers.

The four-electrode impedance techniques were able to simultaneously monitor two variables (CSA and pressures) in sphincters region and hollow organs. These two variables can be used to describe mechanical wall properties (261).

The impedance planimetry probe in Figure 1.14 is capable of simultaneously measuring single CSA and pressure inside the bag. This dynamic assessment of anal sphincter function is of value in terms of investigating anal sphincter physiology and anal incontinence (245-247). With impedance planimetry,

both opening and closing pressures of the anal canal sphincters as well as distensibility at only single radial position could be measured (262).

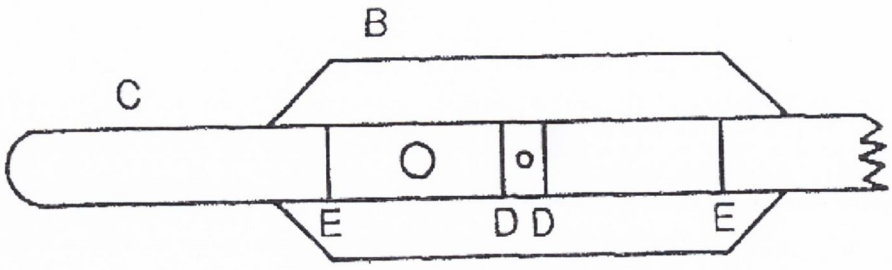

Figure 1.14: Schematic representation of the dynamic manometry probe for measurement of pressure and cross sectional area in the anal canal. B=bag, C=probe, D=detecting electrode, O=infusion channel. CSA is estimated from measurement of electrical impedance of the fluid inside the bag.

1.11.3.4 Errors and Limitations

There were limitations relating to the incomplete data about the anal continence mechanism, especially in regards to the fact that the length of measurements was not capable to describe the active and passive mechanical properties of the anal sphincters because of (i) the difficulties of placing the measurement electrodes at the point of most interest, and (ii) the fact that distending bag in the anal sphincter zone tends to displace during the measurements (166, 167).

1.11.4 Functional lumen imaging probe (FLIP) technique

The Functional Lumen Imaging Probe (FLIP) is a new novel technique that was created by McMahon *et al* and has the ability to functionally image and evaluates the Oesophagus Gastric Junction (OGJ) with the latter evaluation being carried out using impedance planimetry technique. This multi-electrode technique can use CSA and pressure data to calculate the distensibility of the sphincter at different degree of opening. See Figure 1.15.

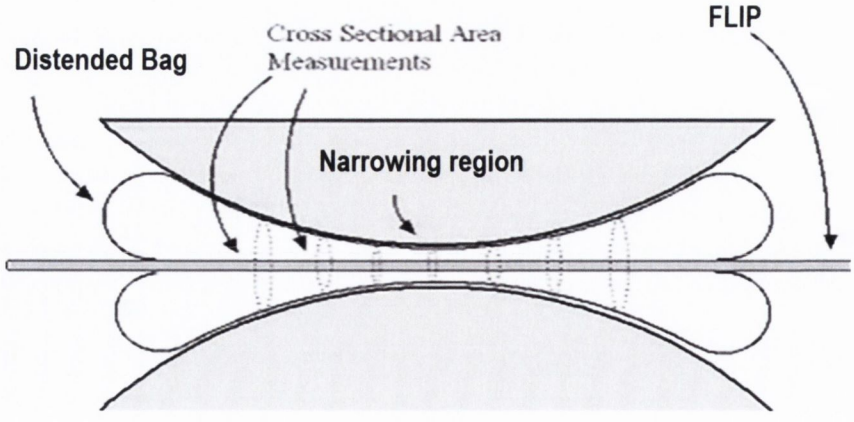

Figure 1.15: The FLIP is using impedance planimetry technology to measure 8 CSAs along the geometry of the narrowing region of a bodily sphincter (modified from scientific paper).

In 2004, a FLIP probe with three measuring electrodes was used to distend the OGJ for the first time (263). Limitations reported in this study included the inability of three CSA measurements to profile the OGJ. To address this deficiency a FLIP probe consists of 8-sensing electrode pairs was designed and constructed.

The custom made FLIP assembly is 72 cm long with a 1.6-mm outer diameter. A noncompliant polyesterurethane bag up to a fill-in volume of 60 mL mounted on the distal 12 cm of the probe was designed to assume a cylindrical shape 28mm long with maximal diameter of 3.2 cm, when fully distended, between the tapering cone-shaped ends sealed at the assembly. The minimal-to-maximal detectable CSA range was 38.5to 578 mm^2. Within the bag 8-sensing electrode pairs spaced 4 mm apart for impedance planimetry measurements. An impedance planimeter system (see figure 1.13) was used to generate an excitation constant current of 100μA at frequency of 5kH. When the bag is filled with a 0.225 g/l saline solution, a fixed current is then passed through the excitation electrodes with the change in voltage being simultaneously recorded for all the electrode pairs. The impedance depends on the measured voltage, which increased as the bag filled with saline. The channels in the probe can be perfused with saline and are used to make manometric pressure measurement using a low-compliance perfusion system connected to external pressure transducers housed within the recording unit that measured bag pressure. The volume of conductive solution injected from syringe to the bag is controlled via a syringe pump unit(264).

Measurements from the 8 sensing electrode pairs and pressure transducers are collected at a sampling rate of 10Hz to allow the readings taken from the FLIP probe to then be compared with a calibration curve in order to convert them to CSA measurements. Using the assumption that the sphincter has a circular CSA, each of the CSA measurements can then be used to obtain diameters at the location of each electrode pair using a custom made data acquisition software system programmed in Labview (National Instruments, Austin, TX) (265).

From the diameters and the knowledge gained of the electrode locations a video clip can be created which subsequently illustrate the dynamic movement of the OGJ walls. The data saved from the probe measurements can also be used to model flow through the OGJ junction by using computational flow dynamics (266).

The probe and the pressure transducers are pre-calibrated using polymethylmethacrylate (Perspex) cylindrical tubes with CSAs of 38.5, 73.8, 132.7, 201, 83.5, and 572 mm^2. During the calibration, the probe is placed in the largest cylinder, i.e 572mm^2, so that the bag is completely inside the tube and kept parallel to the cylinder wall. The bag is inflated with a 0.225 g/l saline solution until it fitted tightly to the inner walls of the tube. The Labview software was set in calibration mode. The

calibration voltage is automatically recorded for 10 s, and the average voltage recorded is converted to the CSA value of the respective tube in square millimetres. This procedure is repeated for each tube in descending order of size. Accordingly, a calibration curve which is stored is created for each CSA using a linear point curve-fit method. The calibration process is repeated before each study (265). Before use, the air is removed from the FLIP assembly by using an automated purge sequence.

1.11.4.1 FLIP for Evaluation of the Oesophago-Gastric Junction (OGJ)

OGJ is the sphincter that is located between the oesophagus and the stomach. The accepted understanding of OGJ is that it closes off to stop the refluxing of stomach contents into the oesophagus. This closing off must be related to a narrowing of the lumen which can be represented by CSAs measured in this region. FLIP technique can distinguish between toned and relaxed sphincter by placing a fixed rate volume infused bag in the junction and monitoring the pressure and CSA in the bag (237). Patient with GORD have a reduced sphincter tone typically exhibit increased sphincter distensibility; which means that the narrowest CSA within the lumen increases throughout the distension and bag pressure remains low. In contrast, patient with achalasia have increased sphincter tone and typically exhibit reduced sphincter distensibility(265).

1.11.5 EndoFLIP® system

The Endoscopic Functional Luminal Imaging Probe (EndoFLIP®) System (Crospon Ltd., Galway, Ireland.) is the commercially developed FLIP. The EndoFLIP® uses impedance planimetry to determine 16 intra-luminal adjacent CSAs within a cylindrical bag placed in a tubular organ during volumetric distension, whereas the pressure transducers provide the corresponding bag pressure.

The EndoFLIP® assembly is 240cm long with a 3mm outer diameter. A noncompliant bag (up to a fill-in volume of 40mL) mounted on the distal 14cm of the probe was designed to assume a cylindrical shape 7.0cm long with maximal diameter of 2.5cm, when fully distended, between the tapering cone-shaped ends sealed at the assembly. The detectable CSA range was 10 to 450 mm. the probe consist of 17 ring electrodes 4mm apart and housed by 6.4cm non-compliant bag. When a constant current induced in the two excitation electrodes, the voltage measured across each of the 16 adjacent pairs of electrodes was proportional to the impedance between them. The impedance across each segment was inversely proportional to the CSA of the bag. Two low-compliance perfusion channels were connected to an external pressure transducers housed within the recording unit that measured bag pressure of the probe (267).

Measurements from the 16 electrode pairs and pressure transducers were displayed with a sampling rate of 10Hz. Before the use of the probe, both probe and pressure transducers were pre-calibrated during the manufacturing process (CSA resolution 0.8 mm^2, accuracy ±0.8 mm^2; intra-bag pressure resolution 0.1 mm Hg, accuracy ±0.8 mm Hg) (264).

An automated purge was performed to remove the air from the EndoFLIP® assembly. the infusion of the conductive saline and the pressure baseline were set up using the touch screen on the EndoFLIP® recording unit (267). The cross sectional area (CSA) measurements from the EndoFLIP® system correlate well with manometric pressure in OGJ. (268).

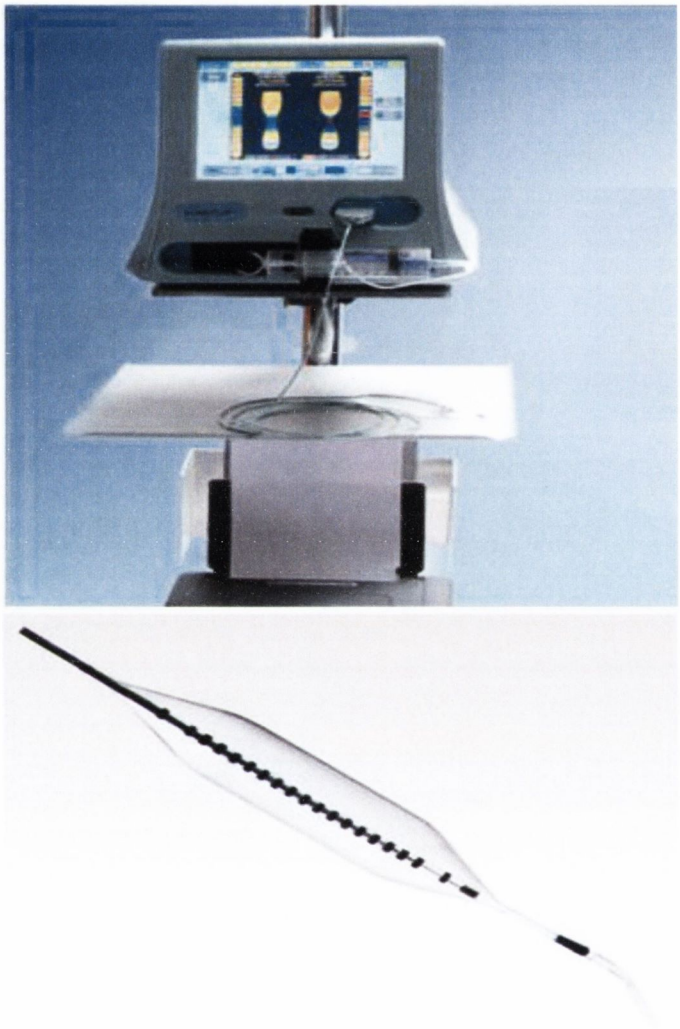

Figure1.16: Once the bag is filled with a conductive solution, 16 intra-luminal cross-sectional areas (CSA) are measured within the central part of the bag by the impedance measuring electrodes, whereas the pressure transducers provide the corresponding intra-bag pressure. The volume of conductive solution injected from the syringe to the bag is controlled via the touch screen on the recording unit. The screen displays the calculated CSAs as a cylinder of varying diameter in real time along with the corresponding bag pressure. (*Photograph courtesy of Crospon Ltd., Galway, Ireland.*)

1.11.5.1 Clinical Utility of EndoFLIP®

The EndoFLIP® technique can provide clinically useful information regarding the profile of the OGJ in healthy volunteers and in patients with GORD and achalasia. It has also been utilised to determine the success of fundoplication surgery in the treatment of GORD (269, 270). Recently, EndoFLIP® has been used intra-operatively by surgeons to monitor the effectiveness of a OGJ myotomy and fundoplication surgeries (271). Three dimensional reconstructions of the OGJ obtained before and after these procedures visualise geometric changes in the sphincter and establish change in

compliance post-surgery. EndoFLIP® has since been used to measure distensibility in other anatomical lumens including the upper oesophagus in patients with eosinophilic oesophagitis, the upper oesophageal sphincter, the sphincter of Oddi and gastric bands in bariatric surgery (272-274). Recently, EndoFLIP® has been used to distend the anal canal in health and FI group. it was suggested that the technique of distending the anal sphincter to measure its ability to resist distension can provide a useful method to study the components of anal sphincters in normal subjects and in patients with FI where the anal sphincters structure and motility are affected .The middle part of the anal canal was found to be the least distensible in health (257, 258).

1.12 Conclusion

Faecal incontinence (FI) is the inability of the anorectal region to control defecation. It is a common condition with a prevalence ranging from 2.2%-15% in the Western World and with the prevalence increasing with age (275). Knowledge of the interaction of muscles, in particular the internal (IAS) and external anal sphincters (EAS) and the puborectalis muscles are essential for diagnosis and treatment of FI (276). Although many tests such as anorectal manometry and endo-anal ultrasound are now established to measure the continence mechanism in clinical practice, the clinical value of most tests is controversial. In addition the lack of a standardised normal material makes interpretation difficult. Other tests currently remain as research techniques limited to a few specialist centres. Such tests include vector-volume manometry, prolonged rectosigmoid manometry and magnet resonance imaging. Consequently only a proportion of the factors contributing to the preservation of the continence are routinely assessed, despite the high prevalence of faecal incontinence (277).

The functional lumen imaging probe (FLIP) is a new technique was developed by for studying the luminal geometry of the oesophagogastric junction (OGJ) in health and disease (278-280). FLIP provides dynamic functional imaging based on impedance planimetric measurement of up to 16 serial cross-section areas (CSA) inside the distending bag (281, 282). CSA-pressure curves were superior to manometry for determining OGJ characteristics and provide much better information on its action (283, 284) . FLIP may potentially be used to study other sphincters and narrowing zones in the human body. Studies are already ongoing in relation to real-time measurements during endoscopic and surgical procedures with the purpose of providing information about the size during procedures such as gastric banding.

As a result of the work that has been carried out previously the studies in this work sets out to adapt the functional lumen imaging probe in order to measure the biomechanical properties and morphological changes of the ano-rectal region during distension and provocative manoeuvres. This study will use the components of incontinence by means of distension technique as a method in the biomechanical evaluation and diagnosis of ano-rectal dysfunction. This is may be expedient since

movement of stools within the anal canal during defecation and faecal incontinence is dynamic process. Furthermore, identifying the biomechanical properties of the muscle structure at different locations in the anal canal would benefit healthcare setting involved.

Chapter Two: Developing a Functional Lumen Imaging Probe (FLIP) For Evaluation of the Anal Sphincters

2.1 Introduction

The FLIP technique can be used to study sphincters and narrowing zones in the human body (see section 1.11.4.). One application that would have huge interest clinically is that of the anal sphincters due to the high prevalence of FI and other ano-rectal diseases (see section 1.8.1). This chapter is elaborate on the promising effort of the researcher to adapt the FLIP, with the aim of measuring distensibility of the anal sphincters as well as the morphological changing during different manoeuvres.

Subsequently, three experiments were conducted with the FLIP prototype 1, FLIP prototype 2 and FLIP prototype 3 respectively. In the first experiment, FLIP prototype 1 was a custom made probe which consists of 8 electrodes 5mm apart. The impedance planimeter was the only available tool for data acquisition and data analysis. During the second and the third experiments with prototype 2&3, EndoFLIP® recording unit was launched. FLIP prototype 2 consisted of 16 electrodes, 5mm apart, with distal end attached to a balloon to distend the rectum. FLIP prototype 3 is the EndoFLIP® probe that was designed for OGJ evaluations.

In this chapter, the first research question within this thesis will be addressed. Namely, the FLIP design for safe and accurate anal sphincter evaluation is investigated. Methodological and conceptual issues are inherent in anal sphincters evaluation studies due to the unique anatomical shape and physiological action of this area.

2.2 Experiment 1: Designing a FLIP probe for evaluating the anal sphincters (FLIP prototype 1): in vitro & in vivo pilot study

2.2.1 The objectives

The objectives for this study were to: (i) develop a functional lumen imaging probe (FLIP) capable of measuring anal sphincter function; (ii) design a FLIP bag; (iii) calibrate and verify the deigned probe, in a bench-top experiment; and to (iv) ensure that the FLIP could be positioned and safely distended in the anal canal.

2.2.2 Materials and methods

2.2.2.1 FLIP prototype1 Design

For this experiment, an entirely custom made FLIP assembly with a 1.6mm outer diameter was built. The probe was made of a 3-lumen polyvinyl catheter of 12.5cm length, with 1 lumen each for electrode wires, saline infusion and pressure measurement.

A non-compliant bag was sealed using a heating sealing machine and then was mounted onto the distal end of the catheter. The bag was made from polyesterurethane and was affixed at the both ends of the probe using a medical thread. High viscosity medical glue was applied at the both ends of the bag to prevent any leakage. The maximum volume of the bag was 60ml.

Within the bag, 8 sensing electrode pairs were spaced 5mm apart for impedance planimetry measurements. Two excitation electrodes were 1.5cm apart from the first sensing electrode pairs at each side. (See figure 2.1). Thin elastic copper (PN:S40AOIT5-1.5) ring electrode pairs were fixed around the probe with an inter-electrodes distance of 1mm. The FLIP bag was filled with conductive saline from the infusion channel through five infusion holes spread across the length of catheter where the bag was placed.

Inside the bag a constant current across the sensing electrodes and the voltage measurement at each electrode was proportional to the impedance between the sensing electrodes, which decreased as more saline was inflated into the bag.

A pressure cuff to pressurize the saline solution was perfused to measure the manometric pressure through a channel in the probe using a low compliance perfusion system connected to an external transducer (Edwards, TruWave, Edwards Lifesciences, Irvine, CA, USA)

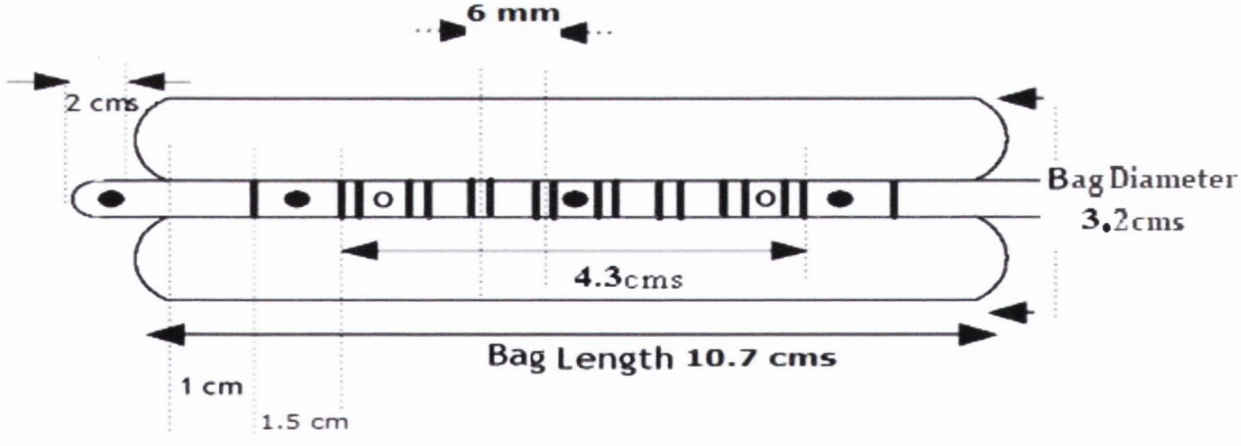

Figure 2.1: FLIP (prototype 1) consists of 8 electrodes. The distance between the middle of adjacent double electrodes is 6mm. Hence, the length of measurements equal to the distance from the first electrode till the eighth electrode (5*7+1*8=43mm). The maximum measured diameter is equal to the maximum diameter of the non-compliant bag which is 30mm).

The bag was made of 35μm thick polyesterurethane, a material that is impermeable to the current and to body fluids. This bag was 11cm long and 30mm of maximum deflated diameter. See figure 2.2.

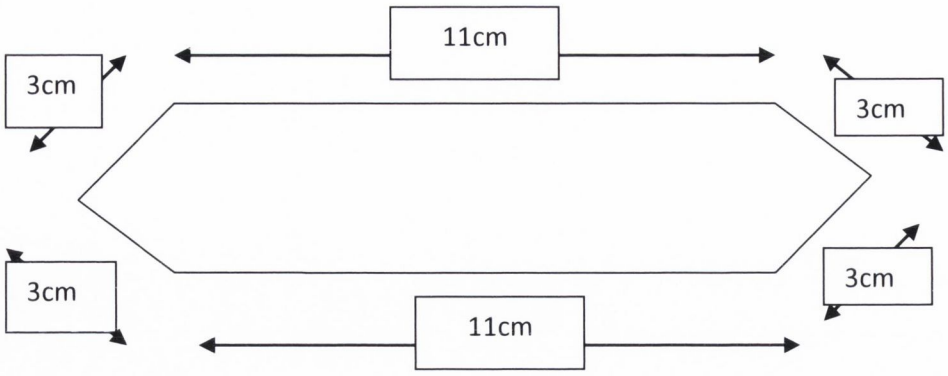

Figure 2.2: The FLIP bag dimensions.

2.2.2.2 Equipment

An impedance planimeter system (see figure 1.12) was used to generate an excitation constant current of 100μA at frequency of 5kH. Data acquisition software system programmed in Labview (National Instruments, Austin, TX) was used to analyse the pressure and cross sectional areas inside the bag. A 100ml syringe driving pump operated at a fixed flow rate of 40ml min^{-1} and was used to inflate and deflate the balloon during the procedure (see sections 1.11.3.3).

2.2.2.3 Calibration of the FLIP

The calibration technique was described in previous studies (263, 264). Briefly, the probe bag was mounted around the FLIP as represented in figure 2.1. The bag was filled with a 0.225 gl^{-1} saline solution. The bag was inflated and deflated with 20ml of saline solution until all the air was removed from the bag. A set of polymethylmethacrylate (Perspex®) cylindrical tubes with cross sectional areas of 45.3mm^2, 76.9mm^2, 111mm^2, 196mm^2, 369mm^2, 607mm^2 were used to calibrate the FLIP. The saline solution was withdrawn through a closed line using a 60ml syringe. The bag was fully deflated and inserted into each calibration tube so that the eight sensing electrode pairs were placed at the centre of the cylinder. The software was set in calibration mode and the probe bag was inflated in each cylinder starting with the largest. The filled amount was secured in the bag using a lock valve in the saline line to prevent any possible air entering the bag or the saline leaking out. (See section 1.11.4). At this point the calibrate button was pressed and the average voltage for 10 seconds (i.e. 100 samples) was recorded and set in a look up table to the value of the CSA of the appropriate calibration tube. This procedure was repeated for each cylinder in descending order. A calibration curve for each CSA of 45.3mm^2, 76.9mm^2, 111mm^2, 196mm^2, 369mm^2, 607mm^2 was generated using a linear point curve fit method.

The pressure transducer used for the perfused system located in the probe bag was calibrated at two points, 0 and 100cmH_2O and then the FLIP bag was emptied of any visible air bubbles. The two point pressure measurements did not feed into the calibration of CSA.

2.2.2.4 Subjects

For this study, two female volunteers (50 and 63 years old) were recruited. Both had a history of faecal incontinence; the first subject had faecal incontinence and the second subject had combined (faecal and urine) incontinence. The study medical history: symptoms (constipation, urinary or faecal incontinence), allergies, past treatments (anal surgery) were obtained and written consent was signed. The protocol was approved by the SJH-AMNCH Joint Research Ethics Committee (see appendix 1).

2.2.2.5 Study protocol

No special bowel preparation or sedation was given to the patients. The procedure was explained to the patients in order to increase their cooperation and comfort level. A digital examination before investigation ensured that the rectum was empty. To insert the FLIP in the anal canal, each patient was positioned on their left side with hips and knee flexed. The bag was then deflated and lubricated. In each patient, the probe was placed so that one sensing electrode pair was seen outside the anal verge.

The patients were allowed 5-10 minutes to become accustomed to the probe before starting the procedure. The FLIP bag was used to distend the anal canal; this bag was filled with a prepared 0.225 gl^{-1} saline solution.

Two preconditioning ramp distensions at fixed flow rate of 40ml/min to a volume of 50ml were performed at the start of the study. The FLIP bag was then filled with 30ml of saline solution and the patient was asked to squeeze as hard as possible for 10-20 seconds. After a relaxation period of 30seconds, the procedure was repeated with 40ml and 50ml step volumes inside the FLIP bag. Finally the FLIP bag was deflated and removed gently, see figure 2.3.

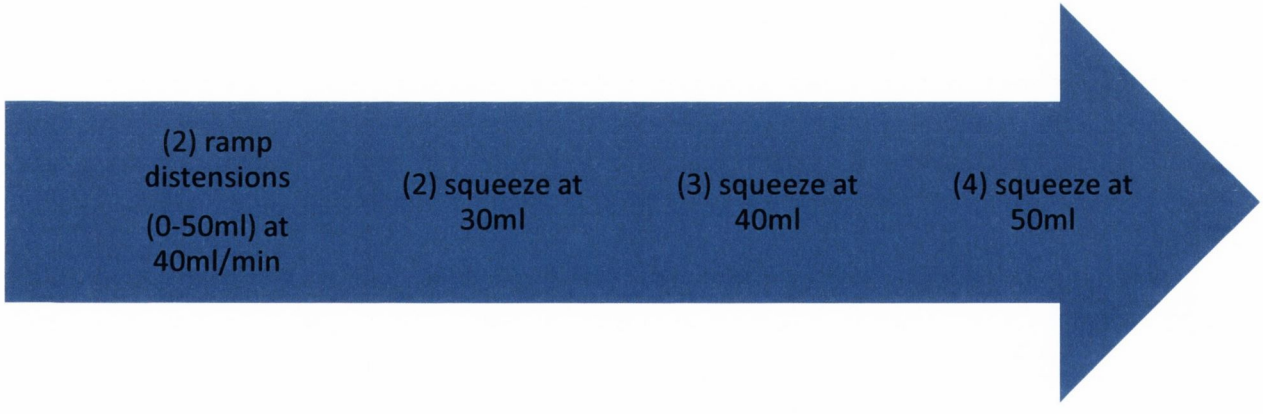

Figure 2.3: study protocol

2. 2.2.6 Data recording

A low compliance perfused system was used to measure the manometric pressure in the bag through a channel in the catheter connected to external transducers (Edwards TruWave, Edwards Lifesciences, Irvine, CA, USA). The probe and pressure transducers were connected to a physiological

measurement system capable of plotting CSAs and pressures in real time, and all data was stored on a computer for off-line assessment and analysis.

2.2.3 Results

Figure 2.4 shows the custom made probe (FLIP prototype 1). This probe consisted of 8 sensing electrode pairs and was designed to assume a cylindrical shape 43mm long with maximal diameter of 30mm when fully distended.

Figure 2.4: FLIP probe (prototype 1) built to be positioned safely in the anal canal cavity. The probe was constructed of a three lumen polyethylene catheter with an outer diameter of 1.6mm.

The voltage measured during calibration at each electrode was recorded (see table 2.1). The average FLIP calibration curve of eight sensing electrode pairs was an exponential curve showing the CSAs represented by the voltage. The variations of the CSA values measured at high CSAs (196, 369 and 607 mm^2) were low and they became less accurate at lower CSAs measured (45, 80 and 111 mm^2). See figure 2.5.

Table 2.1: The voltage measured at each electrode (E) during the calibration of FLIP prototype 1

CSA (mm^2)	(E1) Voltage (μV)	(E2) Voltage (μV)	(E3) Voltage (μV)	(E4) Voltage (μV)	(E5) Voltage (μV)	(E6) Voltage (μV)	(E7) Voltage (μV)	(E8) Voltage (μV)
607	0.333	0.359	0.257	0.335	0.317	0.403	0.308	0.388
369	0.572	0.58	0.352	0.598	0.507	0.627	0.501	0.599
196	1.119	1.121	0.715	1.032	1.187	1.333	1.084	1.265
111	2.543	2.258	1.244	2.239	2.094	2.388	2.034	2.166
76.9	3.562	3.122	2.015	3.078	2.89	3.011	2.612	2.863
45.3	5.573	4.125	2.953	4.262	4.173	4.798	3.752	4.036

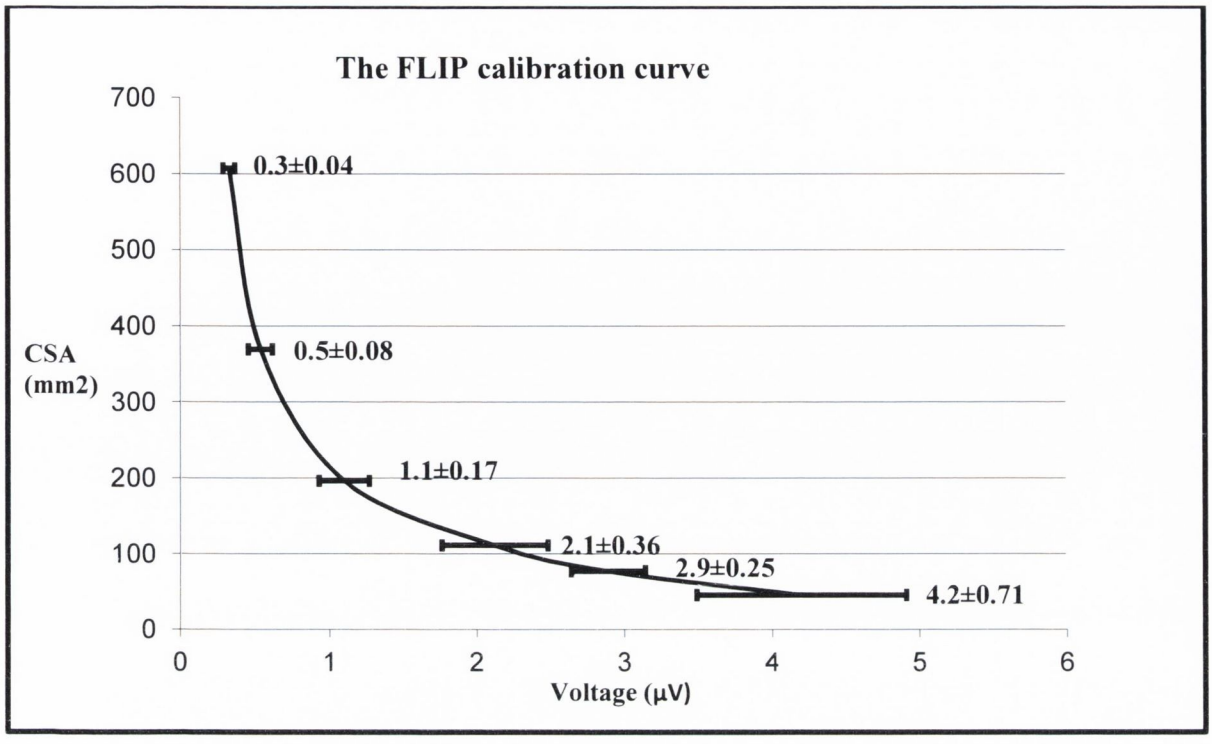

Figure 2.5: Calibration curve showing the CSAs represented by voltages at eight sensing electrode pairs in the FLIP. The horizontal lines represent the variation of the voltage measured over calibration cylinders.

The procedure was well tolerated by both patients and no adverse incidents occurred. There were no complications during the insertion of the FLIP inside the anal canal. See figure 2.6. The bag was moving toward outside anus during the ramp distensions as well as during squeezing manoeuvres.

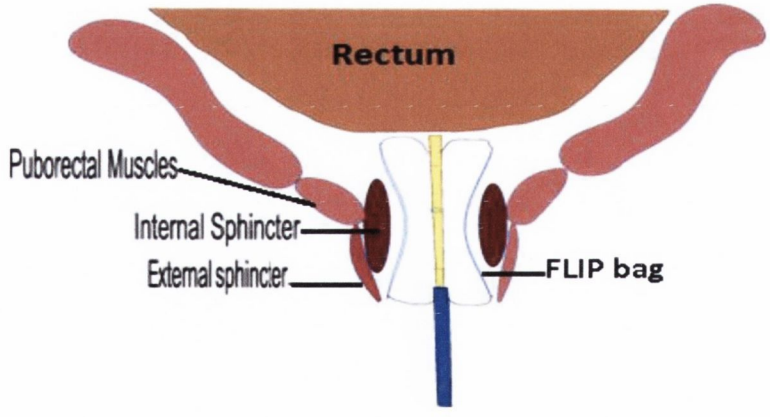

Figure 2.6: a schematic draw of the FLIP positioned in the anal canal

The bag was distended with 20 ml 0.225 gl^{-1} saline solutions and slowly retracted until one sensing electrode pair was visualised outside the anal verge. The bag was deflated and then distended to 10ml, 20ml, 30ml, 40ml and 50ml volumes (ramp distension) within the anal canal (see figure 2.7). The pressure sensor in pilot 2 was not working (see figure 2.7 (B)). In pilot 1, the bag pressure was increasing gradually during the ramp distension. The bag pressure increased from 17mmHg at 10ml

distended volume to 74mmHg at 50ml distended volume. The minimum measured diameter along the anal sphincter increased from 9mm at low volumes to 18mm at the maximum distended volume.

Following the ramp distension, the bag was distended to 30ml, 40ml and 50ml in step distension; at each volume the volunteers were asked to perform manoeuvres (rest and squeeze) (see figure 2.8). However, the hourglass shape of the anal sphincters region was not very distinct during these manoeuvres.

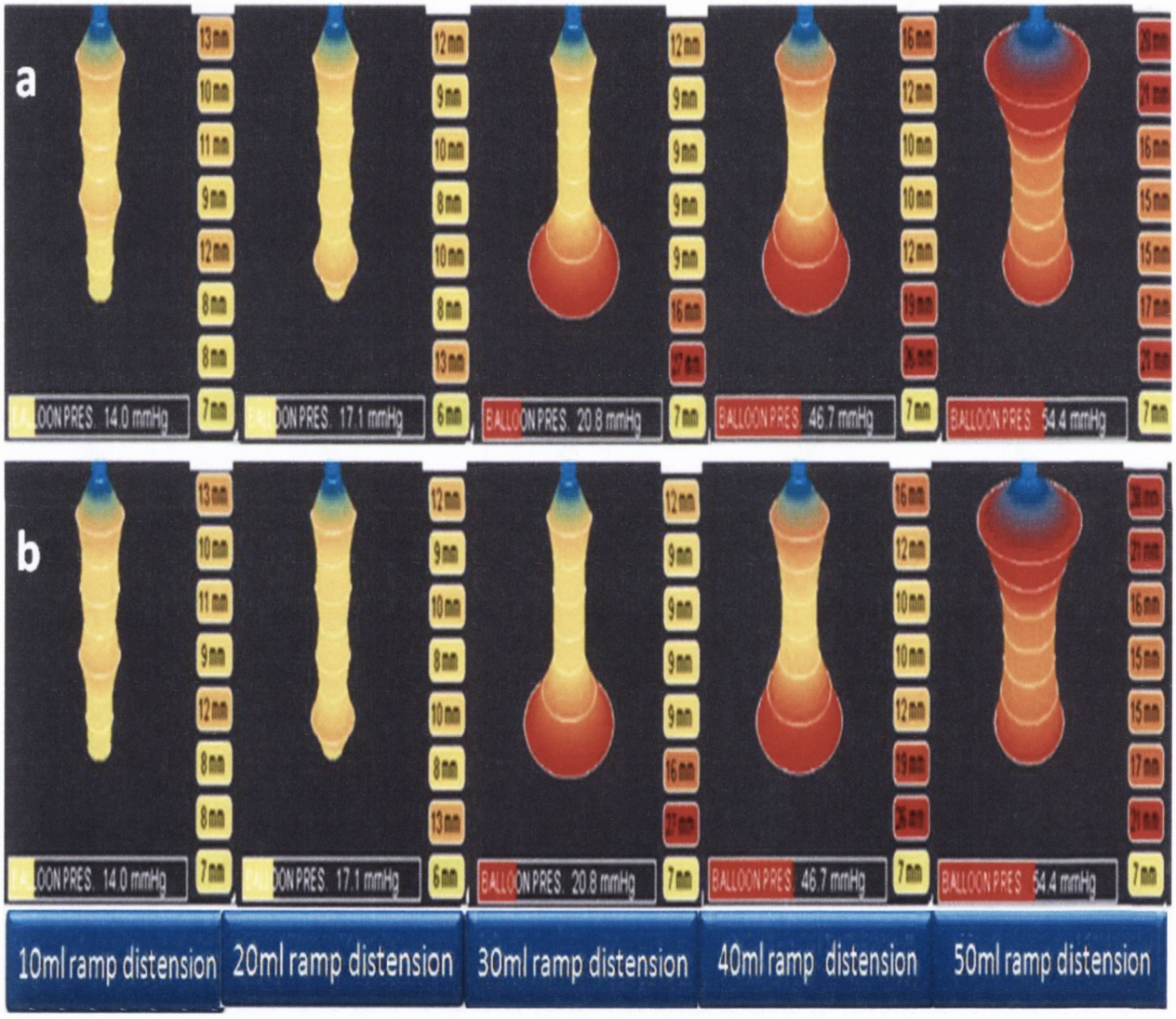

Figure 2.7: Results of the 50ml bag distension from the 8-electrode FLIP. (A) Pilot one and (B) pilot two. Top of each image is towards the rectum and the bottom is more external. Volume increased in steps of 10ml until a volume of 50ml. Diameters are given in the 8 boxes on the right hand side of the FLIP diagrams and bag pressures in mmHg and volume in millilitre are given at the bottom of each diagram.

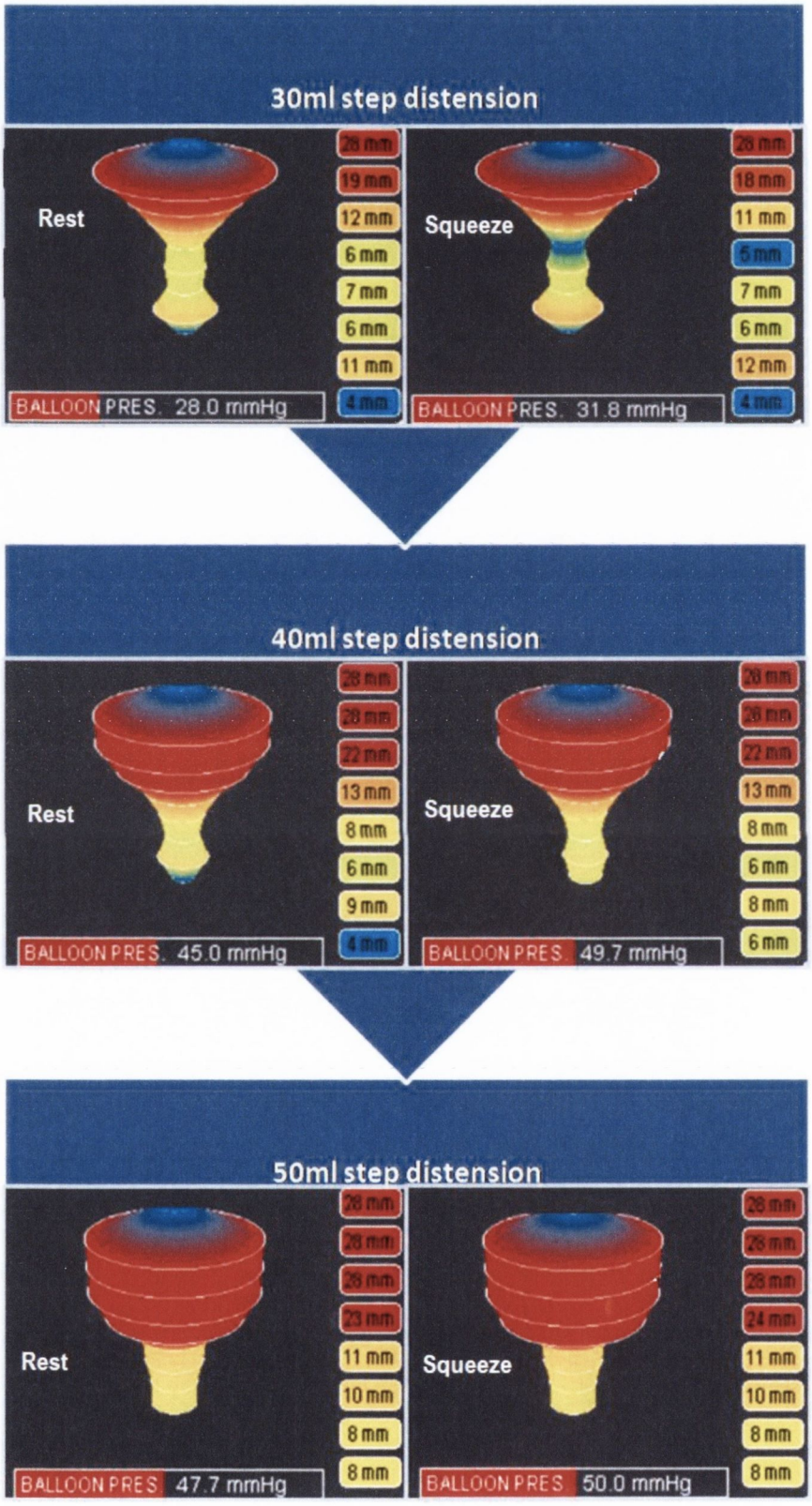

Figure 2.8: Result from the squeezing manoeuvre at 30ml, 40ml and 50ml bag volume in the anal canal. Top of each image is towards the rectum and the bottom is more external. Diameters are given in the 8 boxes on the right hand side of the FLIP diagrams and bag pressures in mmHg are given at the bottom of each diagram.

2.2.4 Discussion

While the original FLIP assume 32mm long cylindrical shape (see section 1.10.4), both anatomical and physiological tests demonstrate the length of anal high pressure zone to be at least 40mm (see section 1.6.1). Experiment 1 was carried out to design a custom made 8 electrodes FLIP probe (prototype 1) which can assume 43mm long cylindrical shape. This was achieved by increasing the spacing between the sensing electrodes from 4mm as in original FLIP design to 5mm.

The calibration was carried out to overcome the variation in the distance between the electrodes as a result of building them manually so that any measuring error from the physical placement of the bag in the cylinder will be stored in the calibration data (265). McMahon et al carried out testing on measure versus set values and demonstrated that there was a very high correlation between them (265).

Two errors were expected on the measurement of CSA. This could be due to the calibration technique or to the manual construction of the probe. Calibration is based on an average value for a 10 s sample (n = 100) from only one distension of the bag in each calibration tube. Consequently, any measurement error from the physical placement of the bag in the calibration cylinders was stored in the calibration data. Because of the manual construction of the probe, the most distal electrodes may receive less saline solution than the more proximal electrodes when the solution infused inside the bag. This may affect the conductivity between the excitation electrodes which are located at the extreme ends of the electrode array (265).

A short recruitment effort indicated that it will be very difficult to recruit healthy volunteers because culturally there is a social stigma attached to studies involving the anus and rectum area. Accordingly two FI patients were recruited for this study.

The FLIP probe (prototype 1) probe was positioned in the anal canal in two patients so that one sensing electrode pair was seen outside the anal verge. There were no complications during the insertion of the FLIP inside the anal canal. The bag could be distended to the required volumes safely without causing any injuries or bleeding. It was concluded that the length may not have been adequate to capture the entire anal canal profile. Therefore, no specific information about the anal canal can be identified using the FLIP probe with eight electrodes. Of note, the bag did not consistently remain in position in the anal canal throughout the study protocol.

Theoretically pressure control study would make more sense. However, in a region of the body which changes rapidly and dynamically, volume changes in the bag would need to occur very fast if pressure is to be regulated instantaneously. In a system where a catheter with a very narrow diameter fill and

empty channel is connected to a relatively large bag volume it would not be possible to accurately measure CSAs and volume which is representative of the contours of the anal canal.

Based on findings from this initial pilot study using FLIP prototype 1, it was proposed that: (i) a FLIP with an increased number of sensing electrode pairs would give a better axial resolution and help with probe movement during the test. (ii) A stretchable balloon at the tip of the FLIP probe positioned in the rectum could be used to study the effect of the rectal distension on the distensibility and the morphology of the anal canal.

2.3 Experiment 2: Designing a FLIP probe for evaluating the anal sphincters (FLIP prototype 2): in vitro & in vivo pilot study

2.3.1 The objectives

The main objectives for this experiment were to: (i) improve the design of the functional lumen imaging probe (FLIP) based on the experiment with (Prototype 1) to be able to measure the entire anal canal profile; (ii) build and test the new probe design, in a bench top experiment; (iii) describe a method for assessing anal sphincters characteristics using distensibility testing; and to (vi) obtain an objective functional real time image of the anal canal during resting, squeezing, coughing, straining, recto-anal inhibitory reflex (RAIR) and rectal sensation tests.

2.3.2 Material and methods

2.3.2.1 FLIP (prototype2) design

For this experiment, an entirely FLIP (prototype 2) was constructed from tin coated copper electrodes mounted on a 4.5mm catheter towards the distal end of the probe. It was designed to measure 16 cross sectional areas, 5mm apart together with pressure inside a saline-filled bag. The FLIP bag was 12.5cm long and was made of 35μm thick polyesterurethane. A stretchable rectum balloon was mounted to the distal end and was used to distend the rectum. The FLIP bag was separated by 5mm from the rectum balloon. The sensing electrode pairs were spaced 5mm apart and the two excitation electrodes were 14mm apart from the first sensing electrode pairs at each side. Inside the bag a constant current across the excitation electrodes caused the impedance to change at each sensing electrode pair and this was proportional to the cross sectional area (CSA) measurements. The bag was filled with a 0.225 g l^{-1} saline solution; the saline solution passes from the catheter through small holes to the lumen of the FLIP bag. The length of the anal canal influences the cross sectional area measurements i.e. this probe is capable of measuring the elongation and shortening of the anal canal during different manoeuvres. The manometric pressure was measured inside the rectum balloon as well as inside the FLIP bag, see figure 2.9.

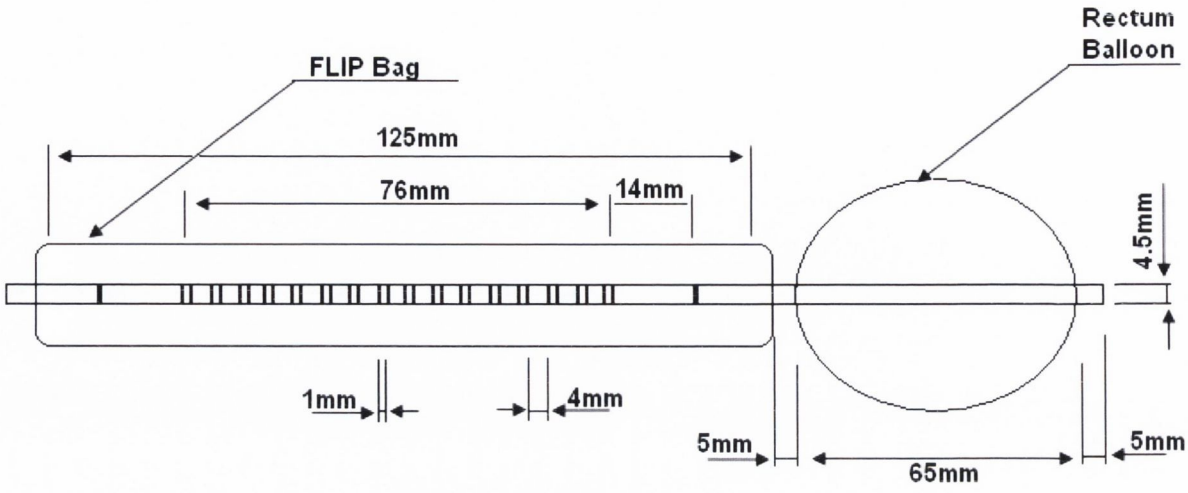

Figure 2.9: Schematic draws of the designed FLIP which was used in experiment 2. It consisted of; a stretchable balloon (rectum balloon) was used to distend the rectum during the recto-anal inhibitory reflex (RAIR) and the rectal sensation tests. The second bag is a non-compliant bag located in the anal canal contains 16 sensing electrode pairs capable of measuring the geometry of the anal canal at different manoeuvres tests. The pressures inside the Flip bag and the rectum balloon were measured.

2.3.2.2 Accuracy test of the FLIP

The accuracy of the FLIP with 16 sensing electrode pairs was measured without a bag. The FLIP was inserted into 7 calibration tubes, each filled with $0.225gl^{-1}$ saline solution with internal diameters of 7, 10, 12, 18, 22, 28 and 35mm. The probe was kept parallel to the cylinder wall and was immersed deep enough so that all of the electrodes — including the most proximal excitation electrode — were in the saline solution.

2.3.2.3 Equipment

A low compliance perfusion system was used to measure the pressures in the rectum balloon and the anal FLIP bag through two separate channels in the catheter connected to external pressure transducers (TruWave, Edwards Lifesciences, CA). The pressure transducers were connected to a measurement system GMC-IP1000 impedance planimeter (Ditens A/S, Aalborg, Denmark) capable of recording pressures in real time and storing data on a computer for off-line assessment and analysis. The pressure transducer was calibrated at two points, 0 and 100 cmH_2O. The custom made FLIP (prototype 2) was connected to a physical measuring system (EndoFLIP® system, Crospon Ltd., Galway, Ireland). EndoFLIP® is Conformité Européenne (CE) marked under the European Device Directive and has been approved for inflation in the oesophagus. Use of custom made probe was covered by an independent indemnity taken out with the relevant insurance company after a risk assessment was carried out by qualified medical device staff.. A 100ml syringe driving pump operated at a fixed flow rate of 40ml min^{-1} was used to inflate and deflate the FLIP bag during the procedure (see figure 2.10).

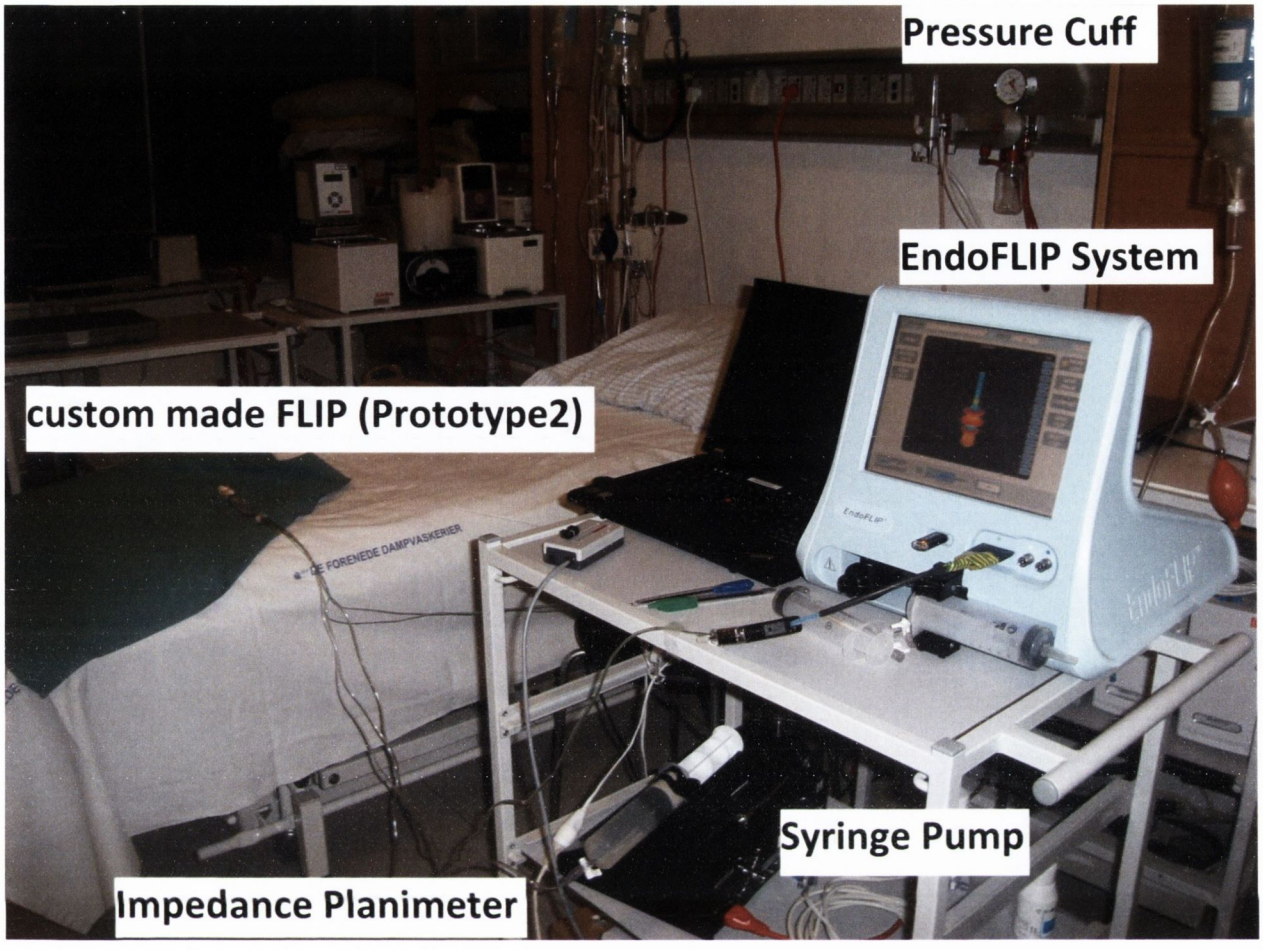

Figure 2.10: This image represents the equipment set up for experiment-2. It consist of an EndoFLIP® system comprising recording unit, GMC-IP1000 impedance planimeter , syringe pump , pressurised cuff and the custom made FLIP (prototype 2).

2.3.2.4 Subjects

Two female and two male volunteers, mean age 24.5 (20-29) years were studied. They were all healthy without a history of gastroenterology disorders. Informed consent was obtained from all. Studies were carried out in Aalborg Hospital in Denmark with ethics approval obtained (See appendix 1).

2.3.2.5 Study protocol

No special preparation of the bowel was needed and no sedation was given to the volunteers. A digital examination before investigation ensured that the rectum was empty. To insert the FLIP in the anal canal each volunteer was positioned on his/her left side, with hips and knee flexed. Both rectum balloon and FLIP bag were deflated and lubricated. In each volunteer, the probe was placed anally so that 4 CSAs measurements could be observed to be outside the anal verge. See figure 2.11.

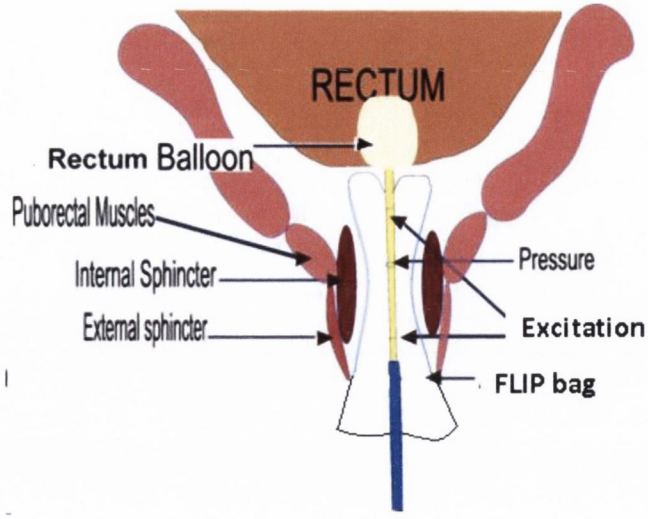

Figure 2.11: A schematic draw of the FLIP prototype 2 in position

The subjects were allowed 5-10 minutes to become accustomed to the probe before starting the procedure. During the first phase of the study protocol, the rectum balloon was completely deflated and the FLIP bag was inflated as the following:

- At least two preconditioning distensions (0-50ml of saline) were performed by infusion the FLIP bag at rate of 40ml min^{-1} .
- Then the FLIP bag was filled with 30ml step volume of saline and the volunteer was asked to complete a series of manoeuvres (rest, squeeze, cough, straining). After relaxing for 30 sec, the volunteer repeated the procedure with 40ml in the bag with 1min rest between each manoeuvre.

During the second phase of the study protocol, both rectum balloon and FLIP bag were inflated as the following:

- To measure RAIR, the rectum-balloon was inflated with 30, 40, 50 and 60ml of air at volume of 30ml and 40ml step distension of saline in the FLIP bag.
- To measure rectal volume sensory threshold, the rectum balloon was filled with air in slow increments till the maximum tolerable volume (MTV) was determined. The FLIP bag was distended with 30ml step volume during this test.

At the end of the procedure, rectum-balloon and FLIP bag were both deflated and removed gently (See appendices 2&3).

2.3.2.6 Sensory assessment

As safety precautions, and to control the study, the sensory intensity during bag distension was assessed using an electronic visual analogue scale (VAS) (GMC-IP1000 impedance planimeter) during the experiments. (0 = no sensation. 5 = pain threshold, 10 = unbearable pain). The volunteers were instructed on how to use the scale during the preconditioning distensions.

2.3.2.7 Data analysis

EndoFLIP® provides sixteen measures of CSA (mm^2) at a frequency of 10Hz during volume distensions. The Impedance planimeter provides a measure of FLIP bag pressure and rectum balloon pressure at the same frequency. The data from both devices was analysed using Open-lab programme. The starting time as appeared on the EndoFLIP® screen and Open-lab was recorded at the start of the study at the same time. This time was used to correlate the CSA (mm^2) and pressure (mmHg) measurements throughout the collected data.

Minimum CSA (mm^2) and pressure (mmHg) at 10ml, 20ml, 30ml, 40ml and 50ml distended volumes were calculated during the ramp distension test. The resting pressure (mmHg) and minimum CSA (mm^2) squeezing pressure (mmHg) and minimum CSA (mm^2), coughing pressure (mmHg) and minimum CSA (mm^2) and straining pressure (mmHg) and minimum CSA (mm^2) were calculated at 30ml and 40ml step distensions in manoeuvres test.

During recto-anal inhibitory (RAIR) tests, minimum CSA (mm^2) were measured at 30, 40, 50 and 60ml volumes of air filled in the rectum at two pressure points: (i) Maximum pressure increase (P MAXi); (ii) Minimum pressure decrease (P MINi). During rectal volume sensory threshold test, the pressure (mmHg) and the minimum CSA (mm^2) were measured at: (i) first sensation rectal volume, (ii) constant sensation rectal volume (urge), (iii) maximum tolerable sensation rectal volume (MTV).

2.3.3 Results

2.3.3.1 Probe construction

Figure 2.12 (A) represents the new design of the FLIP (prototype 2). It consists of: Rectum balloon; FLIP bag; 16 sensing electrode pairs; 2 excitation electrodes; air filling lumen; saline solution filling lumen; 2 pressure measurement holes to measure the balloon pressure in the rectum balloon and the bag pressure in the FLIP bag. The distance between the FLIP bag and the rectum balloon was enough to prevent any effect when both were inflated to their maximum capacity in future clinical tests, see figure 2.12 (B).

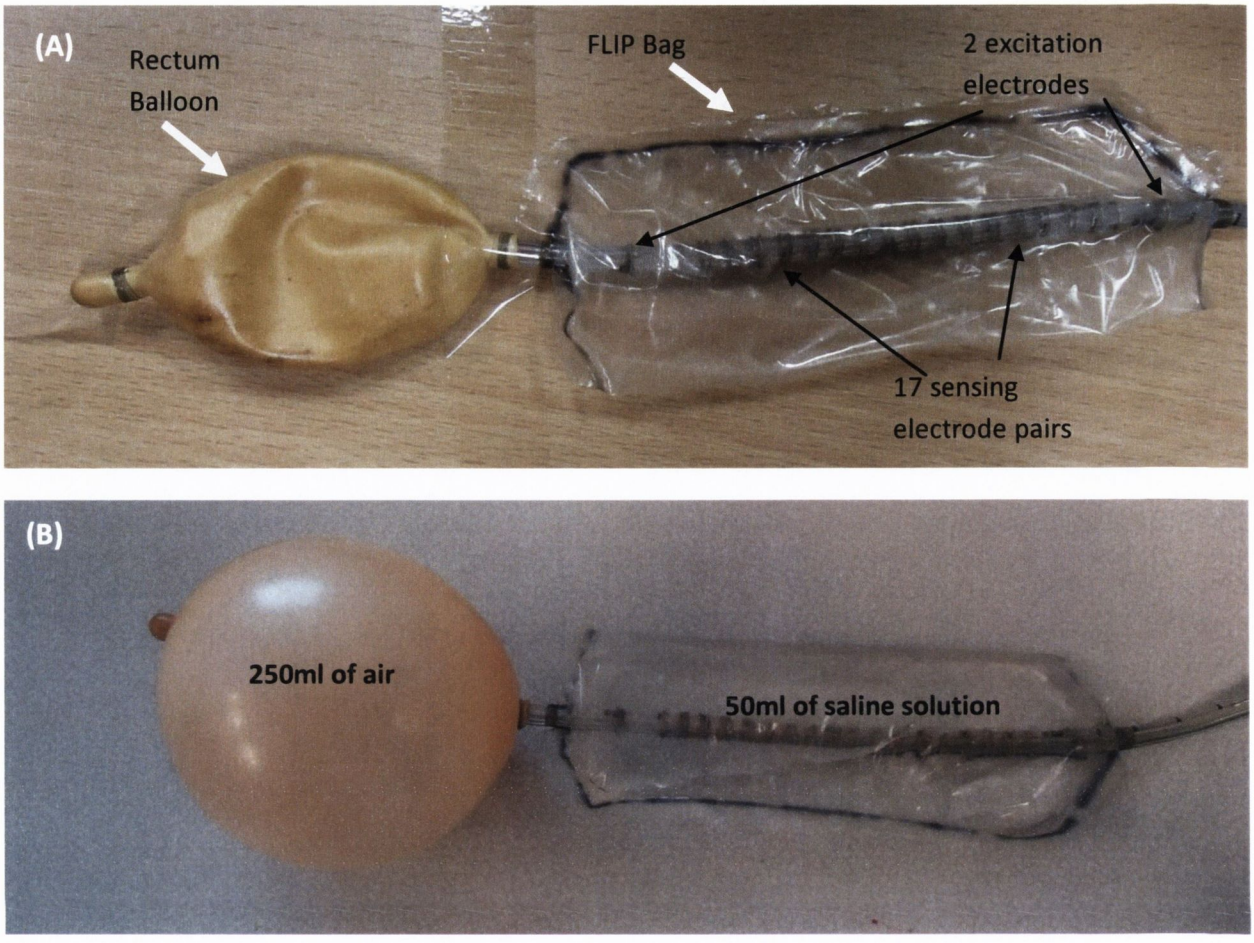

Figure 2.12: (A) FLIP (prototype 2) design for ano-rectal evaluation. (B)The rectal balloon was filled with 250ml of air and the FLIP bag was filled with 50ml of conductive saline

During the accuracy test, the FLIP recordings were found to give a 10% approximately lower reading using the standard system. Hence a correction factor of 1.1 times the displayed diameter was imposed.

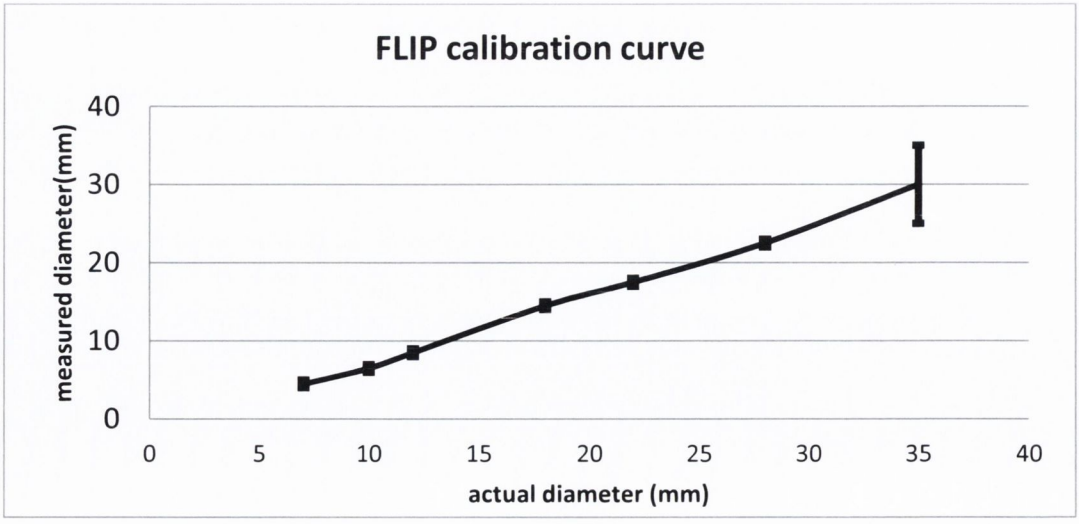

Figure 2.13: A graph represents the relation between the measured and actual measurements during accuracy test, the fitting equation was 1.1 * displayed diameter+2.5.

2.3.3.2 Ramp distension

During the anal ramp distension from 0-50ml, minimum CSA (mm^2) and bag pressure (mmHg) measurements were obtained at 10, 20, 30, 40 & 50ml bag volumes across subjects. See figure 2.15. Mean increases in FLIP bag pressure and CSA during ramp distensions are detailed in Table 2.2. Figure 2.14 represent the relationship between the pressure and volume of the FLIP bag during ramp distension.

Table 2.2: Change in anal canal Cross-Sectional Area and FLIP bag Pressure During 10ml,20ml, 30ml, 40ml and 50ml ramp distensions (N=4).

healthy	Mean of Min CSA (mm^2)±S.D. (mm^2)	Mean of bag pressure (mmHg))±S.D. (mm Hg)
10ml	27.1±6.2	9.5±2.5
20ml	26.9±5.8	16.0±4.6
30ml	28.1±5.7	21.4±4.8
40ml	28.4±5.3	25.5±7.6
50ml	32.1±6.2	49.2±7.31

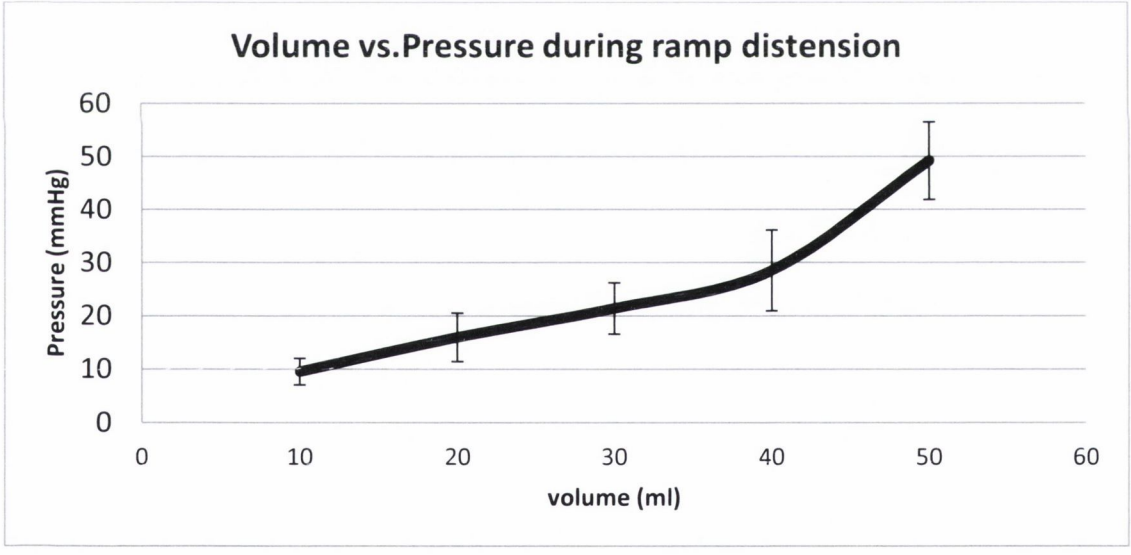

Figure 2.14: A graph represents the relation between the average pressure and volume of the FLIP bag during ramp distension. A dramatic increase in the average pressure occurred at 50ml volume.

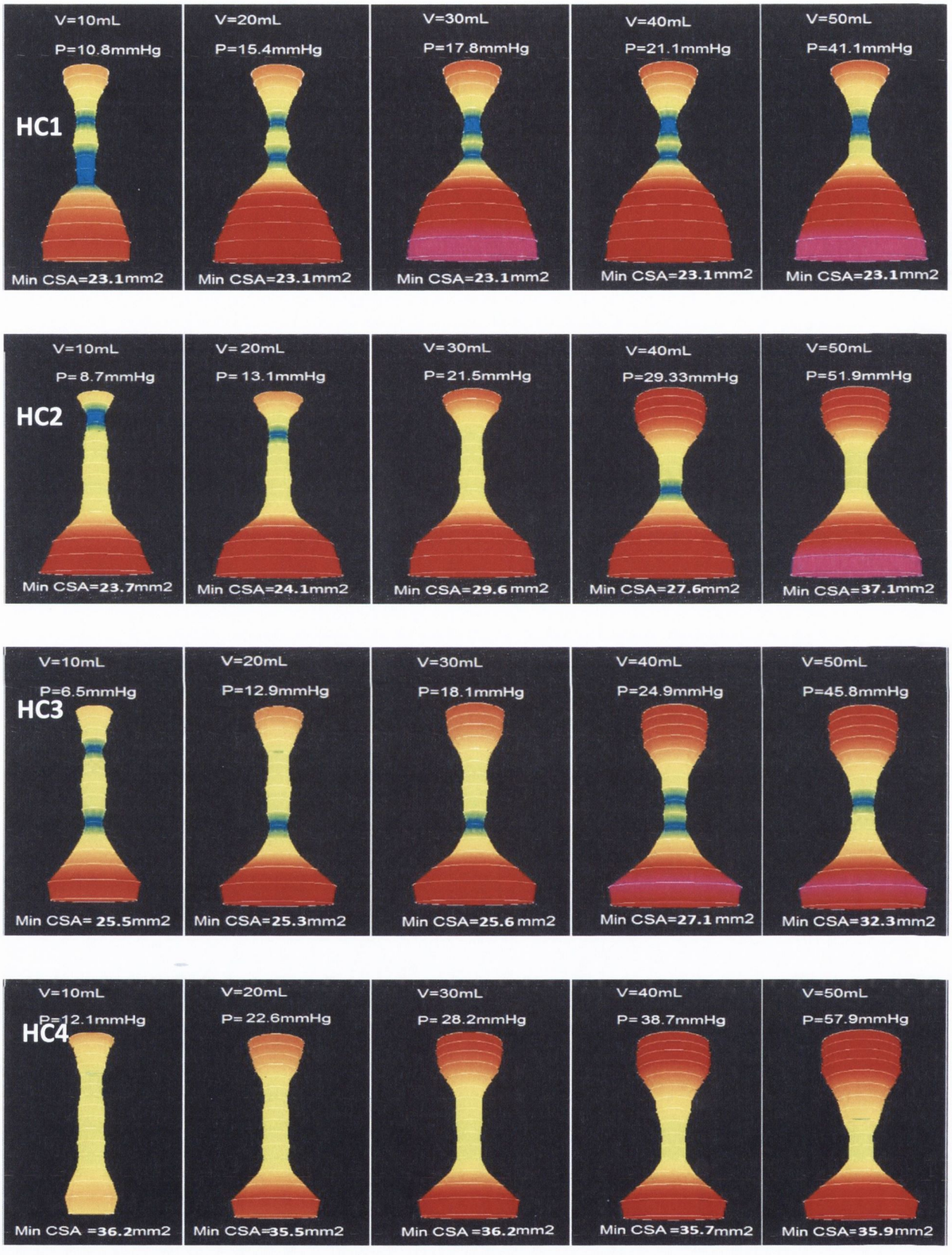

Figure 2.15: Geometric profile of the anal canal during ramp distension from four healthy volunteers. A narrow zone is formed along the anal canal during the ramp distension. A wide zone formed near top as more volume is distended in the bag. Top of each image is towards the rectum and the bottom is more external. The volume in each image represents the volume in the bag.

The anal distensibility as a function of FLIP bag pressure and the minimum CSA for the four healthy volunteers is shown in figure 2.16. During ramp distension, the minimum CSA was approximately constant related to the pressure increasing in the FLIP bag.

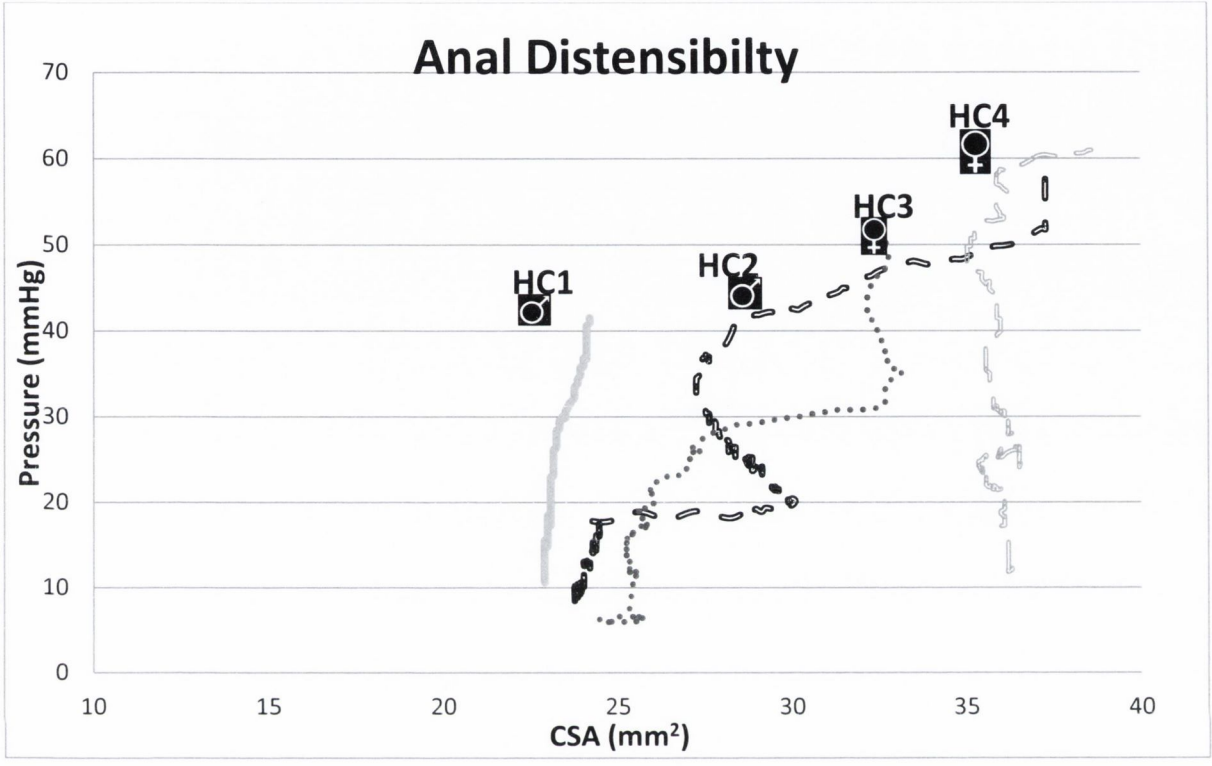

Figure 2.16: The pressure-CSA relation at the narrowest point in the anal canal for four healthy controls (HC).

2.3.3.3 Rest, squeeze, cough, strain

The changes in the mean minimum CSA (mm^2) and FLIP bag mean pressure (mmHg) of the anal canal during rest, squeeze (sqz), cough (cgh) and straining (str) at 30ml and 40ml step volumes are summarized in table 2.3.

No change was observed in the mean of minCSA during squeezing and coughing manoeuvres when compared to the mean of minCSA at rest. The mean of minCSA during straining increased by $5mm^2$ and $9mm^2$ at 30ml and 40ml respectively compared to the resting status. The mean pressure increased by different amount during squeezing, coughing and straining manoeuvres and was the highest during coughing manoeuvre at both volumes.

Table 2.3: Change in anal canal Cross-Sectional Area and FLIP bag Pressure during rest, squeeze, cough and strain manoeuvres at 30ml and 40ml step volumes (N=4).

FLIP bag volume	P rest ±S.D. (mmHg)	CSA rest ±S.D. (mm^2)	P sqz ±S.D. (mmHg)	CSA sqz ±S.D. (mm^2)	P cgh ±S.D. (mmHg)	CSA cgh±S.D. (mm^2)	P str ±S.D. (mmHg)	CSA str ±S.D. (mm^2)
30ML	26.1±3.1	23.9±0.8	45.2±6.1	23.0±1.0	53.0±9.1	23.5±1.5	40.3±11.1	28.1±7.2
40ML	38.1±6.5	24.2±3.4	59.7±15.6	23.6±3.4	77.9±16.6	23.5±1.0	53.0±23.5	33.6±16.6

2.3.3.4 Recto-anal inhibitory reflex (RAIR) test

During the RAIR test, minimum CSA (mm^2) was obtained at the max bag pressure (PMAX) (mmHg) and min bag pressure (PMIN) (mmHg) when the balloon rectum was inflated with 30ml, 40ml, 50ml and 60ml of air respectively at 30m, 40ml step FLIP bag volume, see figure 2.17. The RAIR test for one healthy volunteer at 30ml step volume in the FLIP bag was presented in figure 2.18.

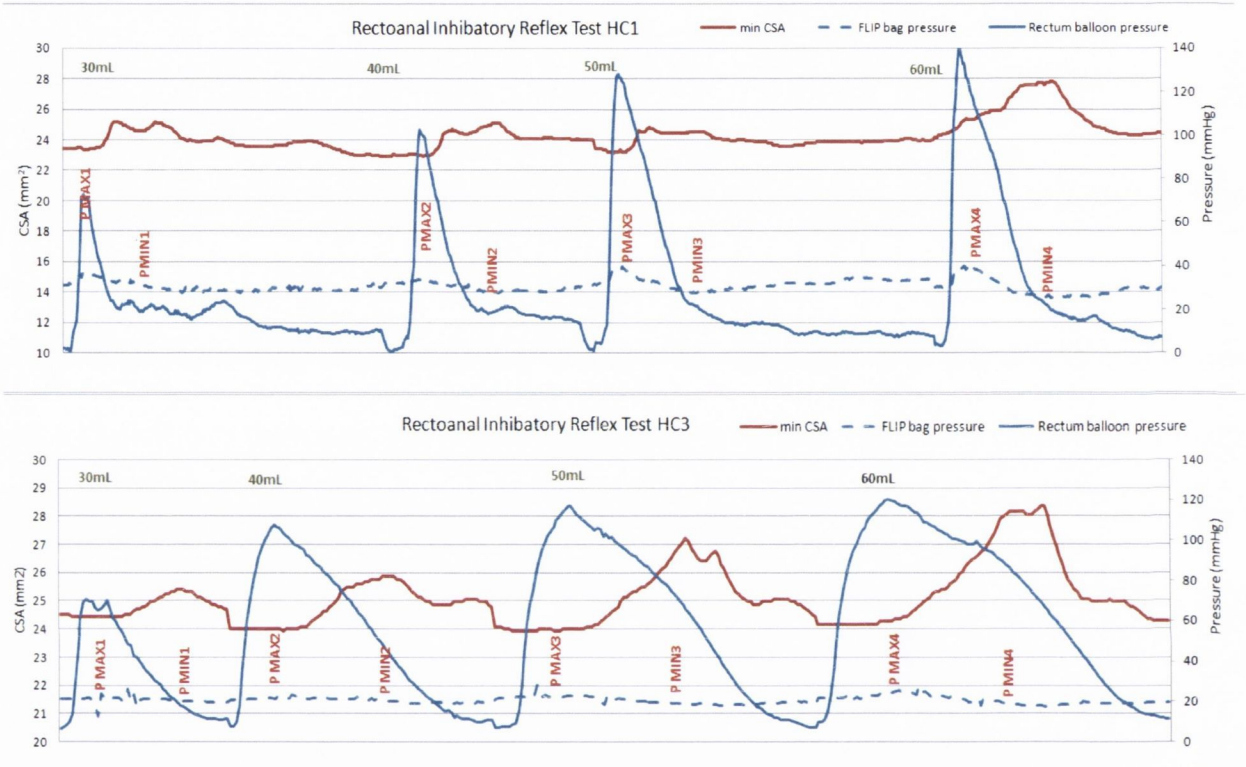

Figure 2.17: A series of rectal distensions were performed to measure the recto-anal inhibitory reflex. The PMAX as well as the PMIN were measured at each rectal distension. This figure represents the RAIR test for two volunteers at 30ml step volume in the FLIP bag. PMAX1 and PMIN1 were calculated when the balloon rectum was inflated with 30ml air; PMAX2 and PMIN2 were calculated when the balloon rectum was inflated with 40ml air; PMAX3 and PMIN3 were calculated when the balloon rectum was inflated with 50ml air; PMAX4 and PMIN4 were calculated when the balloon rectum was inflated with 60ml air.

During the RAIR test, rectum balloon was filled with different amount of air. In figure 2.18, the rectum balloon was filled with 60ml of air. It was observed that the anal bag pressured increased to its maximum value at the same time of rectum balloon inflation. After few seconds the anal canal relaxed and the min CSA increased.

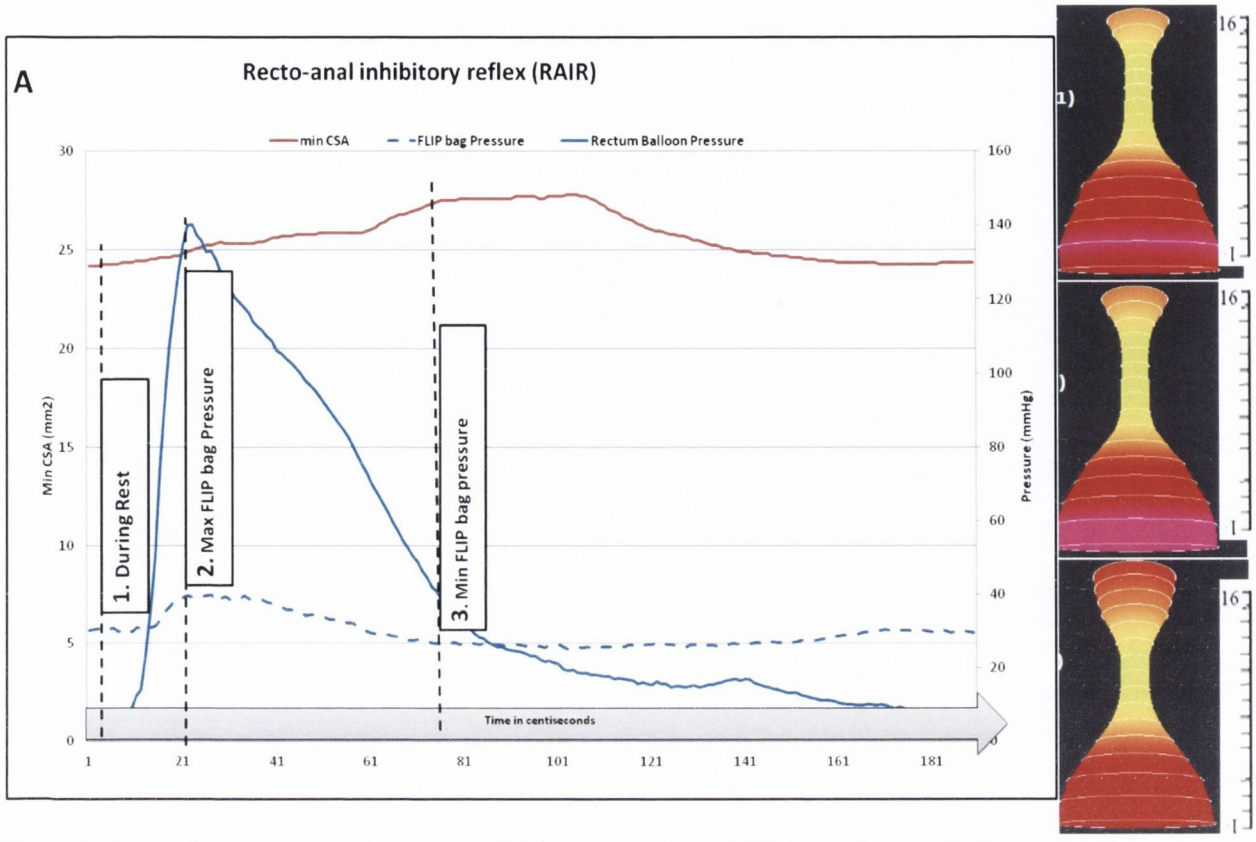

Figure 2.18: This figure represents the recto-anal inhibitory test at 30ml FLIP bag volume. 1. During resting, the rectum balloon was empty; 2. When the rectum balloon was inflated with 60ml of air, both FLIP bag pressure and rectal balloon pressure reached their maximum value; 3. After less than one second, the anal canal relaxed and the FLIP bag pressure reduced to its minimum value.

The changes in the mean PMAXi and mean PMINi when the rectum filled with 30ml, 40ml, 50ml and 60ml volume of air at 30ml and 40ml step distension volumes in the FLIP bag were summarized in table 2.3. It was observed different degrees of relaxation in the anal canal (increase in the min CSA) when the rectum balloon inflated with air. The CSA reach the maximum value when the rectum balloon was inflated with 60ml of air at 40ml step volume in the FLIP bag.

Table 2.4: The average changes in anal canal pressure PMAXi (mmHg) and PMINi during rectoanal inhibitory reflex (RAIR). Average CSA measured at the min bag pressure (P MIN) higher than the CSA measured during resting when the rectum balloon was filled with 30ml, 40ml, 50ml and 60ml air. The measurements were observed at two step volumes of 30ml and 40ml inside the FLIP bag (N=4).

Balloon rectum volume	(CSA, P)	(CSA± S.D., Pressure ±S.D.) At 30ml FLIP bag volume	(CSA± S.D., Pressure ±S.D.) At 40ml FLIP bag volume
0ml	(CSA,P)	(23.6±0.7, 26.1±5.2)	(24.0±0.9, 33.2±6.1)
30ml	(CSA, P MAX1)	(23.5±0.8, 30.1±5.4)	(26.7±3.4, 35.0±3.7)
	(CSA, P MIN1)	(23.4±1.6,23.1±5.1)	(27.7±4.0, 30.0±4.2)
40ml	(CSA, P MAX2)	(23.4±0.6,29.7±4.6)	(25.3±2.1,34.1±7.3)
	(CSA, P MIN2)	(24.3±0.8,22.4±6.0)	(27.5±2.1, 28.5±7.0)
50ml	(CSA, P MAX3)	(23.2±0.6,29.9±6.9)	(25.1±1.6, 35.6±7.3)
	(CSA, P MIN3)	(24.1±1.7,21.8±5.9)	(26.5±2.6, 29.3±6.0)
60ml	(CSA, P MAX4)	(24.0±1.1,31.1±6.3)	(24.8±1.5, 34.8±5.9)
	(CSA, P MIN4)	(25.4±3.1,21.0±5.6)	(28.4±1.9, 27.8±5.9)

2.3.3.5 Rectal volume sensory threshold test

The changes in the minimum CSA diameter (mm) and FLIP bag pressure (mmHg) of the anal canal were measured when the first sensation, constant sensation and maximum tolerable sensation (MTV) were occurred during inflating rectum balloon with air, see figure 2.19.

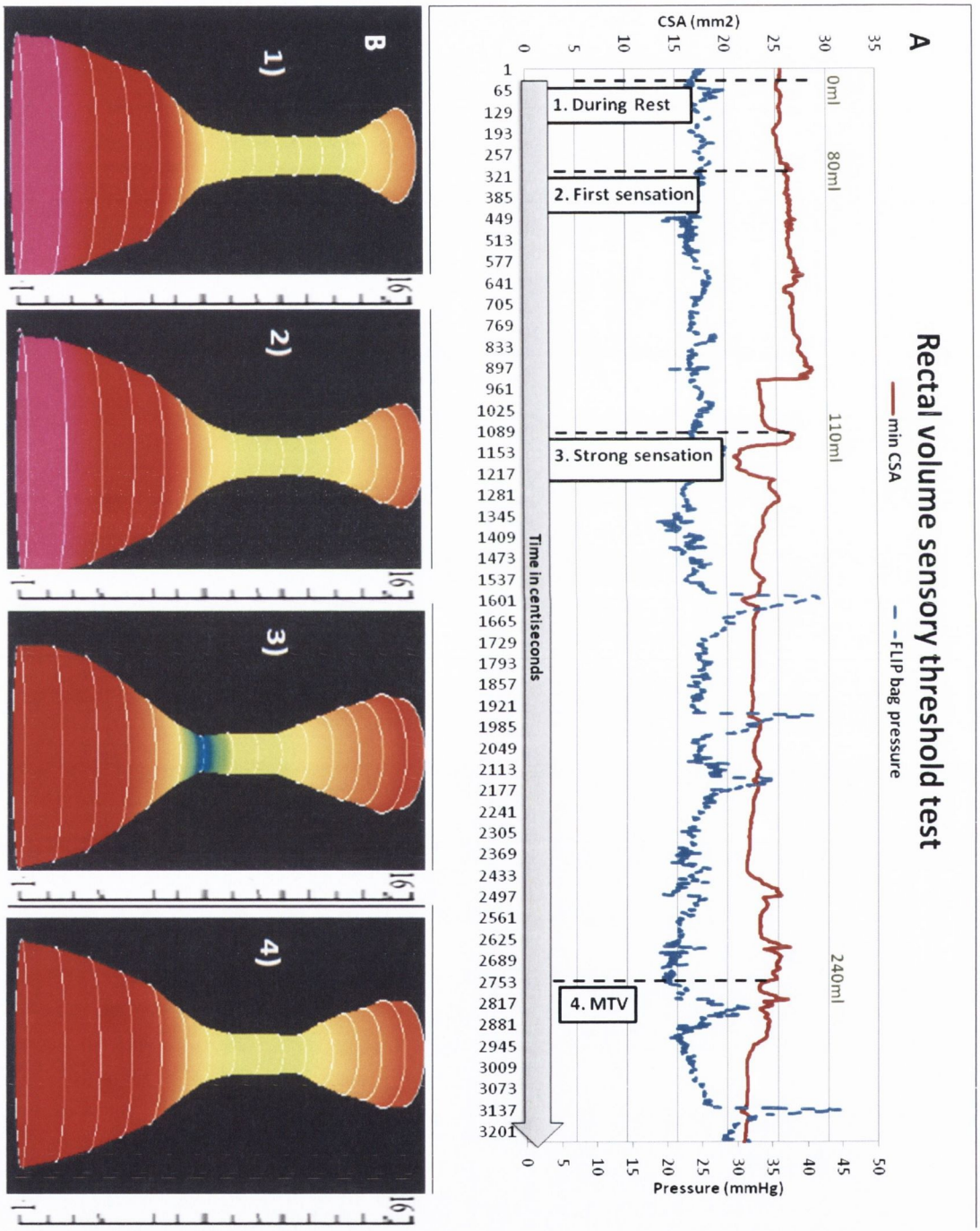

Figure 2.19: This figure represents the rectal sensation test for one subject at 30ml step volume in the FLIP bag. At (A) the changing in the FLIP bag pressure and the minimum CSA during slow rectal balloon distension is plotted At (B) a still images of the anal canal from the EndoFLIP® screen during the rectal sensation test. 1. During resting, the rectum balloon was empty; 2. First sensation occurred at 80ml air inflated in the rectal balloon; 3. Strong sensation occurred at 110ml air

inflated in the rectal balloon; 4. Maximum tolerable volume was 240ml air. (The sudden decrease in minimum CSA at 100ml of air in the rectum balloon is an observed technical error).

The changes in the mean minimum CSA (mm^2) and FLIP bag mean pressure (mmHg) of the anal canal during rectal sensation test were summarized in table 2.5.

Table 2.5: Changes in anal canal CSA and FLIP bag pressure during rectal sensation test. (N=4).

Healthy Controls	Rectum Volume (ml)	CSA (mm^2)	Pressure (mmHg)
HC1			
FIRST SENSATION	85	27.1	19.62
STRONG SENSATION	110	20.8	27.3
MAXIMUM TOLERABLE VOLUME	240	25.6	19.6
HC2			
FIRST SENSATION	40	23.7	25.5
STRONG SENSATION	80	23.7	20.0
MAXIMU TOLERABLE VOLUME	180	27.7	16.9
HC3			
FIRST SENSATION	40	21.6	27.6
STRONG SENSATION	85	20.2	26.2
MAXIMUM TOLERABLE VOLUME	110	26.1	23.6
HC4			
FIRST SENSATION	85	23.7	27.8
STRONG SENSATION	110	24.0	25.9
MAXIMUM TOLERABLE SENSATION	150	31.2	19.5

2.3.4 Discussion

Experiment 2 was carried out using a custom made 16 electrodes FLIP probe (prototype 2). The design of FLIP prototype 2 merges the ano-rectal manometry approach as a gold standard tool for distending the rectum and the findings from experiment 1 which suggested that increasing the axial resolution of the FLIP is essential for the evaluation of the anal canal distensibility and morphology.

In a bench top study, a multi-lumen manometric polyvinyl catheter 18.5cm in length and 4.5mm in diameter was used to build the FLIP prototype 2 because of its adequate diameter and stiffness. The balloon used to inflate the rectum was a 6.5cm long and 250ml max-volume non-latex stretchable balloon. The same balloon was used to distend the rectum in the ano-rectal manometry testing. A non-compliant polyesterurethane 12.5cm long bag housed 2 stimulating electrodes and 16 sensing electrode pairs. The spacing between the rectum balloon and the FLIP bag was enough to prevent any interference between the FLIP bag and the rectum balloon during the distension of either one or both of them.

The in vitro study showed that the custom made probe gives reproducible data. In general the system becomes less accurate the higher the distended diameter. This is because the higher the distended diameter, the lower the voltage drops across a pair of electrodes, and the closer the signal gets to the noise floor of the measurement electronics. The system has an operational range which is related to electrode spacing, drive current and saline concentration. For the standard FLIP system the signal

noise floor is about 32mm (803.8mm^2) at 40° degree, 35mm distended diameter in this case was a very low signal level.

Probe positioning plays an important role in measuring the anal canal distensibility. In FLIP prototype 2 protocols, four measurements were seen outside the anal verge, for that reason, the amount of saline that filled the FLIP bag toward the rectum varies from person to person. The difference in the amount of saline may induce different amount of relaxation due to the initiation of the recto-anal inhibitory reflex. As a result, the measures of CSA and pressure during ramp distension may be limited in terms of their accuracy because of this probe positioning technique. Also, the accuracy of the CSA measurements may be limited because the values recorded of the 'measured' and 'actual' diameters in the accuracy test were unequal.

The subjects were allowed 5-10 minutes to become accustomed to the probe before starting the procedure. During this time the probe was inflated and deflated with water two or three times. This would allow muscles of the anal canal to become accustomed to the probe and therefore help to reach a steady pressure. Other studies indicated that if the probe was inserted in the anal canal it needs a stabilizing period of 30 seconds to achieve resting pressure (171, 285). However, during this experiment filling and emptying the FLIP bag took a finite amount of time to achieve.

The EndoFLIP® system was used to collect the data from the sixteen sensing electrodes. In this system, the probe and the pressure transducers are pre-calibrated during the manufacturing process and required no additional calibration before use. However, the calibration process of the original EndoFLIP ® assumed the minimal-to-maximal detectable CSA range to be 10 to 450 mm^2; i.e. the minimum detectable diameter was 3.5mm, whereas the diameter of the FLIP prototype 2 probe was 4.5mm. Accordingly, the accuracy of the FLIP was measured in order to calculate a correction equation. The later was applied on the data collected by the FLIP prototype 2 during the data analysis of the clinical studies.

Quantitative CSA and bag pressure measurements were obtained at 10ml, 20ml, 30ml, 40ml and 50ml ramp distensions. In figure 2.16, the distensibility of the anal canal was presented as CSA vs. pressure. Intuitively, distensibility graph would be pressure vs. CSA (286) (267). However, the system used to measure anal canal distensibility is in line with other publications in this field (265) (287). Both the distensibility and the morphology of the anal canal play an important role in the anal continence.

This work clearly indicates that this technique is suitable for the squeeze, cough and straining test. Both minimum CSA and the bag pressure were measured during the provocative manoeuvres at 30ml and 40ml step volume. While the measured pressure changes during different manoeuvres, the

measured minCSA was not affected except it increased in straining manoeuvre. This increase in the mean of minCSA can be justified by the relaxation in the anal canal during this manoeuvre.

During the recto-anal inhibitory reflex (RAIR) test, the rectum balloon was rapidly distended to different volumes (30ml, 40ml, 50ml and 60ml). The rapid balloon distension of the rectum mimics the rapid propulsion of liquid faeces or gas into the rectum from the colon and induces an involuntary relaxation of the internal anal sphincter (RAIR). When the rectum balloon was filled with different volumes, there was an instant increase in the FLIP bag pressure PMAX at both step volumes. After approximately one second a relaxation in the anal canal occurred. This was accompanied by a decrease in FLIP bag pressure PMIN for all volunteers. During the rectal sensation test, the rectum balloon was distended slowly; CSA and bag pressure measurements were obtained at the first sensation, strong sensation and the maximum tolerable volume (MTV). An increase in the CSA and a decrease in bag pressure were recorded in three volunteers at MTV.

In each volunteer, the probe was placed so that four sensing electrode pairs were seen outside the anal verge and the remaining 15cm length of the FLIP prototype 2 was positioned inside the anal canal. It was proposed that this method would allow the rectum balloon to be located in the rectum. However, the length of the anal canal is very variable and hence the location of the rectal balloon was not standardized with respect to the upper limitation of the anal canal. This technique of probe positioning may limit the precision of the results of CSA and pressure during RAIR and rectal distension as the balloon might not be located correctly in rectum.

Based on findings from these pilot studies, measurements of minimum CSA and FLIP bag pressure can be obtained during ramp distension. The researcher observed, however, that FLIP bag pressure levels were increased dramatically when the anal canal was distended from 40ml to 50ml. This increase may occured as a result of the voluntary contraction of the external sphincter. Ramp distensions to 40ml FLIP bag volume were deemed to be most appropriate as it keeps the resting status of the region. Additionally, provocative manoeuvres (rest, squeeze, cough and strain) at 20ml, 30ml, and 40ml step distensions will be considered in future studies.

In this experiment, the combination of the FLIP bag for distending the anal canal with the rectum balloon for rectal distension enables the evaluation of the anal canal distensibility and morphology as well as the relation between the motor responses of the external anal sphincter and internal anal sphincter in response to rapid and slow balloon distension of the rectum in normal volunteers. Surprisingly, it was noted that the ramp distension of the anal FLIP bag when the rectum balloon was empty and the rectal distension when the FLIP bag was distended with step volumes had the same effect on the anal canal profile. Table 2.2 and 2.5 indicated that the increase in min CSA at 50ml ramp distension was the same as the increase in min CSA at the max tolerable volume.

The main functions of the anal sphincters are to keep the anal canal tightly closed to maintain continence (see section 1.5.2). It was noticed that a 1mm relaxation in the minimum CSA can cause a major pressure drop in the FLIP bag pressure. When the rectum was filled with 50ml of air at 30ml step distension in the FLIP bag, the min CSA increased from 23.6mm^2 at rest to 24.1mm^2 and the pressure decreased from 26.1mmHg at rest to 21.8mmHg. See table 2.4.

From this, it was concluded that the FLIP probe diameter and FLIP bag volume have the most important impact on the measured geometry of the anal canal. Based on findings from this initial pilot study using FLIP prototype 1&2, a FLIP probe with narrow diameter was proposed for future studies as the probe (FLIP prototype 2) size was of large enough catheter diameter to innervate anal relaxation causing the FLIP bag to slip out easily during the entire experiment even if the bag was empty. FLIP prototype 1 was narrow enough to stay still in position during the clinical study; however, the measurement length was too short to measure the changes in the ano-rectal region.

2.4 Experiment 3: Original EndoFLIP ® (FLIP prototype 3): A pilot study

2.4.1 The objectives

Following on from the findings from the experiment 2, an EndoFLIP® was required for anal sphincters evaluation. The objectives of this pilot study were to (i) establish if the original EndoFLIP® can be safely and accurately positioned and distended in the anal canal region in patients with faecal incontinence; and (ii) to determine if the probe diameter is small enough to measure the minimum CSA as well as to not elicit the relaxation in the anal canal.

2.4.2 Materials and methods

2.4.2.1 Subjects

One female and one male volunteer (54 and 28 years old) were recruited for this study. Both had a history of faecal incontinence. The study medical history including symptoms (constipation, urinary or faecal incontinence), allergies, past treatments (anal surgery) were obtained and written consent was signed. The protocol was approved by the SJH-AMNCH Joint Research Ethics Committee (see appendix 1).

2.4.2.2 Equipment

The EndoFLIP® equipment already described previously (See section 1.11.5) was employed.

2.4.2.3 Study protocol

No special preparation of the bowel was needed and no sedation was given to the volunteers. A digital examination before investigation ensured that the rectum was empty. Each patient was positioned on their left side, with hips and knee flexed. A well-lubricated EndoFLIP® catheter was inserted in the anal canal. To position the EndoFLIP®, the bag was inflated with 10ml conductive solution volume

and slowly retracted until the classical hourglass shape formed in the middle of the anal canal profile. The EndoFLIP® bag was distended by 10ml, 20ml, 30ml and 40ml ramp distension volume at a fixed flow rate of 40ml/min.

2.4.2.4 Data analysis

EndoFLIP® system provides sixteen measures of CSA (mm^2) and bag pressure (mmHg) at a frequency of 10Hz during volume distensions. Minimum CSA (mm^2) and pressure (mmHg) at 10ml, 20ml, 30ml and 40ml distended volumes were calculated during the ramp distension test.

2.4.3 Results

During the anal ramp distension from 0-40ml, minimum CSA (mm^2) and bag pressure (mmHg) measurements were obtained at 10, 20, 30 & 40 ml balloon volumes across subjects. See figure 2.20. The minimum CSA measured in the first volunteers was 12.56mm^2 (4mm in diameter) and in the second volunteer was 13.8mm^2 (4.2mm in diameter).

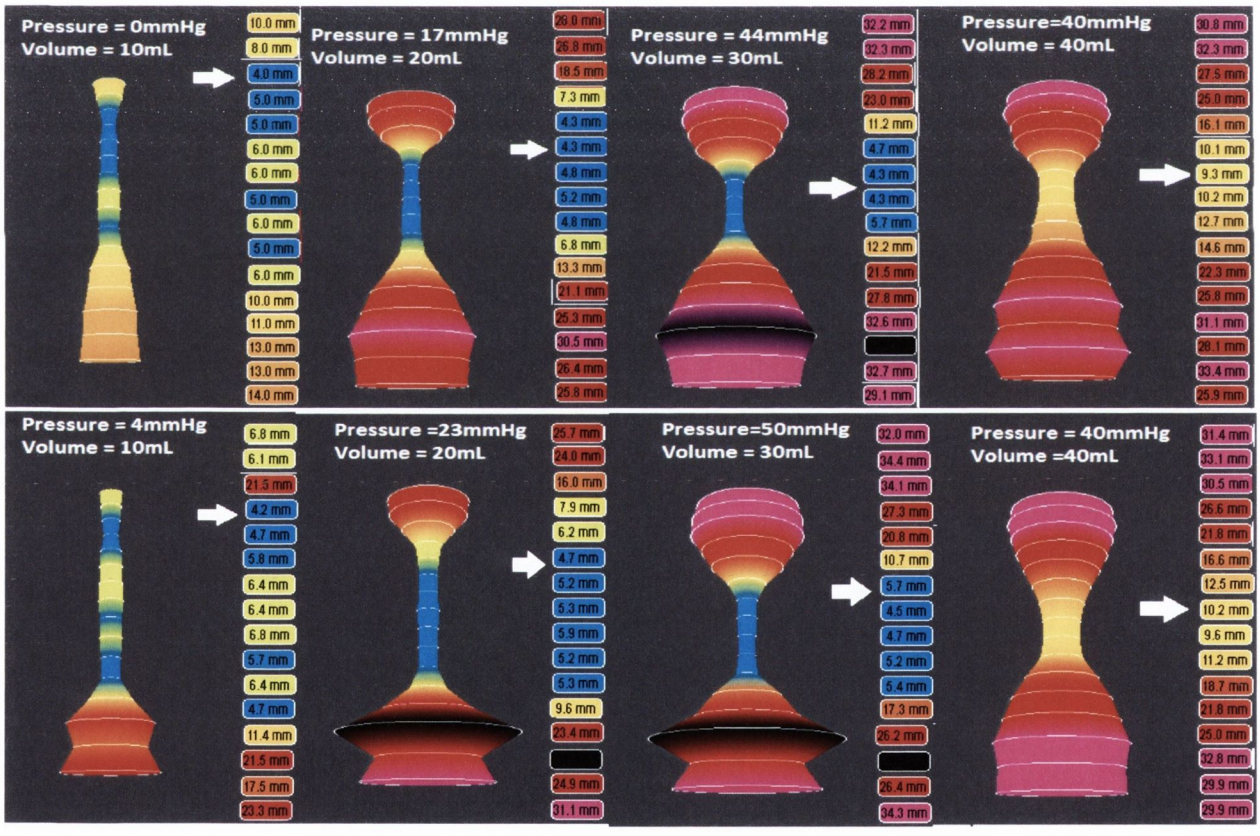

Figure 2.20: A still images from EndoFLIP screen. Diameters are given in the sixteen boxes on the right hand side of the FLIP diagrams and bag pressures in mmHg and volume in ml are given at the bottom of each diagram.

2.4.4Discussion

Using of an EndoFLIP® system, originally designed for OGJ evaluation, to evaluate the anal sphincters was proposed for measuring anal canal distension in Experiment 3. The EndoFLIP® imaging system can provide a real time image of the 3 dimensional reconstructed data from the 16 cross sectional area measurements along the anal canal, so that the entire dynamic changes in the

lumen can be monitored instantaneously. Using the FLIP prototype 3 the minimum diameter measured was 4mm (CSA=12.56 mm^2). This diameter cannot be measured using FLIP prototype 2 especially as the diameter of the probe catheter is 4.5mm (CSA=15.8mm^2). As our measures of distensibility relate to the ability of the anal canal to close fully it is important that using this catheter type probe technique that we measure distension from as close to the closed position as possible. This is way using a probe with a narrower diameter as is the case with prototype 3 is more appropriate for these studies.

EndoFLIP ® (prototype 3), with a length of 10cm and maximal diameter of 2.5cm, was safely positioned and distended in the anal canal of patients with FI without continuous movement along the anal canal and at the same time was capable of displaying the hour glass shape of the narrow region of the anal canal at different volumes. Hence this design was selected as close to optimal for ano-rectal region evaluation.

Based on these initial experiments, researchers considered using the EndoFLIP® to evaluate anal canal distensibility and morphology. The present study shows that it is possible to conduct a test for the components of the anal sphincter, providing data of physiological and clinical interest.

2.5 Conclusion

In this chapter three probes types were tested, FLIP prototype 1, FLIP prototype 2 and FLIP prototype 3 respectively in order to find the best FLIP design to achieve the aims of the thesis. For FLIP prototype 1 the probe length was not adequate to capture the entire anal canal profile. Therefore, it was obvious that anal canal length could not be observed and probe position straddling the length of the canal could not be assured with eight electrodes.

FLIP prototype 2 was found to be too large to innervate anal relaxation causing the FLIP bag to slip out easily during the entire experiment. The diameter of the catheter at 4.5mm did not allow it to measure CSAs that reflect the closure state of the anal canal. As a result of these studies, original EndoFLIP® (FLIP prototype 3) was safely and accurately positioned and distended in the anal canal region in two patients with faecal incontinence. The length and the size of FLIP Prototype 3 were found to be capable of measuring the minimum CSA as well as not elicit the relaxation in the anal canal. For this reason prototype 3 was used for the rest of the studies in this thesis.

Chapter Three: Pilot Studies to Validate Study Protocol and to Define Outcome Measures

3.1 Introduction

The EndoFLIP® was safely distended in the anal canal in a pilot study, as described in chapter two. The physiological interpretation of that experiment was hindered by the absence of any validation components of this technology with other systems. In this chapter, Video-fluoroscopy (see section 1.10.6) and endoanal ultrasound (EAUS) (see section1.10.5) were used to validate these components.

In this chapter, the second research question within this thesis will be addressed. Hence, the quantitative measurements of anal sphincter distensibility and anal canal morphology as measured by FLIP during distension and provocative manoeuvres will be validated.

3.2 Design and Preparation of a Radio-opaque EndoFLIP probe for Validation of Probe Position

3.2.1 Objectives

The main objectives of this bench-top study were to: (i) prepare a radio-opaque solution that is capable of distinguishing the EndoFLIP® bag from the surrounding anal muscles during the video-fluoroscopy study; and to (ii) determine the accuracy of the CSAs measured with the sixteen ring electrodes along EndoFLIP® when radiant conductive solution is used.

3.2.2 Material and methods

3.2.2.1 Equipment

Video-fluoroscopy was used as a validation tool for this study using a Siemens AXIOM Artis dMP multipurpose C-arm X-ray system with dynamic flat detector (30 cm, 40 cm). Images were recorded for later slow motion and millisecond frame-by-frame analysis (frame rate = 25 frames/s) using a Video South Panasonic DVC Pro digital video recorder and 14-inch high-resolution monitor and a high-quality clip-on microphone. The procedure was recorded onto a Panasonic DVC Pro 66L AJ-P66LP videotape (Appendix 5).

The EndoFLIP® (FLIP prototype 3) already described previously in section 2.4 was employed.

3.2.2.2 Preparing a radiant conductive solution

For this test, two sets of contrast agents ionic (Urografin) and non-ionic (Neopam) were diluted with EndoFLIP® conductive solution (See Appendix 4). Each set consisted of 12 different concentrations from 1:1 to: 1:12 contrast agent to conductive solutions. These prepared dilutions from the two contrast agents were poured in plastic cups. An EndoFLIP® probe was immersed in each cup and

placed on the X-ray table in a video-fluoroscopy room, see figure 3.1. X-ray images of the probe inside each cup were obtained for each set.

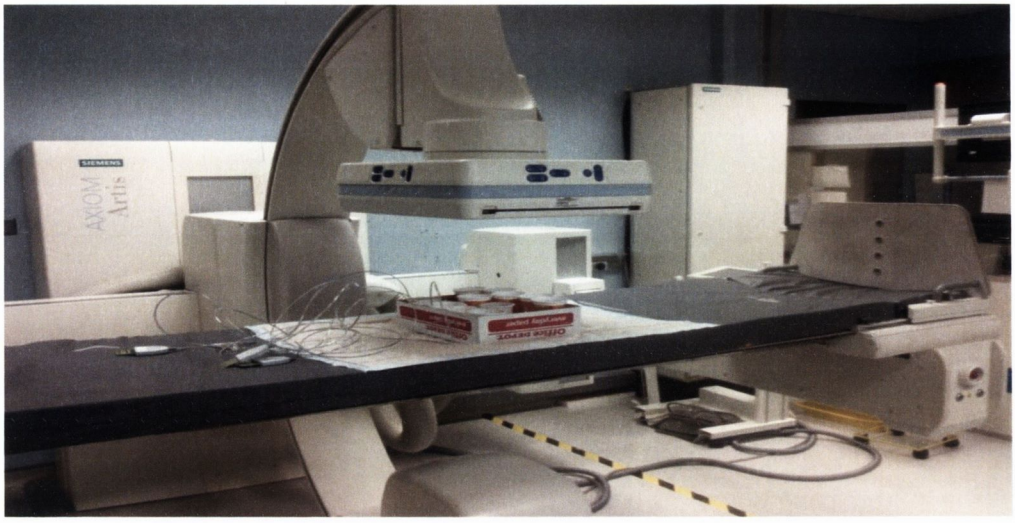

Figure 3.1: EndoFLIP® probes were immersed in each cup of the two sets of radiant dilutions.

Subsequently, the EndoFLIP® bag was placed in a polymethylmethacrylate cylindrical tube and inflated with the prepared dilution that allowed the most visibility of the sixteen ring electrodes. A cylindrical tube was placed in the patient phantom. The patient phantom is a Perspex ® material with similar density to water that is routinely used to simulate a patient in an X-ray beam during testing to mimic human body fluids, see figure 3.2. Another X-ray image was obtained.

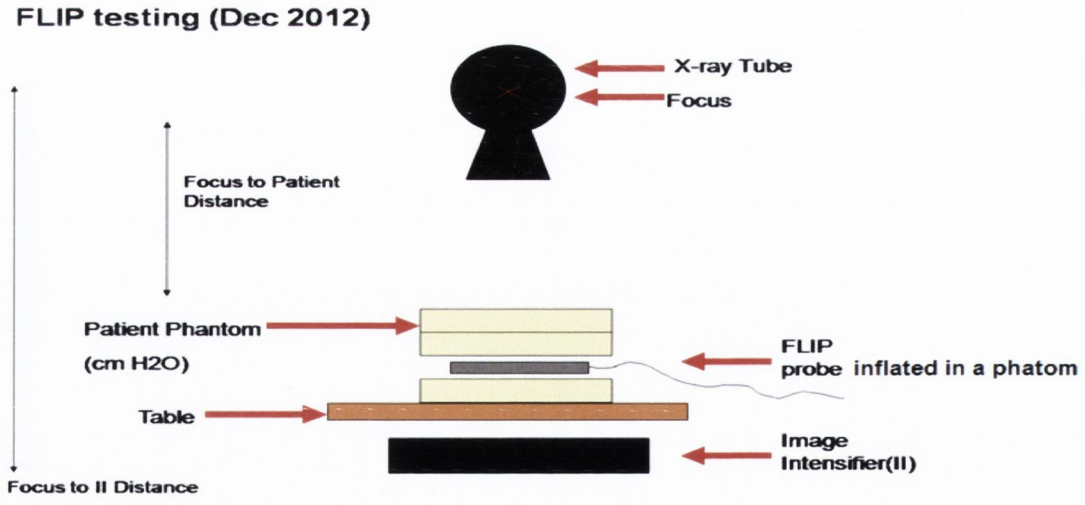

Figure 3.2: An illustration showed the FLIP bag inflated in polymethylmethacrylate cylindrical tube and placed in a patient phantom. The patient phantom is a Perspex® material with similar density to water that is routinely used to simulate a patient in an x-ray beam during testing. It has 10cm of water on top of the probe (towards the tube side of the x-ray machine), and 5cm below (towards the detector side of the x-ray machine) to simulate the probe within a patient body.

3.2.2.3 The accuracy of EndoFLIP® CSA measurements

A set of polymethylmethacrylate (Perspex®) cylindrical tubes with cross-sectional areas of 76.9 mm^2, 111 mm^2 and 369 mm^2 were used to test the accuracy of the diluted contrast agent of 1:12 concentration. The accuracy test was carried out in two steps:

1. The EndoFLIP® bag was deflated and inserted into each cylindrical tube so that the 16 ring electrodes were placed at the centre of the cylinder. The bag was inflated with standard conductive saline. Measurements from the 16 CSA's were saved in a text file.
2. The latter procedure was repeated using the radiant conductive solution (diluted contrast agent).

3.2.3 Results

X-ray images of the EndoFLIP ® immersed in the diluted contrast agents, non-ionic and ionic, at 1:10, 1:11 and 1:12 concentrations is presented in figure 3.3 (a) and (b). The images from the non-ionic contrast agent dilution were darker than the images from the ionic contrast agent dilution. The ionic contrast agent dilution with 1:12 concentration produced the brightest image of the EndoFLIP®.

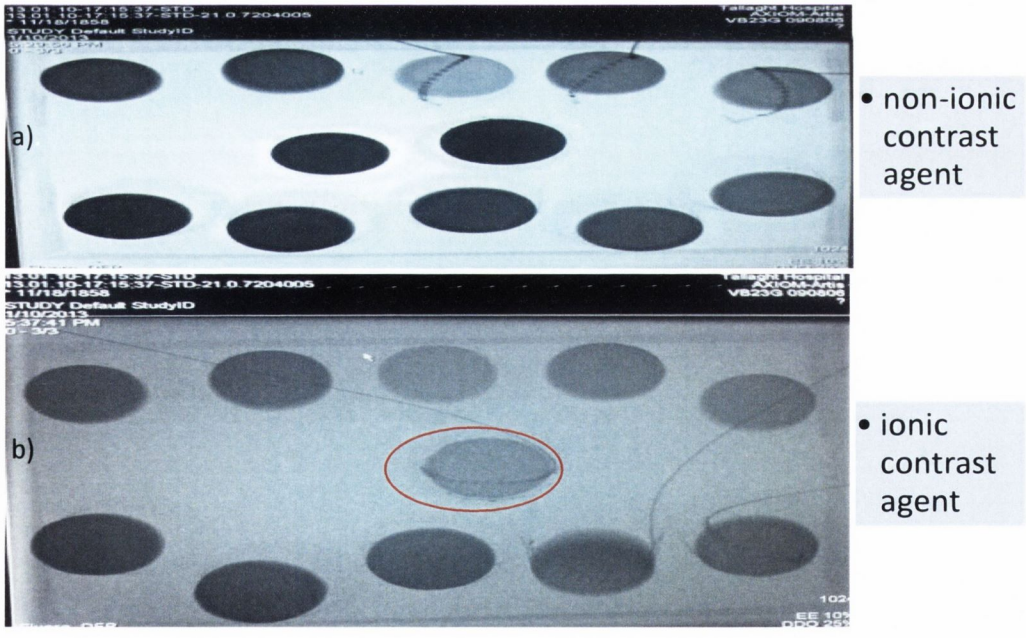

Figure 3.3: An X-ray image of the diluted (a) non-ionic and (b) ionic contrast agents. The highlighted circle represent the FLIP bag immersed in (1:12) diluted ionic contrast agent.

An X-ray image of the inflated EndoFLIP® bag with (1:12) diluted ionic contrast agent inside a phantom placed in a patient phantom was obtained. The sixteen ring electrodes were clearly seen.

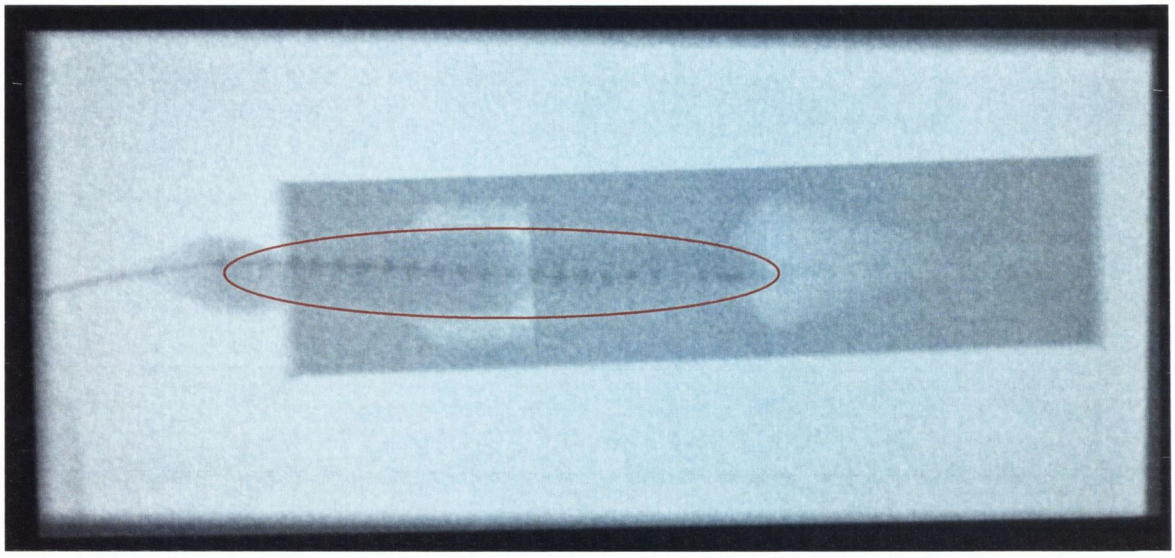

Figure 3.4: An X-ray image of EndoFLIP® bag inflated with 1:12 diluted contrast agent inside a phantom and placed in a patient phantom.

The CSA measurements of the cylindrical tubes using the EndoFLIP® inflated with standard conductive solution in comparison with the same EndoFLIP® bag inflated with diluted contrast agent are presented in table 3.1.

Table 3.1: CSA measurements of EndoFLIP® inflated with diluted ionic contrast agent in the accuracy test.

Actual reading (CSA)mm^2	EndoFLIP® reading with standard conductive solution (CSA)mm^2	EndoFLIP® reading with diluted contrast agent (1:12) (CSA)mm^2
76.9	77.1	76.7
111	111.3	110.4
369	369.3	368.8

3.2.4 Discussion

In this bench-top study, the researchers were able to prepare a diluted contrast agent that is capable of imaging the EndoFLIP® bag during video-fluoroscopy studies. The video-fluoroscopy image of the diluted non-ionic contrast agent (Urografin) with the standard conductive saline at concentration of (1:12) was bright enough to distinguish the EndoFLIP® bag from the dark shade of the anal canal muscles.

In order to validate the place of the FLIP bag in the anal canal we need to use video-fluoroscopy to image the bag during pilot studies. Two contrast agents were diluted and tested to find out the radio-opaque dilution that allowed the most visibility of the bag when inflated in the anal canal as part of the clinical study. Visualization of the bag is important to confirm radiologically the position of the probe during typical measurements in the anal canal and might provide information on the effects of distension on the anatomical and physiological components.

The ano-rectal region anatomically made up of smooth tissue and consisted mainly of muscles that cannot be easily distinguished using the X-ray image alone. The latter dilution was tested using the patient phantom that mimics human body fluids in order to insure optimum visibility of the electrodes. Because we were able to see the 16 electrodes and distinguish the bag in figure 3.4, we expect that the bag would be visible when distended with the radio-opaque solution in the clinical study. Hence, the position of the bag in the ano-rectal region would be defined.

During the clinical pilot study, the starting time and the ending time of the provocative manoeuvres were registered as seen on the EndoFLIP® screen. At the same time, video-fluoroscopy images were marked at the start and the end of each manoeuvre. This allowed us to correlate the images from both techniques with confirmation from a trained radiologist.

Although dynamic MRI (see section1.10.7.1) can provide a very clear images of both striated muscles and smooth muscle of the EAS and IAS respectively when a EndoFLIP® bag inflated in the anal canal, the standard supine position of the patients inside the MRI core would prevent comparisons between the live images of the anal canal profile that appear on the EndoFLIP® screen with the MRI images. Additionally, the insertion of the EndoFLIP® in supine position is inconvenient.

3.3 Pilot study1: Validation of the probe position by Video-fluoroscopy

3.3.1 Objectives

The main objectives of this chapter were to: (i) validate the probe position by distending the EndoFLIP® bag with the radiant conductive solution under video-fluoroscopy; and to (ii) define measurements along the anal canal during ramp distension and provocative manoeuvres.

3.3.2 Materials and methods

3.3.2.1 Subjects

Two volunteers were studied; the first volunteer was a 65 year old male with normal anal sphincters function and the second volunteer was 49 year old female diagnosed with faecal incontinence because of weakness in anal sphincters. An ano-rectal manometry test was performed to measure the volunteer's anal sphincters function before the study. Ethical approval was obtained (Appendix 1). Informed consents were signed by the participants before the procedure.

3.3.2.2 Distensibility measurements

Measurement of anal canal distensibility was made using the original EndoFLIP® probe (FLIP prototype 3) based on the technique described previously, (see section 1.11.5) – figure 3.5a. In brief, the EndoFLIP® catheter had a 3mm outer diameter. A non-compliant bag, 12cms long, which could be filled to a volume of 60ml, was mounted on the distal end of the probe. The bag was designed to

assume a cylindrical shape 7.5cm long with maximal diameter of 2.5cm, when fully distended. The detectable cross-sectional area (CSA) range was 10 to 450 mm^2.

The bag contained 17 ring electrodes spaced 5 mm apart for impedance planimetry measurements. As the bag was filled with a specially formulated conductive solution, the impedance across each segment caused a measured voltage change which was calibrated to represent the radial CSA of the bag at that position. The probe also contained a solid state pressure transducer mounted towards the distal end of the bag 2mm from the last electrode. The transducer was capable of measuring the pressure of the liquid-filled bag during distension.

Sixteen CSA measurements between adjacent electrodes and the data from the pressure transducer were sampled at 10 Hz and stored in the data acquisition system of the EndoFLIP®. The data was displayed in real time as a 7.5cm cylinder of CSAs along its length reflecting diameters estimated from the 16 measured CSAs.

The distensibility measurements were carried out with the radiant–opaque solution that was prepared in bench-top studies (see section 3.2.3) and under video-fluoroscopy (see section 3.2.2.1).

3.3.2.3 Study Protocol

During the study with video-fluoroscopy, the subjects lay on fluoroscopy table and were positioned on their left side, with hips and knee flexed for the procedure. A digital examination before investigation ensured that the rectum was empty. Before using the system, any air was removed from the EndoFLIP® assembly by using an automated purge sequence and the intra-bag pressure was zeroed using a function on the system. The EndoFLIP® probe was inserted anally as illustrated in figure 3.5a. The EndoFLIP® bag was inflated to a small volume (5-10ml) so that three distal measurements (EndoFLIP®$_{DIS}$ end) were seen on the EndoFLIP® display to be increasing in diameter indicating that they had reached the rectum and the probe was held in this position by an assistant's hand. The bag was deflated and the subjects were allowed 5-10 minutes to become accustomed to the probe before starting the procedure.

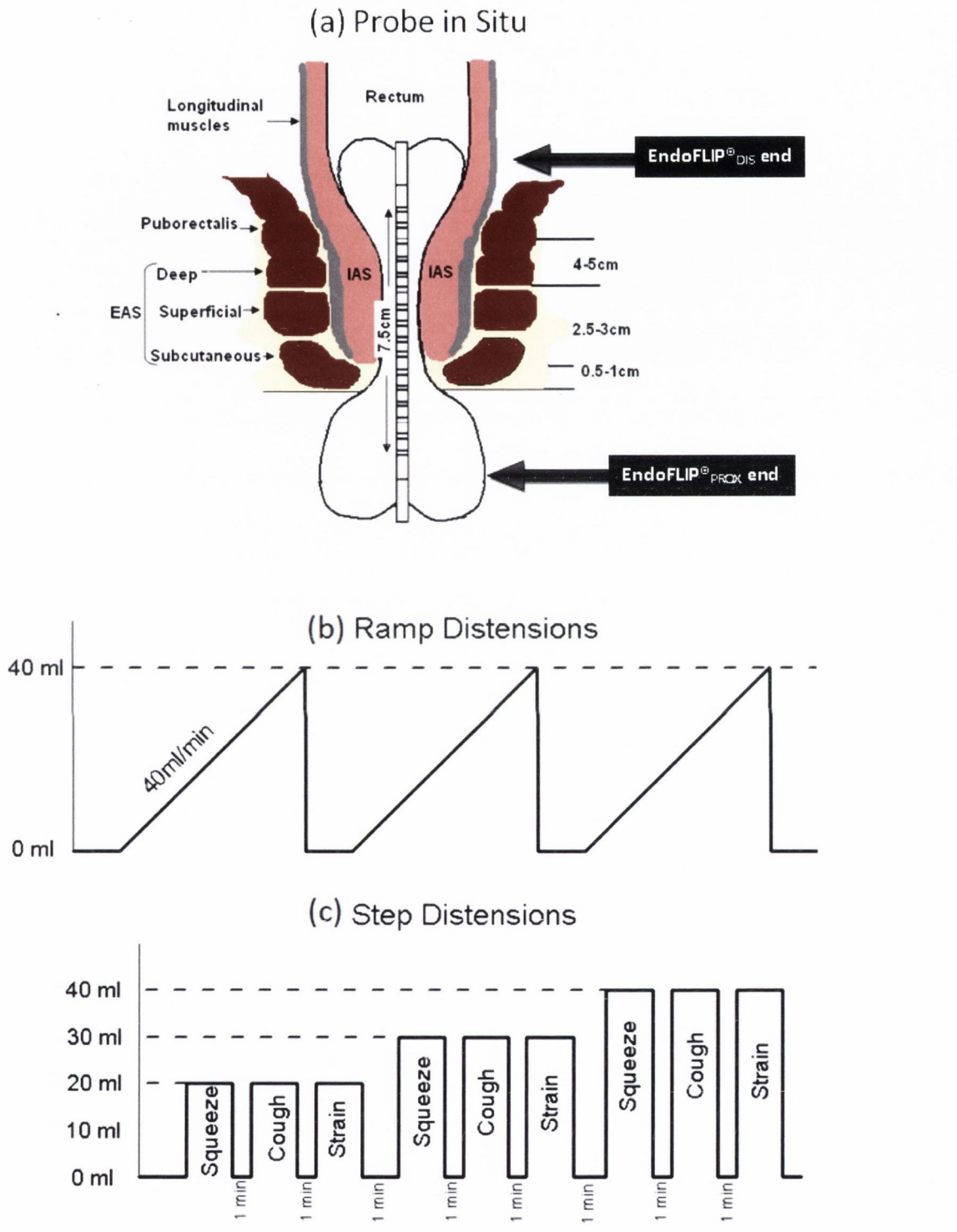

Figure 3.5: (a) Illustration showing the EndoFLIP® in position along the anal canal in the ano-rectal region. EndoFLIP® distal end was positioned toward the rectum and the EndoFLIP® proximal end was positioned outside the anal verge (black arrows). (b) Diagram for volunteer protocol starting with 3 ramp distensions(0-40ml) at 40ml/min. (c) Then three further step distensions at 20, 30 and 40ml, at each step volume the volunteers were asked to perform two provocative manoeuvres (squeeze, cough, strain).

3.3.2.4 Probe Positioning

Once a 10ml volume was inflated into the EndoFLIP® bag, a profile of the narrow zone was observed on the visual display. The 16 CSAs provided estimated diameter measurements of the narrow zone and the wider regions at either ends. The narrow zone is the most constricted part in the anal canal (the lowest of the sixteen CSAs) where the difference between two adjacent estimated diameters was ≤1mm; when the differences exceed 1mm, proximal and distal borders are defined.

The CSA and the corresponding distension pressure measurements were recorded during 3 ramp distensions where the bag was filled with the conductive liquid at a rate of 40ml/min to a volume of 40ml (figure 3.5b) and then a series of provocative manoeuvres were carried out with the bag filled in steps to 20, 30, and 40ml respectively (figure 3.5c). The manoeuvres were squeezing, where the subject was asked to tighten the ano-rectal region as much as they could for 10-20 seconds, coughing, when they were asked to spontaneously cough, and straining, where they were asked to strain as if trying to defecate. There was a 1 minute relaxation between each manoeuvre. The measurements were monitored in real-time to ensure proper bag placement by directly visualizing the functional image on the EndoFLIP® system during all stages of the protocol. If the bag was observed to move along the anal canal, the probe was repositioned and the measurement was repeated.

3.3.3 Results

3.3.3.1 Probe positioning via video-fluoroscopy

The probe position along the anal canal matched the narrow zone that was observed in the EndoFLIP® system. The number of electrode rings that were imaged outside of the anal verge confirmed the shifting in the probe toward the rectum during squeezing and toward the anal verge during straining manoeuvres, see figure 3.6.

X-ray images of the EndoFLIP® bag for FI volunteer during resting and squeezing manoeuvres confirmed that the probe was angulated inside the anal canal and shifted internally toward the rectum as a response to the change in the ano-rectal angle during this manoeuvre, see figure 3.7. The EndoFLIP® bag was curved by 77.4◦ when the bag was distended with 40ml volume, see figure 3.8.

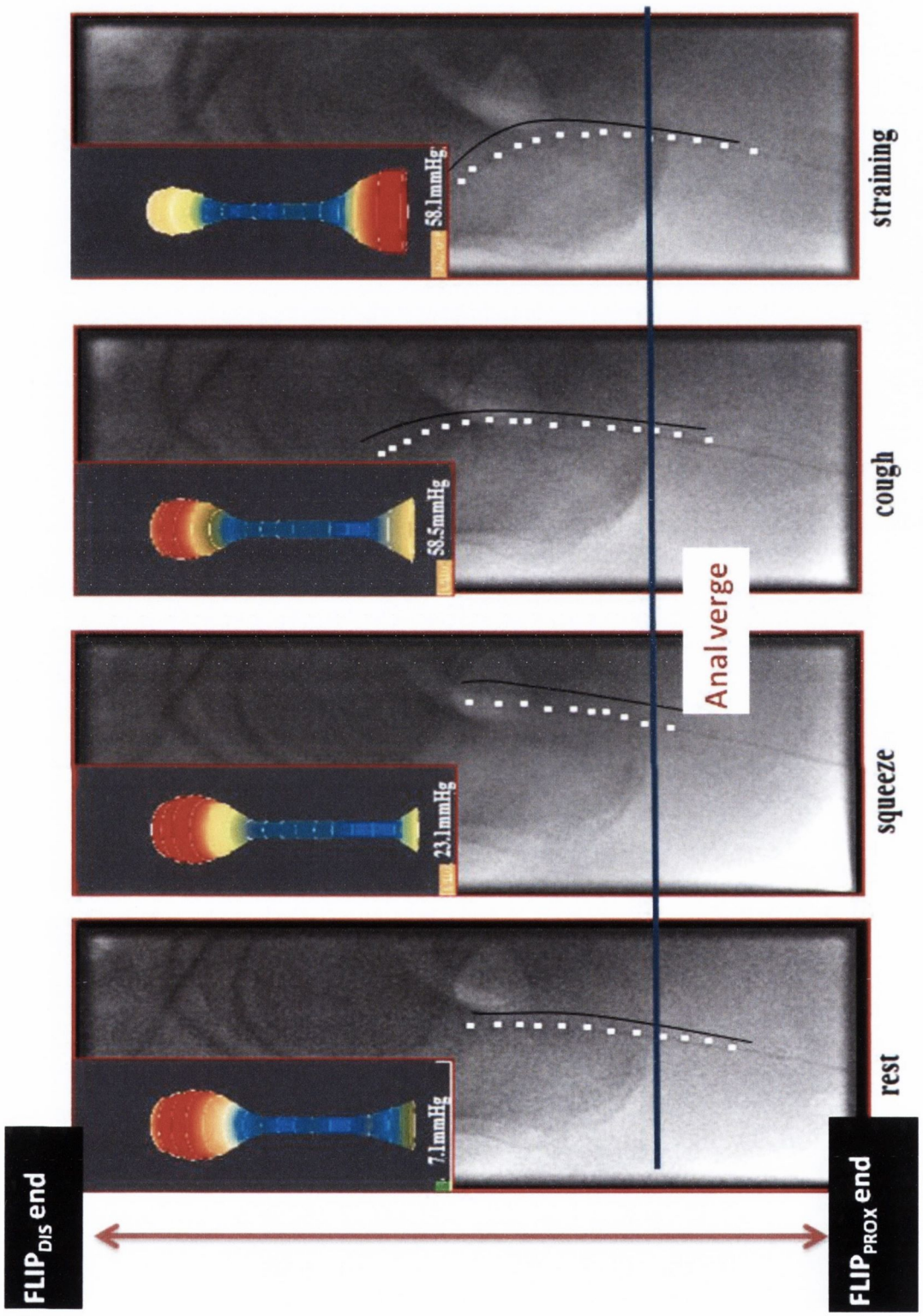

Figure 3.6: An X-ray image of EndoFLIP® in the anal canal of male volunteer during different manoeuvres (rest, squeeze, cough and strain). The blue line represents the anal verge. The number of the electrode rings that were seen below the anal verge was not the same in all manoeuvres. The sixteen ring electrodes are highlighted by the white dots and were not always clearly visible during the provocative manoeuvres.

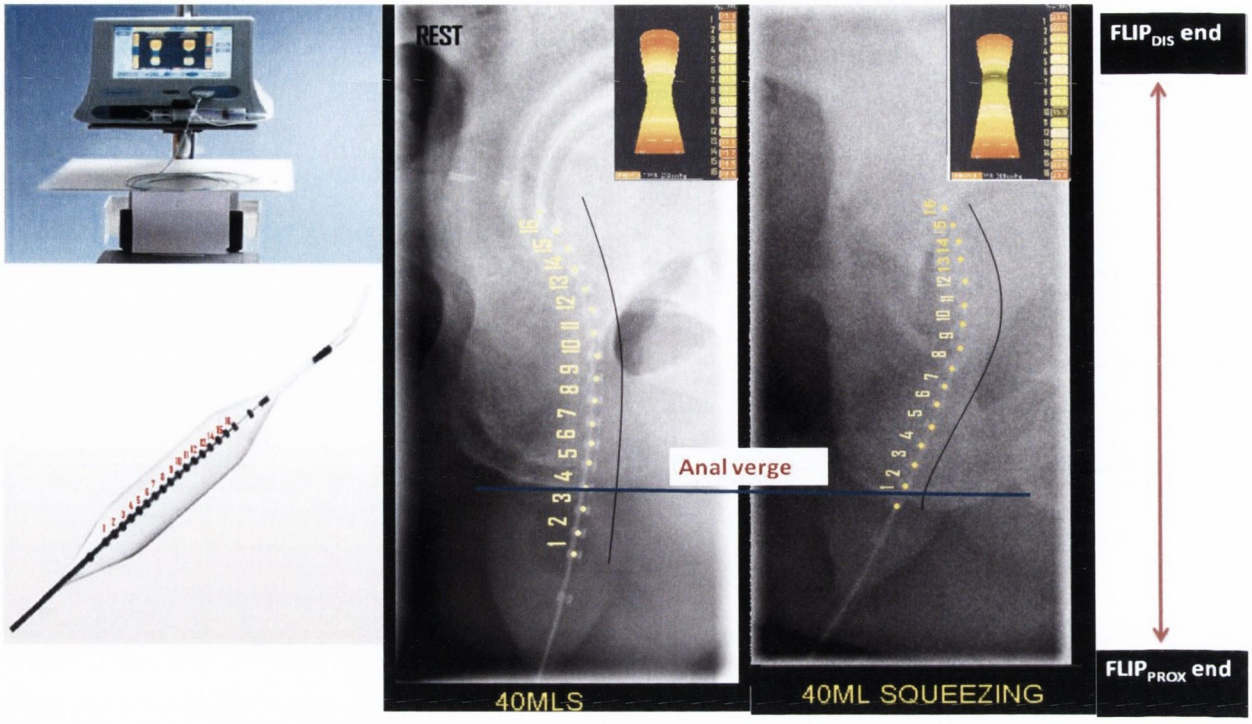

Figure 3.7: An X-ray image of EndoFLIP® in the anal canal of female volunteer during rest on the left and squeezing on the right. The blue line represents the anal verge. The sixteen ring electrodes are highlighted in yellow. The probe was angulated inside the anal canal as an effect of the anatomical structure of the region as highlighted by the thin black arrow. The angle was accentuated during the squeezing which shifts the probe toward the rectum.

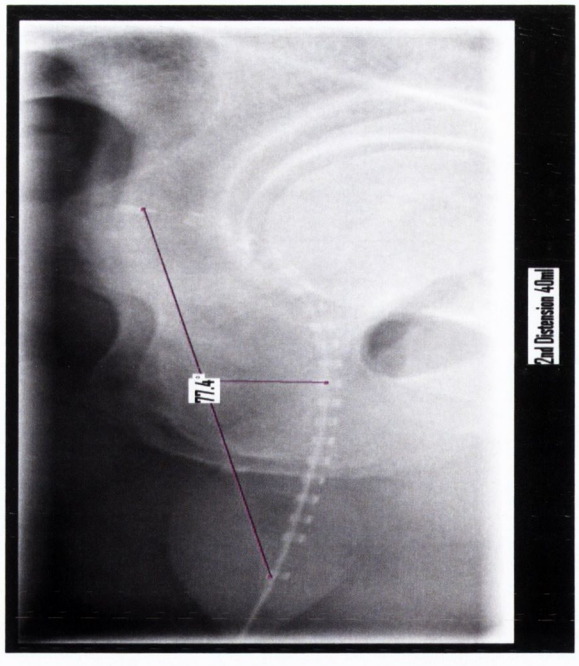

Figure 3.8: An x-ray image of the EndoFLIP® bag when inserted in the anal canal. The bag was distended by 40ml of prepared radiant conductive solution. The figure clearly shows the sixteen ring electrodes inside the probe. The distal end of the bag was located in the rectum as the probe was curved by 77.4◦.

3.3.3.2 EndoFLIP® outcome measurements

During the ramp distension from 0-40ml, a narrow zone was observed along the anal canal profile at 10, 20, 30 & 40ml bag volumes. The average estimated diameters (mm) (calculated from measured CSA (mm^2)) along the narrow zone were calculated at each volume. The length of the narrow zone was estimated as the distance between adjacent electrodes were equal to 5mm. in figure 3.9; at 10ml volume there were 12 electrodes in the narrow zone area. As a result, the narrow zone length is estimated to be (12* 5=60mm). The resolution of the narrow zone length was ±5mm. Also, the bag pressure (mmHg) measurement was obtained.

Figure 3.9 represents the anal ramp distension of a healthy male subject. The narrow zone length, narrow zone average diameter and bag pressure measurements were calculated at each volume: (60mm, 5.9mm, 2.7mmHg) at 10ml; (30mm,5.6mm, 15.6mmHg) at 20ml; (20mm, 5.4mm, 19.5mmHg) and; (10mml, 6.1mm, 39.3mmHg).

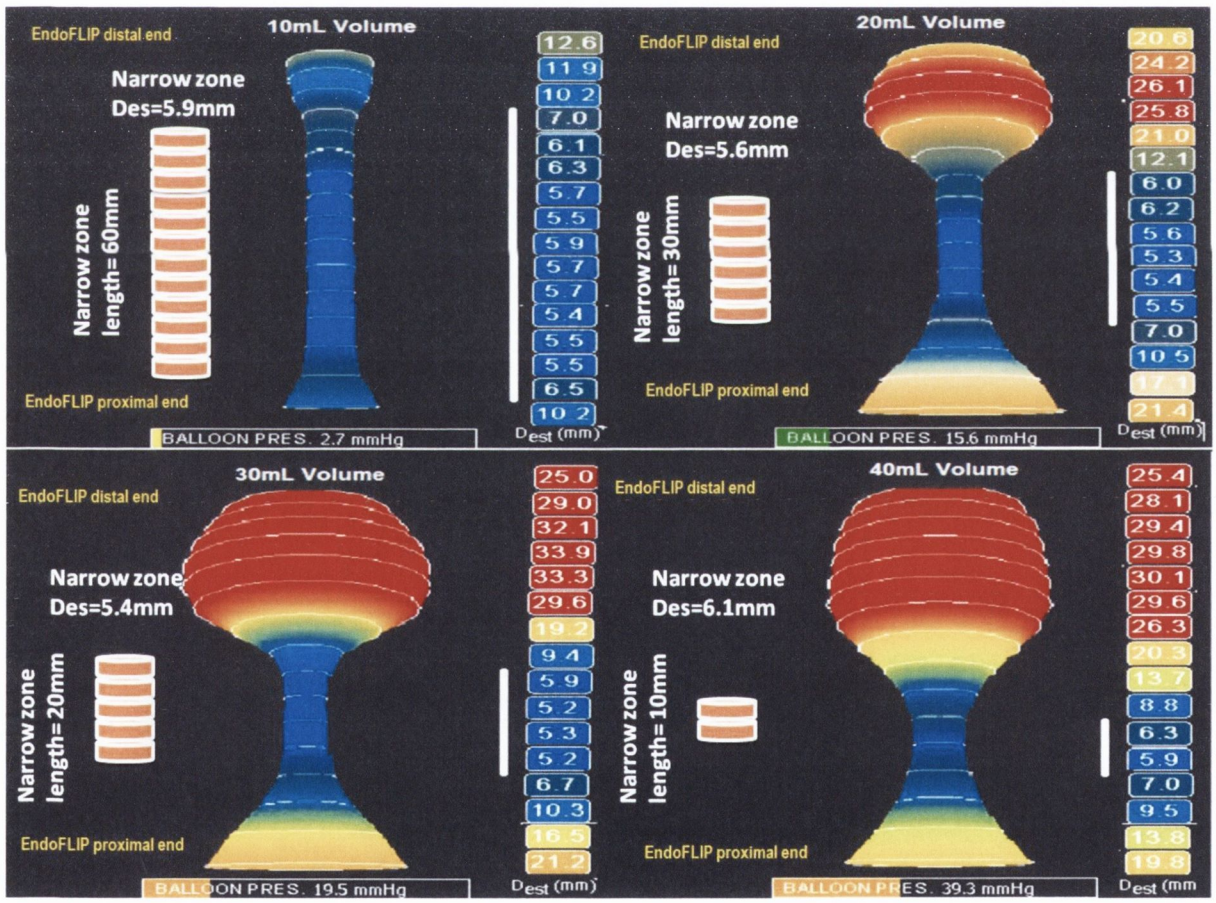

Figure 3.9: Geometric profile of the anal canal during ramp distension of the healthy male subject. The top of each profile is directed toward the distal end of EndoFLIP®. Diameters are given in the sixteen boxes on the right hand side of the FLIP diagrams and bag pressures in mmHg at the bottom of each diagram. The longitudinal white line highlights the narrow zone length at each volume. The orange rings represent the average estimated diameters along the narrow zone. A distinct narrowing towards the centre of the colour profile was observed at higher volumes. EndoFLIP® distal end was positioned toward the upper part of the anal canal and the EndoFLIP® proximal end was positioned toward the lower end of the anal canal.

Figure 3.10 represents the anal ramp distension of female FI subject. The narrow zone length, narrow zone average diameter and bag pressure were calculated at each volume: (35mm, 5.6mm, -3.9mmHg) at 10ml; (15mm,5.8mm, 0.4mmHg) at 20ml; (10mm, 7.35mm, 11mmHg) and; (5mml, 13.3mm, 17.6mmHg).

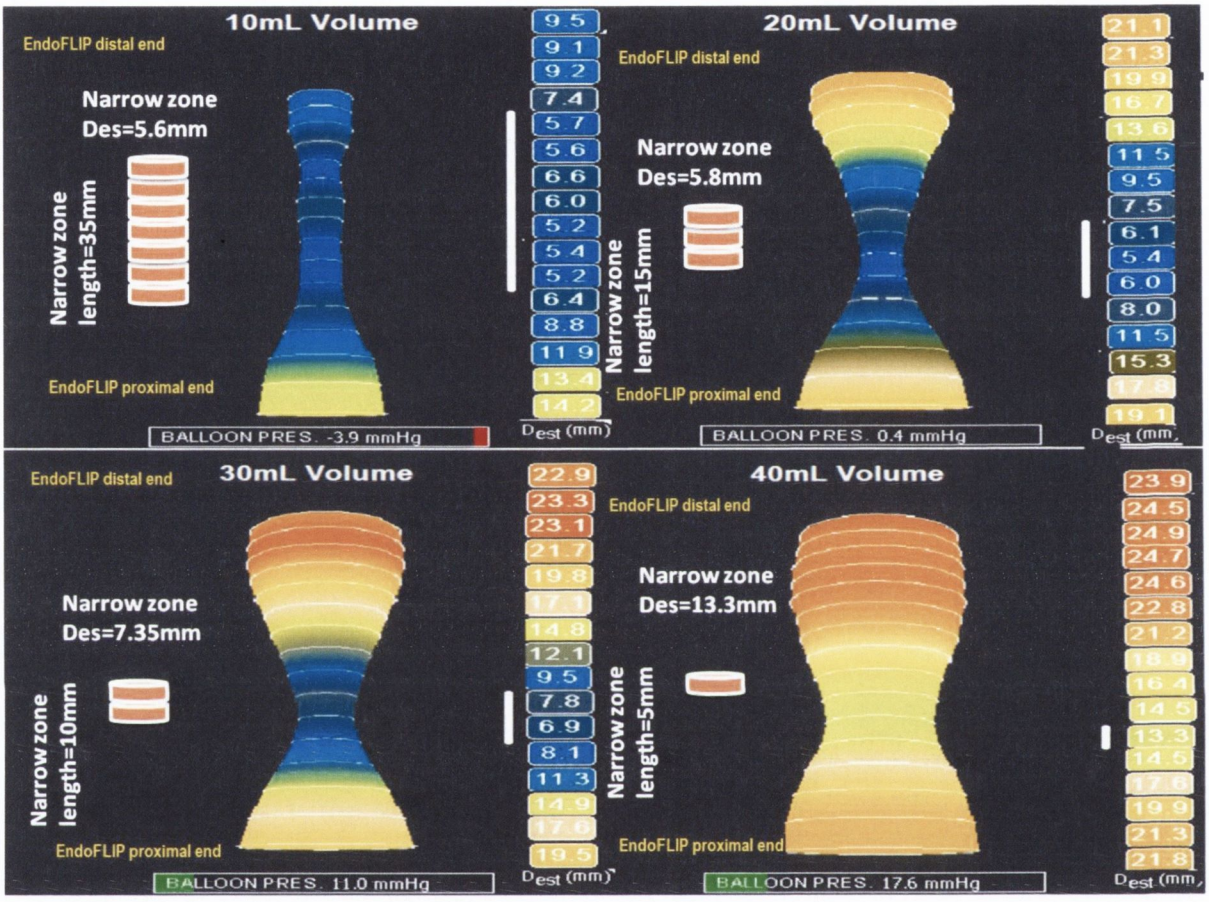

Figure 3.10: Geometric profile of the anal canal during ramp distension of the FI female subject. The top of each profile is directed toward the EndoFLIP® distal end. Diameters are given in the sixteen boxes on the right hand side of the FLIP diagrams and bag pressures in mmHg at the bottom of each diagram. The longitudinal white line highlighted the narrow zone length at each volume. The orange rings represent the average estimated diameters along the narrow zone. A distinct opening in the narrow zone was observed at higher volumes. EndoFLIP® distal end was positioned toward the upper part of the anal canal and the EndoFLIP® proximal end was positioned toward the lower end of the anal canal.

Provocative manoeuvres, rest, squeeze, cough and strain, were performed at 20ml, 30ml and 40ml step distension volumes. During each of these bag distensions, the narrow zone of the anal canal was observed on the EndoFLIP® screen.

Narrow zone length (mm) , narrow zone average estimated diameter (mm), anal bag pressure (mmHg) were measured at 20ml, 30ml and 40ml step volume distensions during resting for both volunteers. During squeezing manoeuvres, the narrow zone length and average estimated diameters were evaluated at the maximum squeezing pressures Psq1, Psq2 and Psq3 at 20mL, 30mL and 40mL step volumes respectively, see figure 3.11, 3.12.

A consistent increase in the narrow zone length during squeezing was observed at all step volumes for the healthy volunteer. See figure 3.11. For the FI volunteer, the narrow zone length during squeezing was longer than narrow zone length during resting at 20ml step volume only (see figure 3.12). The squeezing pressure was higher in the healthy volunteer than in the FI volunteer at all step volumes.

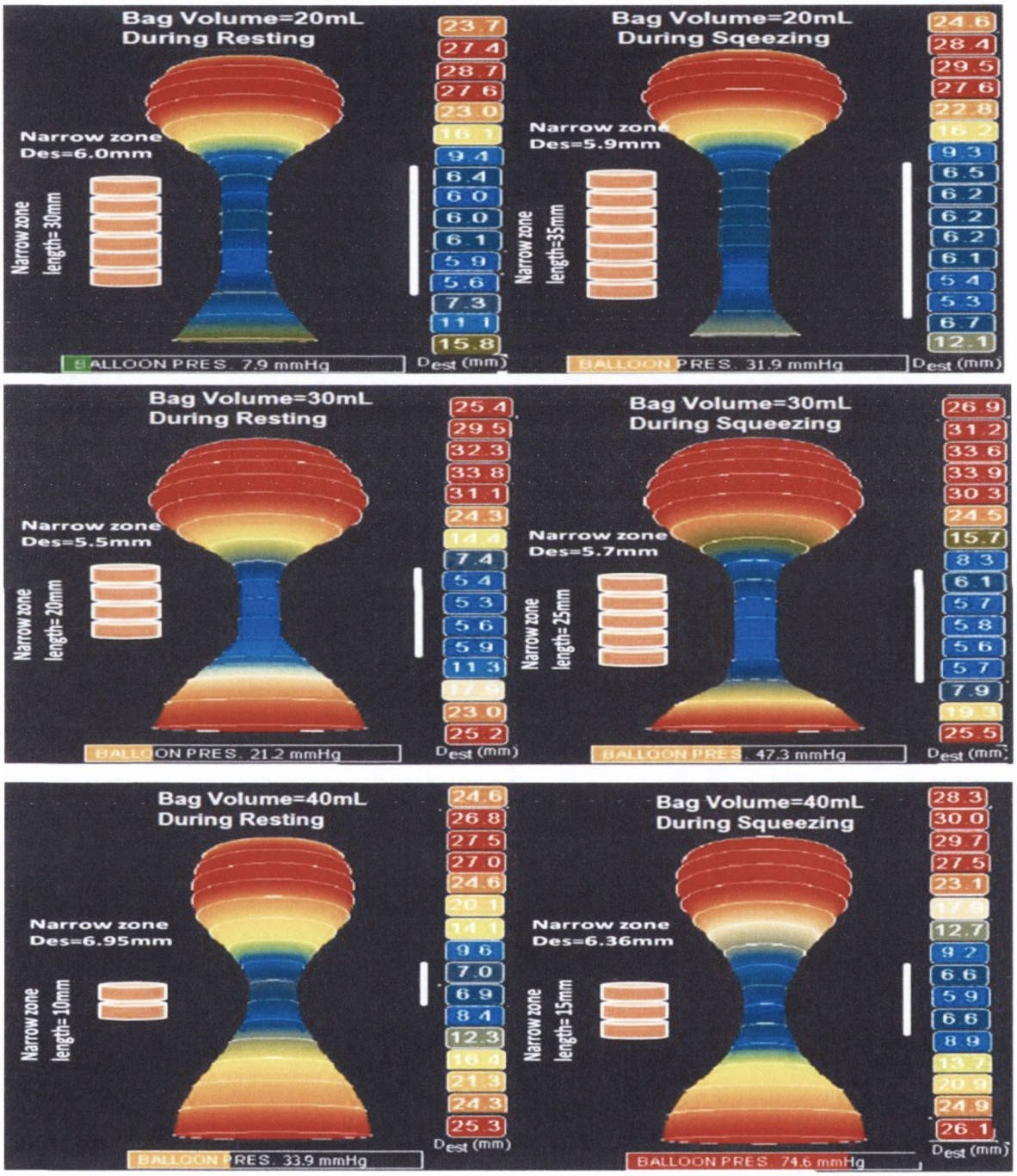

Figure 3.11: Still images from the male healthy volunteer during resting and squeezing at three step volumes 20ml, 30ml and 40ml. Diameters are given in the sixteen boxes on the right hand side of the colour profile and bag pressures in mmHg at the bottom of each diagram. The longitudinal white line highlighted the narrow zone length at each volume. The orange rings represent the average estimated diameters along the narrow zone. The narrow zone was tightly closed during both resting and squeezing manoeuvres. Both narrow zone length and bag pressure increased noticeably during squeezing at each step volume. EndoFLIP® distal end was positioned toward the upper part of the anal canal and the EndoFLIP® proximal end was positioned toward the lower end of the anal canal.

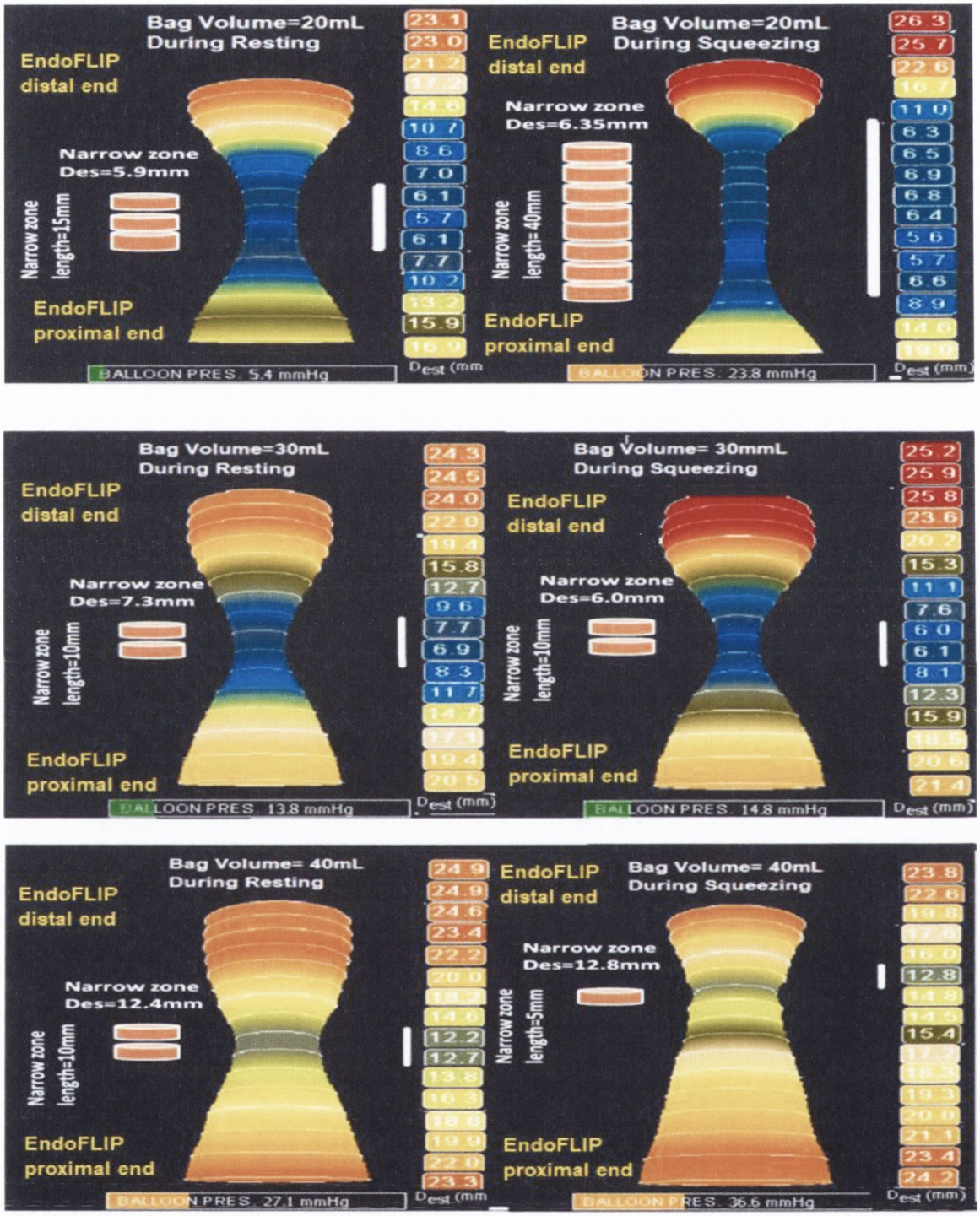

Figure 3.12: Still images from the female FI volunteer during resting and squeezing at three step volumes 20ml, 30ml and 40ml. Diameters are given in the sixteen boxes on the right hand side of the colour profile and bag pressures in mmHg at the bottom of each diagram. The longitudinal white line highlighted the narrow zone length at each volume. The orange rings represent the average estimated diameters along the narrow zone. The estimated diameter of the narrow zone increased significantly at 40ml volume. Narrow zone length increased during squeezing only at 20ml step volume. The bag pressure increased during squeezing at all step volumes. EndoFLIP® distal end was positioned toward the upper part of the anal canal and the EndoFLIP® proximal end was positioned toward the lower end of the anal canal.

During squeezing, the pressure started to increase to its maximum value, then it was stable for a period of time (t) before it reverted back to its resting status, see figure 3.13. The duration between the onset increases in squeezing anal bag pressure (Psq) until the return to the resting pressure was called the squeezing duration time (t); t1 equal to15 seconds was calculated at the 20ml step volume; t2 equal to10 seconds was calculated at the 30ml step volume and; t3 equal to 10 seconds was calculated at the 40ml step volume. Figure 3.13 illustrates how to calculate the squeezing time in one healthy volunteer.

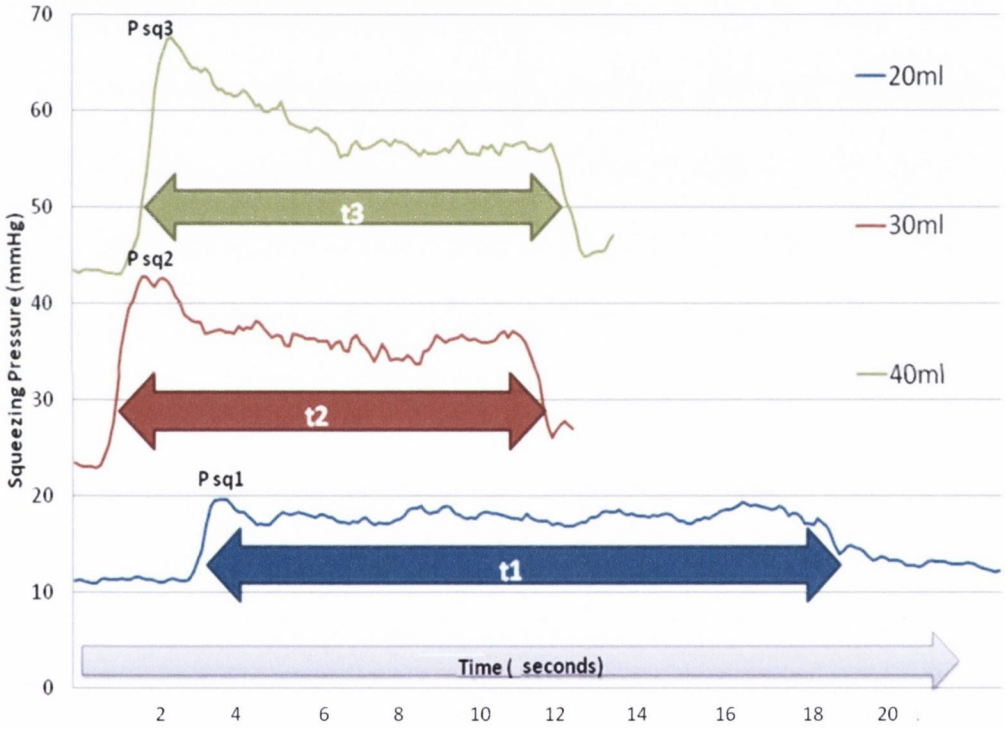

Figure 3.13: Squeezing pressure at three step distensions 20ml, 30ml and 40ml. t1, t2, t3 represents the squeezing duration time at 20ml, 30ml and 40ml respectively.

The sequence of estimated diameter and pressure changes over time during anal coughing and straining is represented in the colour contour plots. During coughing, the bag pressure increased from its baseline to the maximum cough pressure with no change in the narrow zone location along the anal canal in healthy volunteer; however, the bag was ejected outside the anal verge before coughing pressure reached its maximum value in FI volunteer, see figures 3.14.

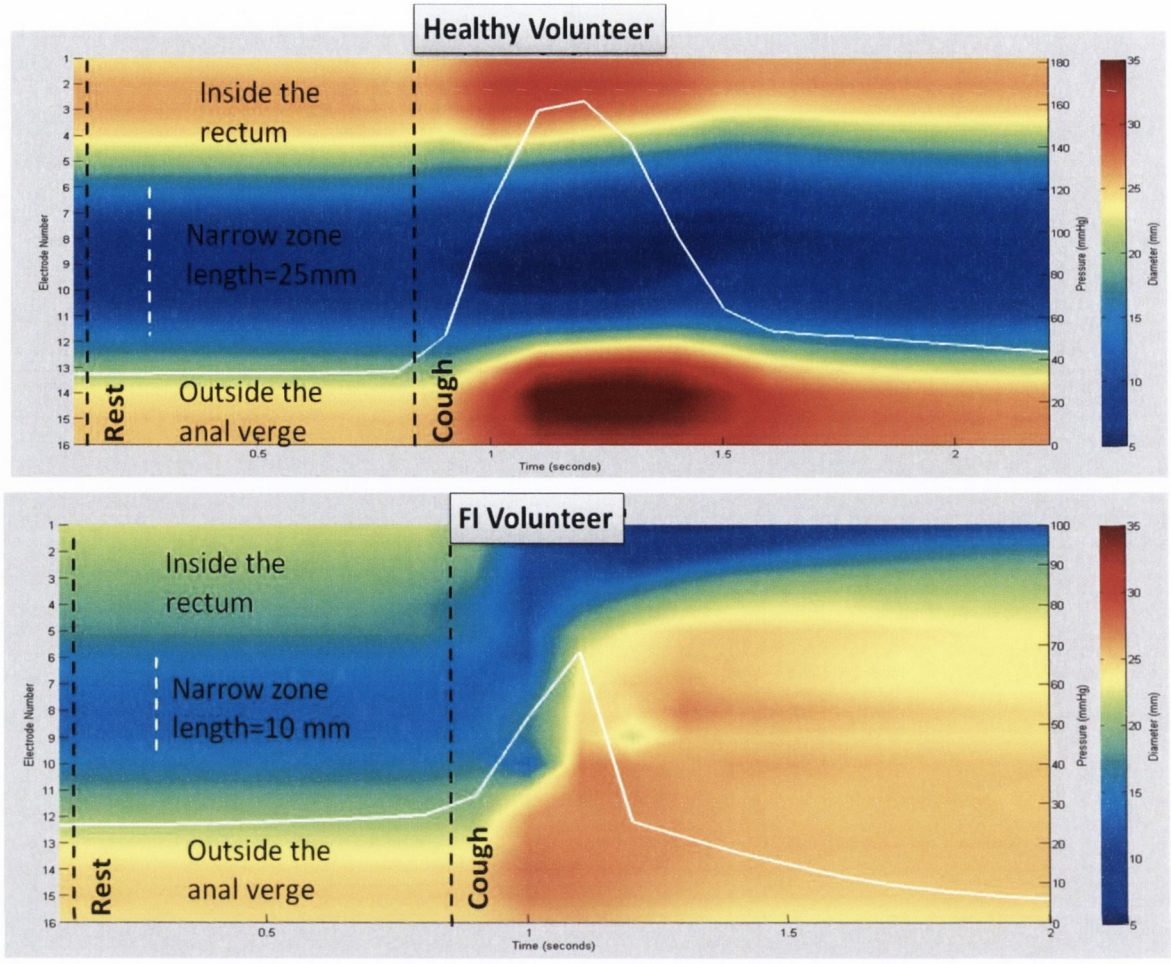

Figure 3.14: The contour plot of the anal canal during a single cough for the healthy male subject and the FI female subject. The white line represents the pressure within the bag (mmHg). The colours indicate the diameter present at specific point in time at specific electrode pair. The red/ yellow colour shows the large diameters of the bag in the rectum (top) and outside the anal verge (bottom). The blue colour represents the narrow zone part of the anal canal. In healthy volunteers the blue zone still in the middle during rest and cough which indicate that the bag still in position. In FI the blue zone is found at rest and moved toward the top during coughing which indicate that the bag was ejected.

During the straining manoeuvre, the bag pressure increased gradually and the probe steadily moved outside the anal canal over a time of 3 seconds; however, the probe was suddenly pushed out of the anal canal in the FI volunteer, see figure 3.14. The time needed to push the EndoFLIP® bag outside the anal canal during the straining manoeuvre was called the evacuation time and was calculated when the bag was inflated with 40ml volume.

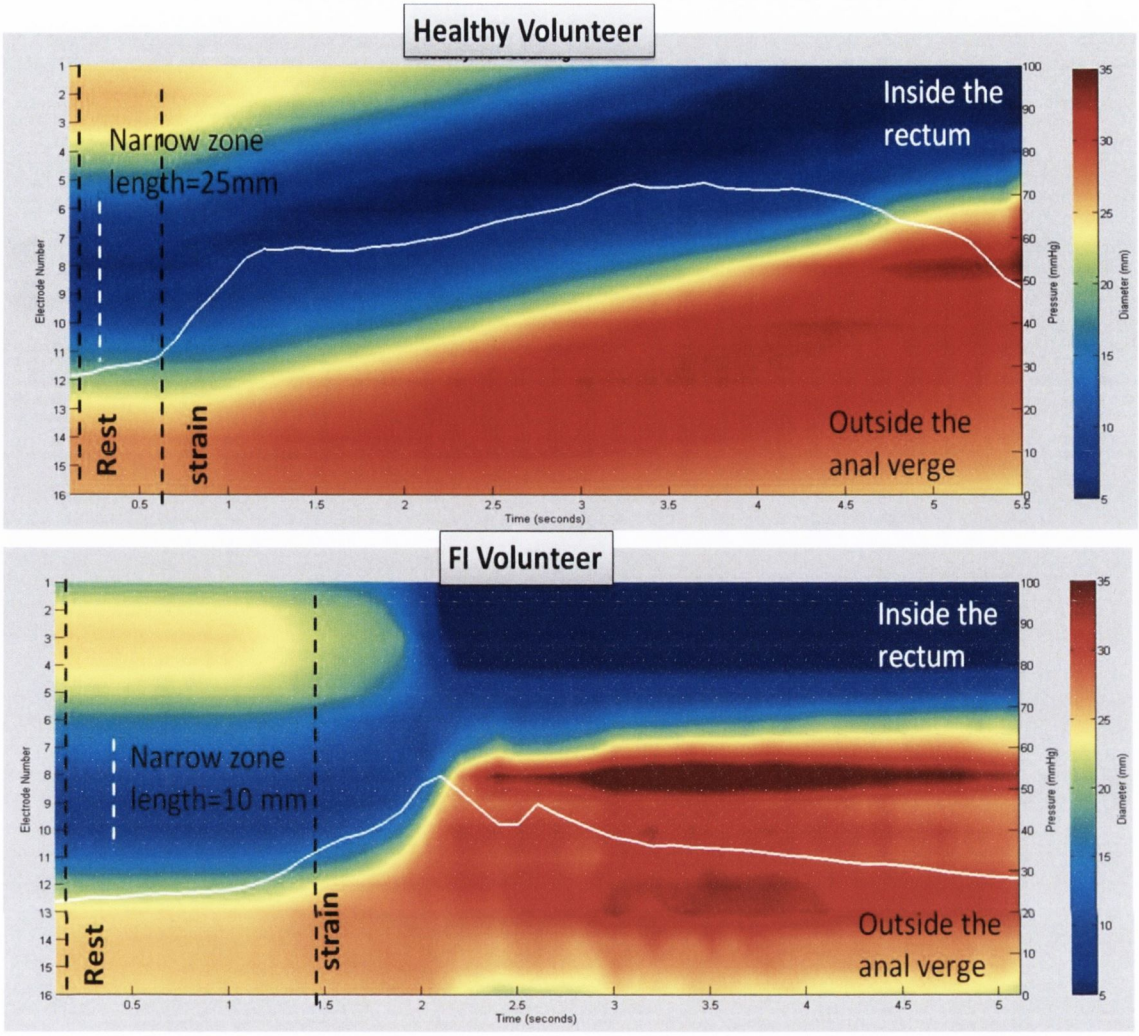

Figure 3.15: The contour plot of the anal canal during straining for the healthy male subject and the FI female subject. The white line represents the pressure within the bag (mmHg). The colours indicate the diameter present at specific point in time at specific electrode pair. The red/ yellow colour shows the large diameters of the bag in the rectum (top) and outside the anal verge (bottom). The blue colour represents the narrow zone part of the anal canal. The blue zone is shifting gradually toward the top in 6 seconds in healthy volunteers and is shifting quickly in 0.5 seconds in FI.

3.3.4 Discussion

The EndoFLIP® bag was inserted into the anal canal and inflated with radiant conductive solution to allow for the monitoring of the bag position on the EndoFLIP® system and under video-fluoroscopy at the same time. Whilst every effort was made to ensure the full observation of the bag during ramp distensions and provocative manoeuvres, some of the X-ray images contained indistinguishable electrode rings along the bag. The most unambiguous images of the EndoFLIP® bag for both volunteers were presented and the electrode rings were highlighted.

During the clinical pilot study, the starting time and the ending time of the provocative manoeuvres were registered as seen on the EndoFLIP® screen. At the same time, video-fluoroscopy images were marked at the start and the end of each manoeuvre. This allowed us to correlate the images from both techniques with confirmation from a trained radiologist.

The probe was found to be curved by 77.4◦ as a result to the anatomical structure of the region. The measured angle of the probe inside the anal canal was very similar to the ano-rectal angle at rest (see section 1.4.3). In addition, this study confirmed the lateral movement of the bag during different manoeuvres even when the probe kept in position.

The distal end of EndoFLIP® bag was inflated in the rectum while the proximal was seen outside the anal verge as a part of the probe positioning protocol. When one of the detecting electrodes was located in the anal canal while an adjacent electrode was outside the anal verge, the slope of the wall between the detecting electrodes might affect the measurements accuracy. However, previous study confirmed that this slope has no significant influence on the CSA measurements (Published Thesis, Regan j. 2014).

Distensibility as described in the oesophago-gastric junction (OGJ) (see section1.11.5.1) is a measure of the minimum CSA to the pressure in mmHg/mm^2. However, the minimum CSA parameter alone was not able to describe the morphological changes along the narrow zone in the anal canal. At low volumes a narrow zone formed along the EndoFLIP® bag in the anal canal. In the healthy volunteer, both distal and proximal ends of the EndoFLIP® bag were relaxed while the central section was tightly closed at high distension volumes. When the distal and proximal ends started to open, the narrow zone length was shortened. Accordingly, the researchers concluded that two parameters can define these changes; the narrow zone average CSA and the narrow zone length.

The narrow zone was defined as the most constricted area along the anal canal where the difference between two adjacent estimated diameters is less than or equal to 1mm. The narrow zone was calculated for each subject during ramp distension and provocative manoeuvres. During ramp distension for example, the sixteen diameters as measured using EndoFLIP® technique were defined at 10ml volume in the bag and the lowest measured diameters were calculated. When the difference between two adjacent diameters were more than or equal to 1mm the proximal and distal borders of the narrow zone were found to be distinct. Once the narrow zone was defined in this manner the diameters were averaged to calculate the average cross sectional area and the length of the narrow zone to the nearest 5mm was estimated as the distance between adjacent electrodes were equal to 5mm. See figures 3.9-3.12. Hence, the anal canal distensibility is a measure of three parameters: estimated narrow zone length (mm), average narrow zone CSA (mm^2) and, bag pressure (mmHg).

Morphological changes in the narrow zone as well as bag pressure were observed during resting and squeezing manoeuvres at different step volumes. Therefore, both narrow zone length and average narrow zone CSA were measured during resting and at the maximum squeezing pressure. The duration of the squeezing was calculated at each step volume. The latter parameters can be measured in health and in disease.

The contour plots were used to present the diameter and pressure during coughing and straining. During coughing, narrow zone average CSA and length were measured if the bag resided in position. On the other hand, only minimum CSA was measured during straining as it is impossible to track the narrow zone during this manoeuvre. In addition, the time need to evacuate the bag was evaluated.

3.4 Pilot study 2: Endoanal Ultrasound validation tool

3.4.1 Objectives

Evaluation of the anal canal muscle structure is based upon history and physical examination, and upon imaging studies using endoanal ultrasound. The main objective of this study was to correlate anal canal muscle structure with the outcome still images from EndoFLIP® at different portions along the anal canal using endoanal ultrasound.

3.4.2 Materials and methods

3.4.2.1Subjects

Two volunteers were studied; the first volunteer was a 75 year old female and the second volunteer was an 85 year old male, both volunteers were diagnosed with faecal incontinence because of weakness in anal sphincters. A standard ano-rectal manometry test was performed to measure the volunteers' anal sphincters function before the study. Ethical approval was obtained (see appendix). Informed consent was signed by the participants before the procedure.

3.4.2.2 Equipment

An ano-rectal ultrasound probe, B-K Medical (6004 10Mz) endo-probe (Sandoften9, 6004 Gentofte, Denmark) was used for the study. This has a mechanically rotated 10-MHZ single crystal protected by a hard TPX plastic cone (rigid balloon), see figure 3.16.

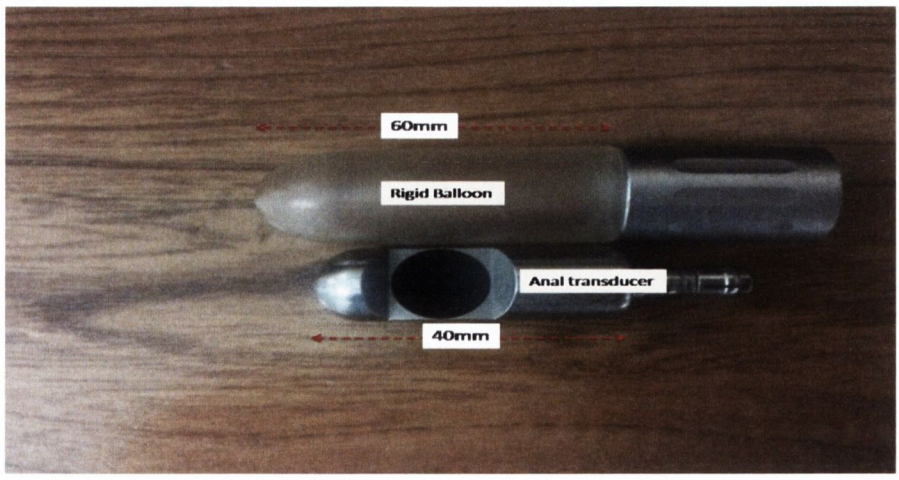

Figure 3.16: The ultrasound probe was assembled by filling the small rigid balloon (60mm) on the end of the ultrasound probe with degassed water then covering the end of the probe with a latex balloon. The evaluation of the anal sphincter was done using special radial rotary transducers (15mm) spinning 360° around a fixed shaft.

The EndoFLIP® equipment set up already described previously (See section 1.10.5) was employed.

3.4.2.3 Study protocol

The protocol for the ramp distension using EndoFLIP® was implemented as described previously in pilot study 1(see section 3.3.2.3). See figure 3.5.

For the EAUS study, the volunteer was lying in left lateral position as this minmics deformity of the upper canal and PRM. The EAUS probe (40mm) was gently inserted and rotated until the 'U' shape of the PRM is visible. An image at 30mm depth was recorded (marked as A in figure 3.17). The probe was slowly withdrawn around 10mm down the canal as multiple images were taken to record the overview of the overlapping of the anal sphincters (marked as B in figure 3.17). Then, the probe was withdrawn around 10mm and the image of the EAS was recorded (marked as C in figure 3.17). After that, the EndoFLIP® was positioned in the anal canal and the number of electrodes that were seen outside the anal verge was counted. See figure 3.17 (ii). The EndoFLIP® was distended with 40ml conductive solution at rate of 40ml/min. the anal profile at 20ml, 30ml and 40ml was defined.

The anal canal profile which was recorded at a distance of 30mm from the anal verge was compared with the ultrasonic images of the anal canal that was recorded at 30mm, 20mm and 10mm distance from the anal verge.

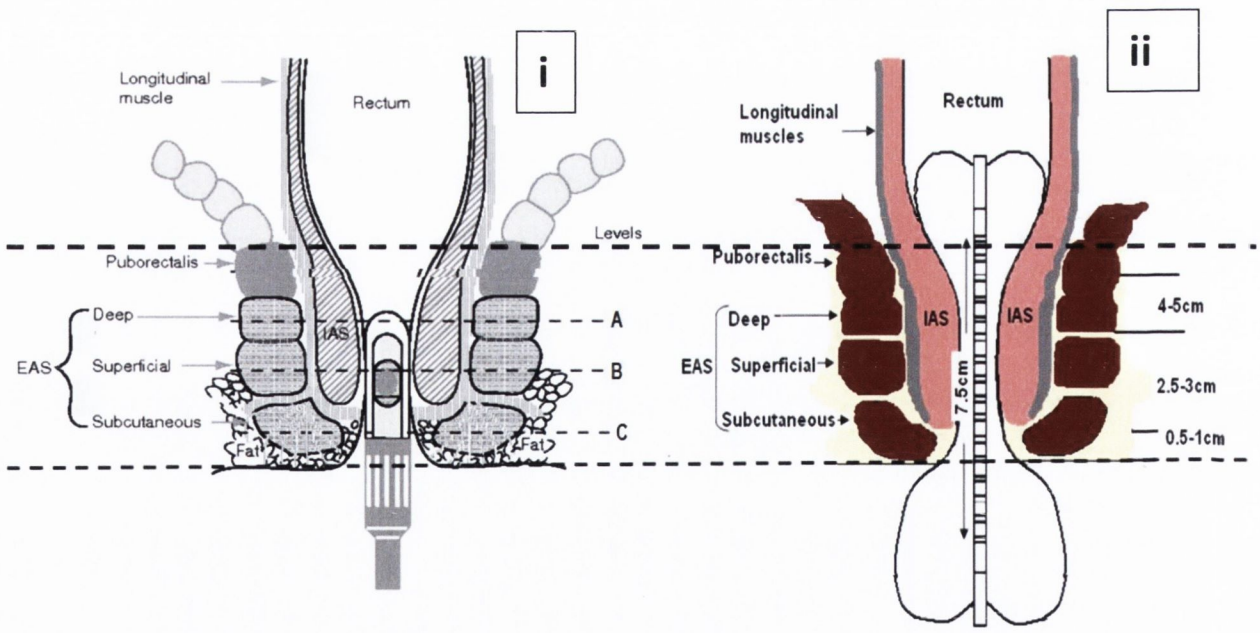

Figure 3.17: A comparison between the anal canal (upper (A), middle (B) and lower (C) levels as measured by the ultrasound probe (i) and the anal canal profile as measured by the EndoFLIP® (ii). The EAUS probe was used to scan the muscular structure around the probe in the upper part (A) of the anal canal (40mm in depth from the anus). Then the probe was withdrawn down approximately 5-10 mm toward the middle of the anal canal (B). Then the probe was withdrawn down again approximately 5-10 mm toward the lower end of the anal canal.

3.4.3 Results:

For two FI volunteers, anal profile images at 20ml, 30ml and 40ml ramp volumes were compared with ultrasonic images of the upper (A), middle (B) and lower (C) portions of the anal canal (see section 1.10.5). Distinct ultrasonic images were observed at each of the three sections along the anal canal. In the upper section, the PRM which composed of striated muscles appeared as a non-complete circle of white shade. The IAS which composed of smooth muscles appeared as a thick black ring inside the white shade. In the middle section, the EAS formed a complete ring of white shade. The IAS contrasted sharply against the EAS and appeared as a black ring. In the lower section, the white shade was the thickest as the subcutaneous EAS defined the lower part of the anal canal .See figures 3.18 &3.19.

As the distance between the ultrasonic images at each portion is approximately 5-10mm, the structure of the muscles along the narrow zone can be defined. Portion A was referred to the distal end of the narrow zone, portion B was referred to the middle part of the narrow zone and level C was referred to the proximal end of the narrow zone. The anal verge was the reference point for the two techniques.

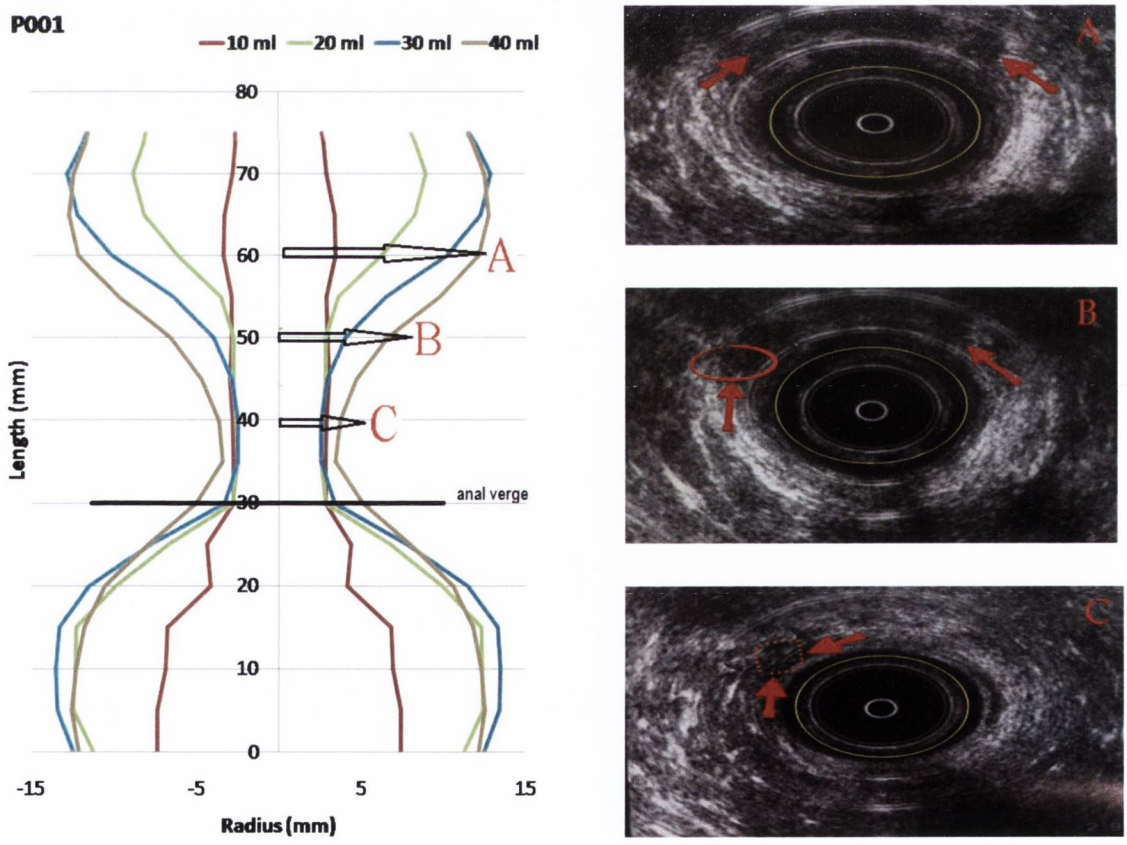

Figure 3.18: A representation of images for female patient (p001). On the left (EndoFLIP®), anal canal profiles at 10ml, 20ml, and 30ml and 40ml ramp distensions. On the right (2.EAUS) A, B and C represented the upper, middle and lower portions of the anal canal as measured by the Endo-anal Ultrasound. On the right the muscular images measured by the ultrasound probe at three levels. The muscular image consisted of a striated muscle sling (white shaded) and a thick circle of smooth muscle (black shaded) around the probe. The red arrows indicated the striated muscles sling ends and the yellow

circle highlighted the smooth muscle structure. The small dotted red circles at B&C represented a muscular defect in the striated muscles.

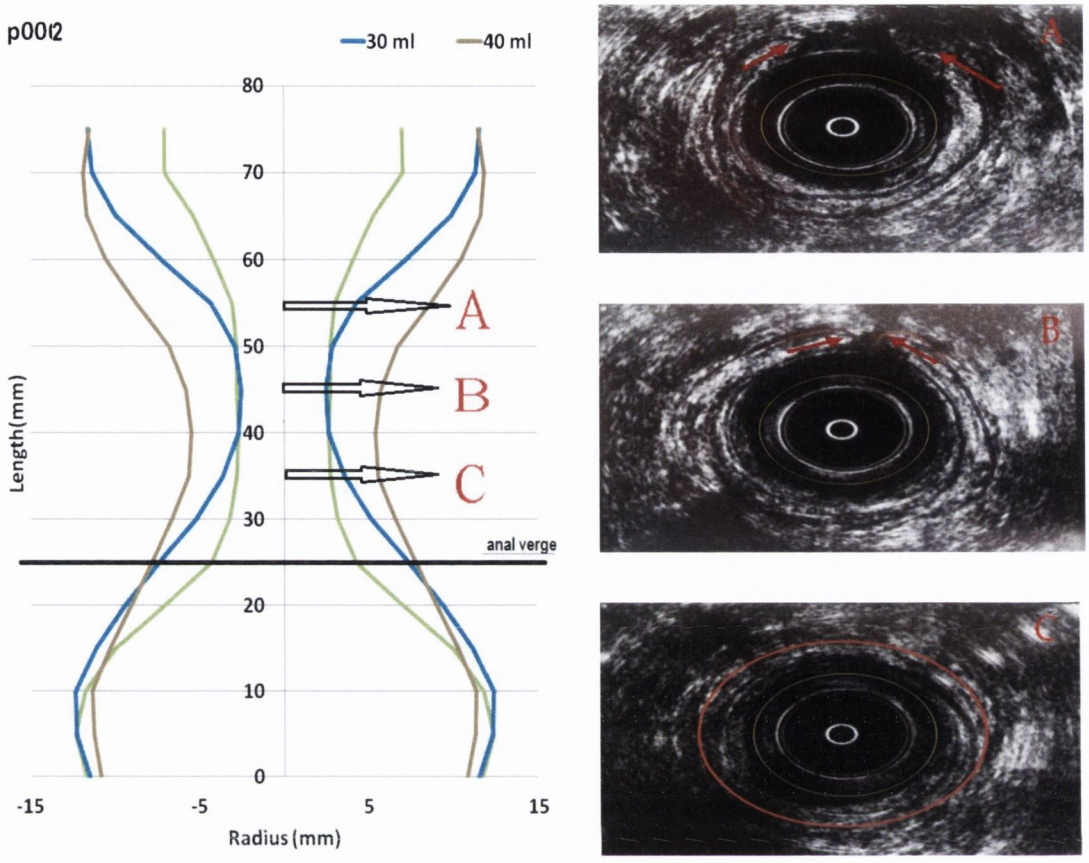

Figure 3.19: A representation of images for female patient (p002). On the left (EndoFLIP®), anal canal profiles at 10ml, 20ml, and 30ml and 40ml ramp distensions. On the right (2.EAUS) A, B and C represented the upper, middle and lower portions of the anal canal as measured by the Endo-anal Ultrasound. On the right the muscular images measured by the ultrasound probe at three levels. The muscular image consisted of a striated muscle sling (white shaded) and a thick circle of smooth muscle (black shaded) around the probe. The red arrows indicated the striated muscles sling ends and the yellow circle highlighted the smooth muscle structure.

3.4.4 Discussion

This preliminary study measured the muscle structure of the anal canal at three portions: the upper, the middle and lower. It was observed during ramp distension study with EndoFLIP® that the narrow zone formed along the anal canal shortened and was moving toward the centre of the anal profile. See figures 3.18, 3.19. Endoanal ultrasound was therefore essential in defining the muscle structure of the most restricted part of the anal canal (narrow zone) at different volumes; 20ml, 30ml and 40ml.

In this pilot study, The still images of the anal canal as measured by the EndoFLIP® at different distended volume were used to define the images of the endoanal ultrasound at upper , middle and lower anal portions. In brief, the PRM and IAS demarcate the upper portion of the anal canal. The former blends into the EAS in the middle part of the canal, forming a complete ring anteriorly and the IAS is thickest in the middle portion of the anal canal. The subcutaneous EAS, lying below the termination of the IAS, defines the lower portion of the anal canal.

The structure identified with endoanal ultrasound was compared to findings with FLIP supposedly at the same location. In previous study, subcutaneous ultrasound of FLIP bag distended in the anal canal had confirmed that distending the muscles of the pelvic floor can displace the probe (272). This was considered as a source of error in this study and it was suggested that large number of subjects are needed for validation of the probe positioning technique.

After comparing the ultrasonic images of the three portions of the anal canal with their locations on the narrow zone measured with EndoFLIP® at 20ml, 30ml and 40ml distension volumes, it was observed that the two anal sphincters IAS and EAS, together with PRM were acting in a complementary way in maintaining continence depending on the amount of volume inside the anal canal. The muscle structure of the three portions (A,B &C) were responsible for the narrow zone at low volume , and as more volume was inflated, the narrow zone tended to move to the middle of the anal canal which represents the most overlapping of the EAS and IAS at the middle portion (B).

3.5 Conclusion

Previously in chapter two, the researcher discussed an approach of measuring the anal canal distensibility using the EndoFLIP®. In this chapter two imaging techniques, video-fluoroscopy and ultrasound, were carried out to confirm the outcome from EndoFLIP® as an imaging functional technique. A radiant-opaque solution was prepared with specific concentration to visualize the EndoFLIP® bag along the anal canal during the video-fluoroscopy study and at the same time was capable of measuring the CSAs measurements using EndoFLIP® technique.

Video-fluoroscopy was capable of confirming the positioning technique as described in the EndoFLIP® protocol. In fact, it was able to demonstrate that the bag was shifting during the distension and the manoeuvres as a normal response to the changes in the ano-rectal angle. The researchers introduced a new definition of distensibility in the anal canal that can measure the morphological changes in the area in two directions and can overcome the limitation the EndoFLIP® displacement along the anal canal.

This work correlates the narrow zone that formed along the anal canal at different volumes with the muscular structure of the anal canal at each volume. EAUS studies in this work confirm that the changes to the ano-rectal region during distension with EndoFLIP® correlate well with muscle structures observed with ultrasound, furthermore, verifying the position of EndoFLIP® in the ano-rectal region and its potential for demonstrating function in health and disease.

Chapter Four: Clinical Applications of the Functional Lumen Imaging Probe in the Ano-Rectal Region

4.1 Introduction

The role of EndoFLIP® in clinical practice for use in the ano-rectal region evaluation has been investigated recently. New studies have demonstrated safe insertion and distension of the EndoFLIP® bag in the anal canal under video-fluoroscopy (see chapter three) and examined the capability of FLIP technique in measuring anal sphincters distensibility in healthy volunteers and patients with FI (256-259). Based on these initial studies, researcher hypothesizes that EndoFLIP® may provide new information on ano-rectal region characteristics during distension and provocative manoeuvres testing.

The control data of the anal sphincter distensibility and anal canal morphology in adult healthy volunteers as measured by EndoFLIP® during distension and the anal provocative manoeuvres will be discussed in this chapter to address the third research question. After that, anal sphincter distensibility and anal canal morphology at rest and during provocative manoeuvres will be evaluated using EndoFLIP® in a population of FI with anal sphincter dysfunction. In addition, the quantitative measurements of anal sphincter distensibility and anal canal morphology measured by EndoFLIP® will be correlated to outcomes from ano-rectal manometry. These measurements will address the fourth research questions.

4.2 Study One: Identification of Component Muscle Function in the Ano-rectal Region of Healthy Controls using EndoFLIP® Distension

4.2.1 The objectives

The aim of this study was to identify morphological and pressure changes in the ano-rectal sphincter region by means of EndoFLIP® and to infer the biomechanical action of the region based on measured results.

The specific objectives of this study were; (1) to measure anal sphincter distensibility and morphology at rest and during provocative manoeuvres using EndoFLIP® in a group of healthy non-elderly adults and (2) to uncover any gender differences in anal sphincter distensibility and morphology during distension and provocative manoeuvres test using EndoFLIP®.

4.2.2 Materials and methods

4.2.2.1Subjects

Twenty-one healthy volunteers 36.5±2.5 years (mean ± SEM) were studied, 11 female (2 multi-parous) with no history of gastroenterology disorders. None of the subjects were taking any medication and had no medical conditions or previous surgery related to bowel disorders. Ethics was approved (see appendix1) and the studies were carried out at Aalborg Hospital in Denmark. Informed consent was signed by all participants before the procedure.

4.2.2.2 Equipment

The EndoFLIP® equipment already described previously (See section 3.3.2.2) was employed.

4.2.2.3 Study protocol

The protocol for this study is outlined in section 3.3.2.3.

4.2.2.4 Statistical analysis.

Statistical analysis was performed using a software package PASW statistics 18.0 (SPSS statistics). As a first step, numeric values were analysed for the presence of normal distribution. In cases of normal distribution, values are stated as mean ± SEM. Distensibility data was analysed by using ANCOVA on mean pressure and CSA's. The manoeuvres data was analysed using two ways ANOVA and included all individuals. The paired t-test was used to calculate the probability of the increase in the narrow zone during squeezing and coughing manoeuvres.

4.2.3 Results

All subjects underwent the planned procedure and tolerated the probe well for the duration of the study. There were no adverse incidents. Healthy control no. 15 was excluded despite a successful study as it was discovered that she had a problem with constipation which was only revealed after the study was completed.

The EndoFLIP® was positioned in the anal canal as described previously (section 3.3.2.4) at the start of the study, see figure 4.1.

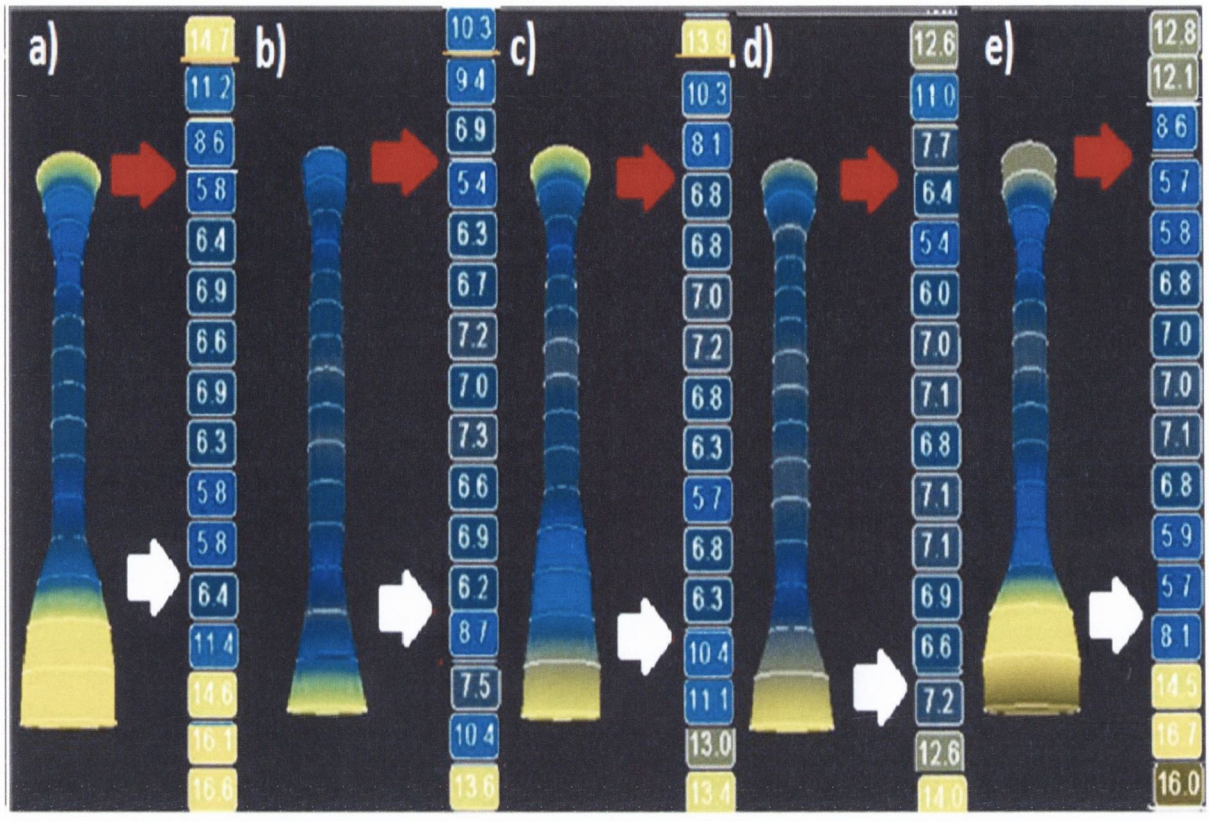

Figure4.1: Probe positioning in five volunteers. The red arrow and the white arrow pointed to the distal border and the proximal border of the anal canal profile respectively. The EndoFLIP® was retracted in the anal canal till thee CSA measurements were seen outside the distal border. The number of CSA measurements seen outside the proximal border varied according to the narrow zone length; it can contain 3-CSA measurements as in volunteers (b&d) or 4-CSA measurements as in volunteers (a&e).

4.2.3.1 Ramp Distensions

Initially during ramp distensions there was some variability in pressure readings but once the volume in the bag reached 10ml the pressure changes were consistent and smooth. The pattern of geometric changes in the ano-rectal region during the ramp distensions was very consistent across all 20 subjects. This is indicated in figure 4.2 where four examples of the geometric patterns represented by the functional images are shown at bag volumes from 10ml to 40ml. In these diagrams the top of each image is towards the rectum and the bottom of each image is outside the anal verge. D_{est} is the estimated diameter from the measured CSA. All subjects had a pattern of two narrow regions at either end of the anal profile at a volume of 10ml developing into a cylindrical channel at some point between 10 and 20ml and then by the time the bag filled to 30ml a single narrow region formed in the centre of the profile.

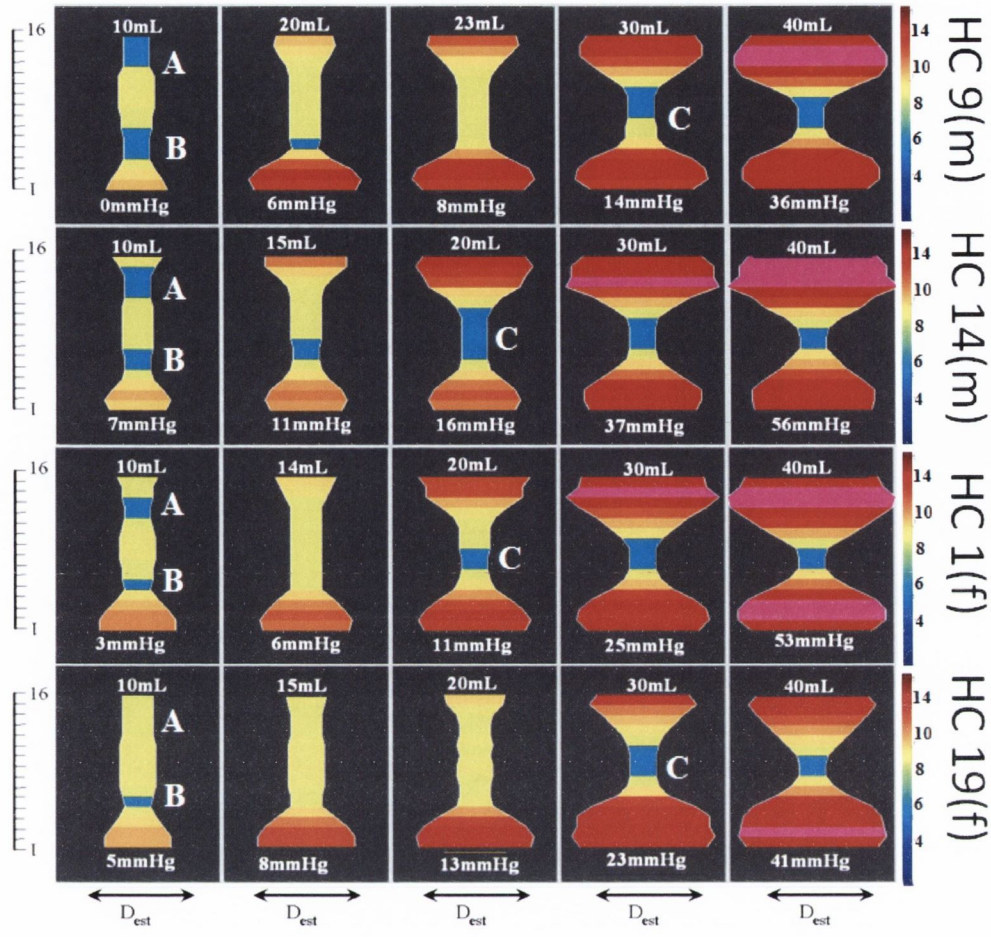

Figure 4.2: Still images of geometric changes in the ano-rectal region during distension for four volunteers. The top of each image (the upper anal canal) is towards the rectum and the bottom (lower anal canal) is outside the anal verge .The x axis represents the distended profile diameter. The y-axis on the left side represents the position of sixteen sensing electrodes across the EndoFLIP® bag with electrode 1 located toward the anal verge. The y-axis on the right side represents the changing in diameter in millimetres compared with the change in colour in the anal profiles as appeared on the EndoFLIP® display. Three main regions shown with the annotations A, B & C were formed during volume distension in the anal canal. A & B illustrate narrow regions formed at low volumes in the upper and lower segments and C illustrates a distinct narrowing towards the centre (middle segment) of the colour profile at higher volumes.

Figure 4.3 shows a scatter plot of pressure in the bag against the CSA of the minimum CSA measured in the anal profile for males and females. The pressure at which the CSA starts to increase is indicated as the opening pressure on the plot. The opening pressure in females was twice that obtained in males (11 versus 5; $P<0.001$). Once opening had occurred the rate of change of pressure with CSA was $0.086 mmHg/mm^2$ for females and $0.077 mmHg/mm^2$ for males. The distensibility index was calculated as the minCSA divided by the intra-bag pressure. There was a statistically significant difference between the distensibility index between males and females ($p>0.001$).

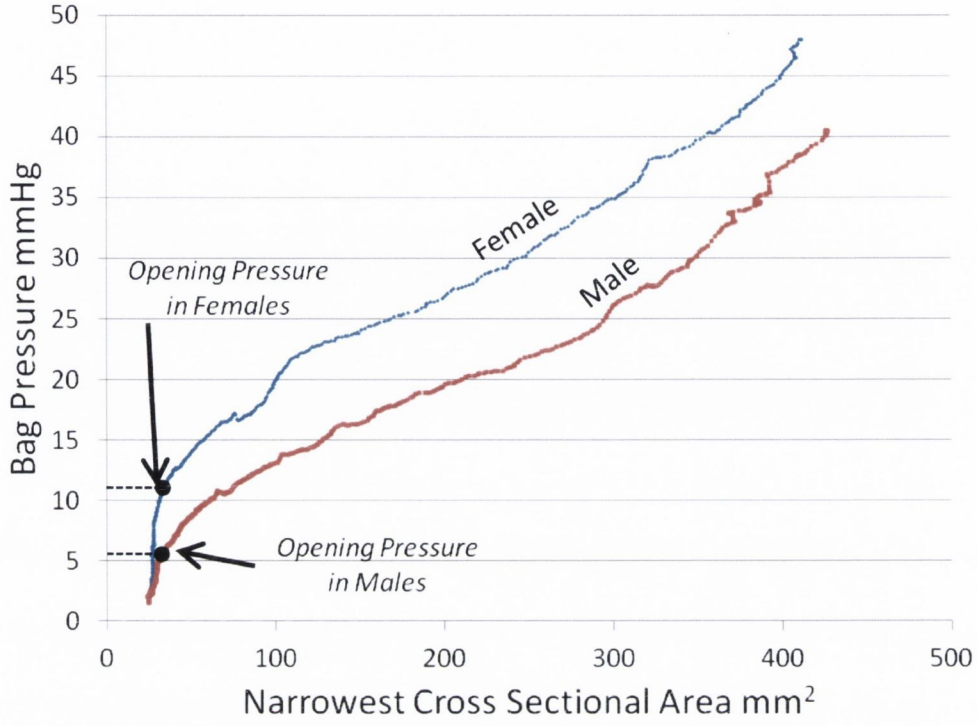

Figure 4.3: Distensibility plot of the upper part of the anal canal (pressure plotted against the minimum cross-sectional area) for ramp distensions at 40ml/min for the male and female healthy controls. The distensibility index was 0.086 for females and 0.077 for males. The opening pressure for females was higher than for males.

4.2.3.2 Squeeze Manoeuvres

The geometric pattern was observed to have subtle but distinct changes during "squeeze" manoeuvres when compared with the 'at rest' state. Figure 4.4(a) shows changes in the profile with a volume of 20ml in the bag between at rest (blue outline) and squeeze (red line) for a representative subject. The points at where the greatest changes occurred were at the two ends of the anal canal. The CSA measurement at which the greatest difference occurred at either end of the canal were selected. See figure 4.4(a). In this case the more proximal (δ prox) was toward the proximal end of the EndoFLIP® was located at measurement detector D6 and the more distal (δ distal) toward the distal end of the EndoFLIP® at detector D14. Figure 4.4(b) shows these diameter estimates (D_{est}) represented as their true CSA values. This indicates that during the squeeze manoeuvre, which correlates with the increased pressure shown by the blue tracing, the proximal anal canal CSA (δ prox – red line) initially reduced dramatically but within 4 seconds it has relaxed again to reach close to its normal value. In the meantime the distal anal canal CSA decreased a little slower initially when squeeze occurred. It maintains this decrease for the duration of the squeeze and only begins to increase to its normal CSA value after the squeeze pressure has ended. Figure 4.4(c) shows the CSAs between the δ prox and δ

distal points. They were observed to increase in CSA while the δ points were decreasing. The narrow zone shortened when squeeze occurred at higher bag volumes. This pattern was found in all healthy controls.

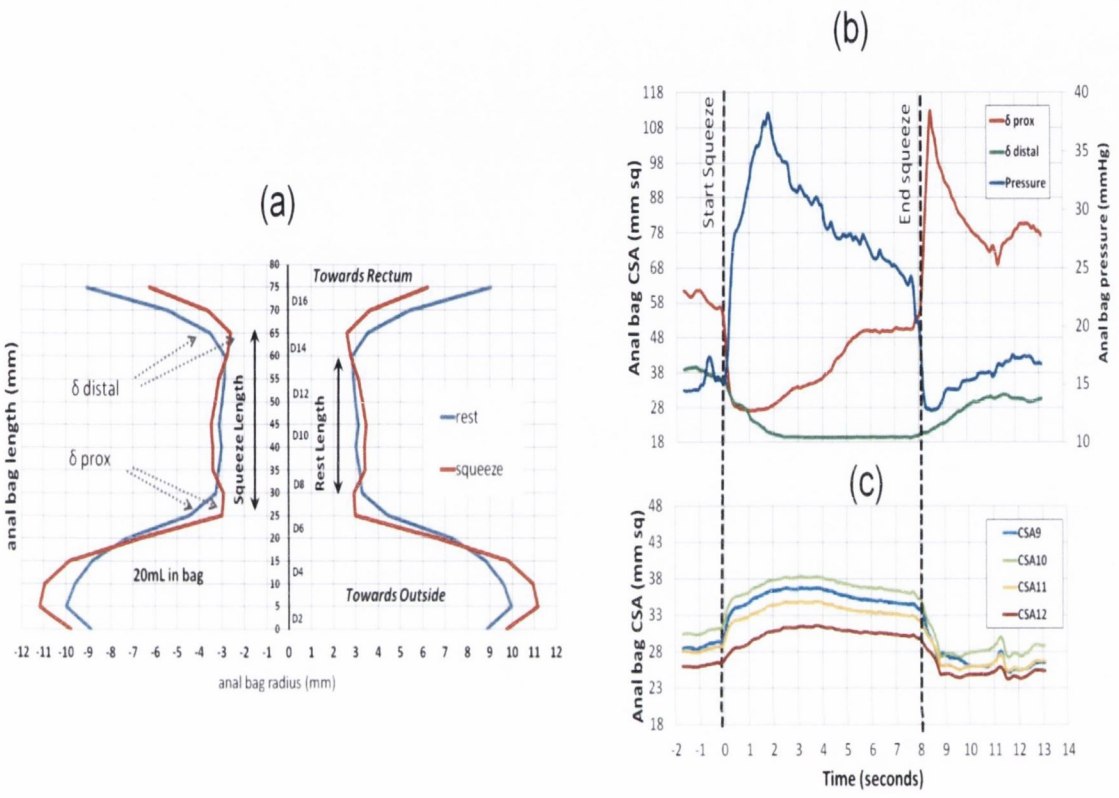

Figure 4.4: (a) The changes in the anal profile during squeezing at 20ml in the EndoFLIP® bag between at rest (blue outline) and squeeze (red line) for one health control. This image was used to determine the anal canal borders. The changes at both borders are represented by δ prox (proximal border toward anal verge) and δ distal (distal border towards rectum) which are as a result of changes in the CSA values at the relevant detectors. This figure suggests that the anal canal is narrower at rest than during squeezing and the distended narrow zone is longer during squeezing than at rest. (b) CSA for δ prox and δ distal and bag pressure for the same volunteer as shown in (a). (c) CSA measurements along the anal canal that indicted by CSA9, CSA10, CSA11 and CSA12 lie between the δ prox and δ distal CSA measurements for the same volunteer.

4.2.3.3 Cough Manoeuvres

The changes in the geometric pattern were more subtle during cough manoeuvres compared to squeeze manoeuvres. This is illustrated for one subject in figure 4.5(a). However when we observe these changes in time (figure 4.5(b)) it can be clearly seen that both δ proxy and δ distal reduce, in this case for the duration of the cough indicated by the pressure increase. In fact, in this example there is a double peak indicating a secondary cough 5 seconds after the first one and showing a repeat pattern of pressure increase and δ proxy and δ distal decrease.

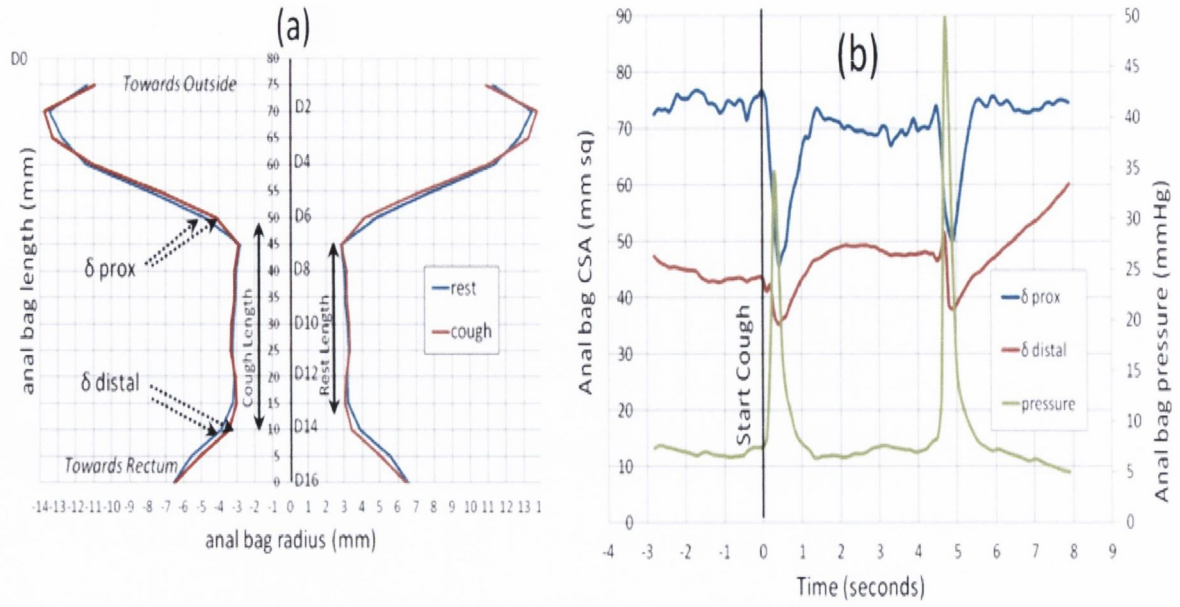

Figure 4.5:(a) The changes in the anal profile during coughing at 20ml in the FLIP bag between at rest (blue outline) and cough (red line) for one healthy control. This image was used to determine the anal canal borders. The changes at both borders are represented by δ proxy (proximal border toward anal verge) and δ distal (distal border towards rectum) which are shown as changes in the CSA values at the relevant detectors. (b) CSA measurements for δ proxy and δ distal and bag pressure for the same volunteer as shown in (a).

The pressure in the anal bag during cough was higher than during squeeze at different bag volumes (P<0.001) (figure 4.6). The pressure was significantly lower at 20ml (p=0.005).

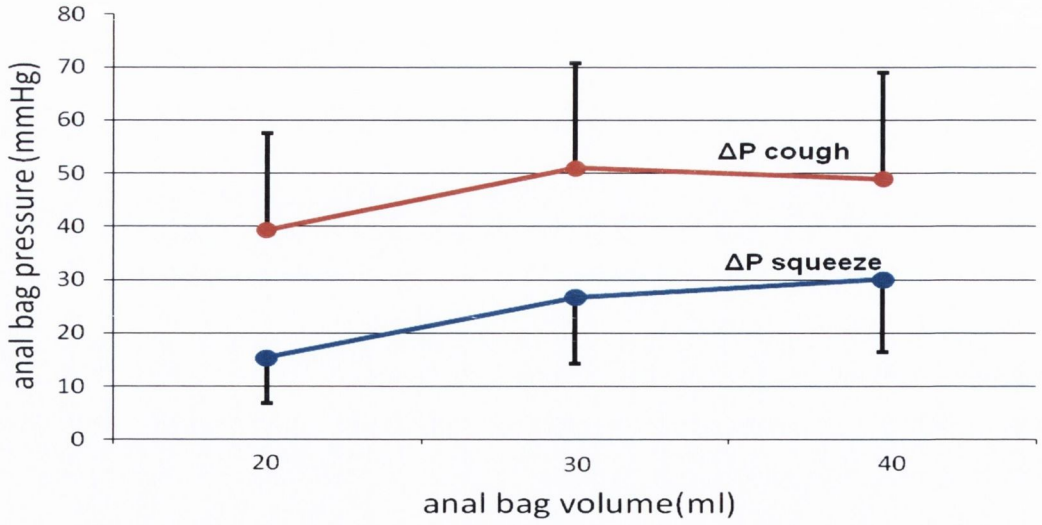

Figure 4.6: Average pressure plotted against volume during squeezing and coughing (n=20). Anal canal pressure during coughing was higher than during squeezing at all three step volumes. Also the squeezing and coughing pressure at 20ml was significantly lower than the pressure measured at 30 and 40ml.

4.2.3.4 Straining Manoeuvres

The geometric pattern was observed to have distinct changes during "straining" manoeuvres when compared with the 'at rest' state. It consisted mainly of two steps, the pressure increased at the start of the manoeuvres and then the conductive solution in the distal end of the EndoFLIP® moved to the proximal end causing a morphological changing in the distal and proximal ends of the narrow zone (an increase in δ proxy and a decrease in δ distal). See figure 4.7. A straining time was defined as the duration between the pressure at its maximum value and the change in the anal canal morphology. See figure 4.8. The straining time for males was (3.8±3 sec) significantly higher than the straining time females (0.52±0.9 sec), $p<0.001$. Besides, the increase in straining pressure for males (32.8±18mmHg) was higher than the increased in straining pressure for females (13.8±6.7) mmHg, $p<0.001$.

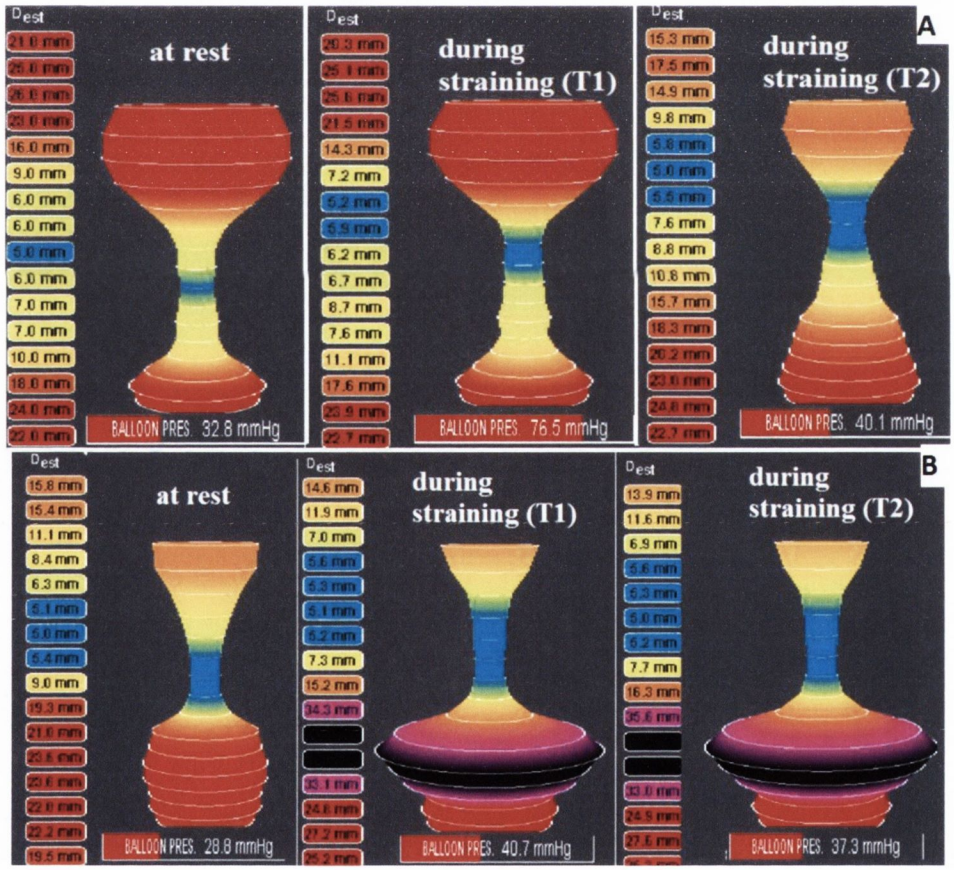

Figure 4.7: The changes in the anal profile during straining at 30ml in the FLIP bag in (A) one male and (B) one female healthy subject. The straining manoeuvre started with a sharp increase in the bag pressure at time T1 and then a change in the morphology of the anal canal occurred at T2. The straining time was defined as the time between T1 and T2.

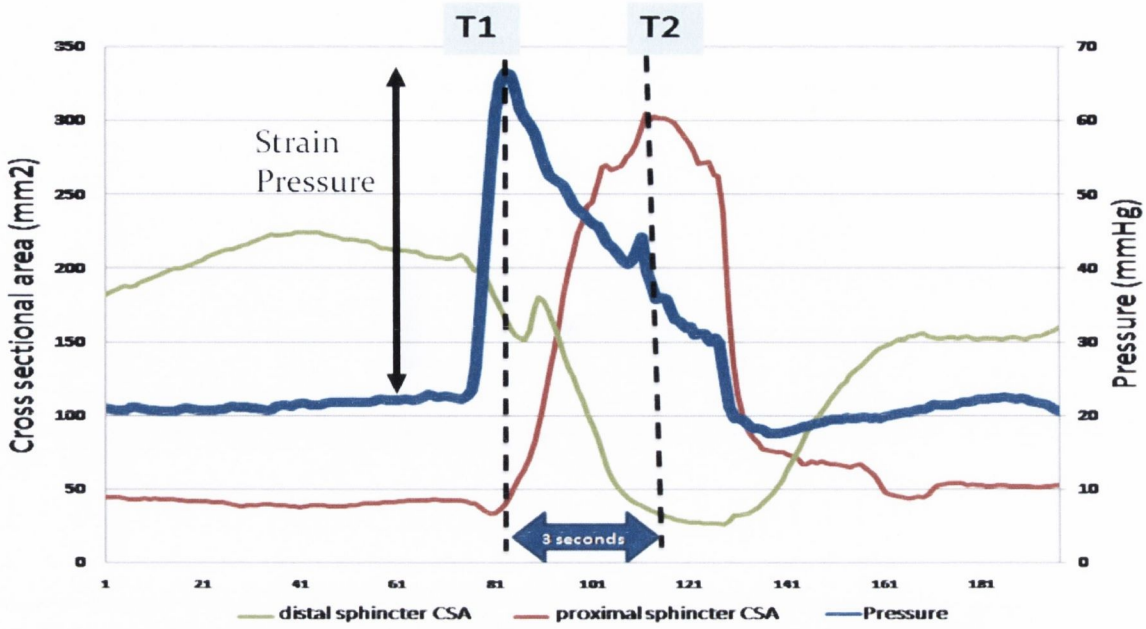

Figure 4.8: This figure illustrated the EndoFLIP® bag pressure as well as the proximal and distal CSAs of anal canal narrow zone for one subject during straining manoeuvre. The bag pressure at rest (blue line) increased to 65mmHg when the straining started. After 3 seconds the proximal CSA (red line) increased and the distal CSA (green line) decreased. There was a time (T) between the T1 (the time when the pressure reaches its max value) and T2 (the time when the anal profile change). Straining time= T2 – T1.

4.2.4 Discussion

This study represents a novel application of a technique originally developed for use in the upper gastrointestinal tract. The study demonstrates that controlled volume distension produces a remarkably similar profile for a group of 20 volunteers in a rapid, safe and effective manner. This data represents the distensibility of the anal canal region in a straightforward manner. The effects of provoking squeeze, cough and straining on the dynamics of the anal canal can be measured.

Positioning of the EndoFLIP® was important to define a common baseline between all twenty volunteers; it guaranteed that the same amount of volume was inserted distally towards the rectum area, so that the relaxation in the distal end of the anal canal during bag distension would be uniform for all volunteers. Also the physiological variation in the distended narrow zone length between gender would not affect the results obtained using this technique.

The bag fills first in low-pressure zones, primarily outside the anal verge at both ends. Also the bag filling may to some degree induce recto-anal inhibitory reflexes (as discussed in section 3.4)

While the interaction of the muscle components in the ano-rectal region is quite complex, it is amazing to see how they maintain a narrow zone in the anal canal during distension. At low volume the upper part of the anal canal was tonically contracted. In most subjects there was also an observed contraction towards the lower (toward anal verge) end of the profile. When the volume increased to

between 15ml and 25ml a full relaxation was observed indicated by the absence of blue colour on figure 4.2 which is representative of very small CSAs. At larger volumes (> 25ml) a narrow region formed in the centre of the profiles indicated by the blue region. This could be a semi-voluntary inhibition that ensures defecation is controlled. The changes in the biomechanical properties along the anal canal during control volume distension in healthy volunteers group was observed by Luft et al. They confirmed that dynamic properties during distension vary at different location in the anal canal (272).

In the present study the opening pressure of the min CSA of the anal canal was defined, see figure 4.3. The results tell us the pressure at which the upper part of the anal canal opens is lower in males than in females. Several studies have reported that manometry pressure varies between male and female. Normally the male resting and squeezing pressure are higher than the female pressure. In this study opening pressure of males was lower than female. This is contradictory to the literature but this can be related to the study size. A larger group of subjects in future work will confirm or reject this finding.

Squeeze is a standard provocative test from manometry. With EndoFLIP® the information we get from squeeze represents changes in the geometric profile of the canal. We can see that squeezing does not have a uniform effect along the anal canal profile; two distinct narrow regions formed (δ distal) and (δ proxy) which suggests two groups of muscles are involved in squeezing. Moreover these muscles are under voluntary control. It is more pronounced towards the proximal end close to the anal verge. See figure 4.4(a).

This work confirms that contraction during squeeze is not as a result of tightening throughout the anal canal. In effect the squeezing at both ends of the canal probably traps the liquid in the FLIP bag which accounts for the increase in CSA recordings at detectors D9, D10, D11 and D12 confirming the effects of the proximal end and the distal end. The liquid will take the path of least resistance and move to the part of the bag experiencing the least force, this may be a source of error and limits interpretation of the physiological significance of this observation. See figure 4.4.

Straining manoeuvre involves changes in the ano-rectal angle and anal canal length. During this study, distinct anal canal morphology was detected during resting and straining manoeuvres. Also it was observed that a specific time needed for this change. This time was defined as straining time (see figure 4.7). Using the straining time definition the researcher was able to observe a significant difference between male and female (287). We suggest that using this new technique, further information on the passage of stool through the ano-rectal region, as well as the size and consistency can be obtained. The time and pressure measurements show objective physiological differences between males and females which can be related to the anatomical structure differences between genders.

4.2.5Limitations of the current study

While every effort was made to ensure there was little or no lateral movement in the probe during this study, we accept that there may be some small amounts of movement particularly with high volume and high pressure in the bag.

The pressure seemed not be stable within and between subjects at very low volumes in the bag. The likely explanation is that because the pressure sensor is located towards the distal end of the bag, when there is very low or no liquid volume in the bag it is possible that this sensor's pressure reading would be more indicative of the immediate squeeze on it, than of the average force inside the bag. For this reason data is only reported at 10ml or greater inside the bag.

The physiological interpretation of this experiment is hindered by the absence of any validation components of this technology with other systems such as ano-rectal manometry or endo-anal ultrasound. Hence no specific anal components can be determined using this technology but comparative studies in the future may add new insight.

4.2.6 Conclusion

This study is one of the first to use a distending technique to identify changes in the ano-rectal sphincter. Further studies comparing manometry and EndoFLIP® in healthy subjects and patients are necessary to identify the strengths and limitations of EndoFLIP® versus manometry. The technique may have a role in testing sphincter competence in disease groups. With the correct set up it may be possible to visualize ano-rectal function rapidly without specialized training and in the treatment room in the hospital. Studies in patient groups such as those suffering faecal incontinence will provide further information to determine the practical use of the technique as an aid to the clinician.

In chapter three the researcher discussed the limitation of this study and measurements were established using video-fluoroscopy and EAUS.

4.3 Study two: Evaluation of the continence mechanism using EndoFLIP®: Clinical outcome from healthy control and FI groups

4.3.1 Objectives

The main objectives were to: (i) carry out a study with FI patients diagnosed with impaired sphincter function; (ii) apply the outcome measurements that was verified in previous chapter (chapter three) on both healthy (the healthy group in study one, section 4.2) and FI group data; (iii) identify geometric changes in the narrow zone region by a distension technique and provocative manoeuvres and to simultaneously assess circumferential dimensions at several positions within the anal canal at

different volumes; (vi) to compare narrow zone pressure and morphological changes from FI group during distension and provocative manoeuvres with narrow zone pressure and morphological patterns in a control group previously evaluated; and to (v) correlate the narrow zone pressure and morphological findings during resting and squeezing manoeuvres from FI group to resting and squeezing pressure measured with ano-rectal manometry.

4.3.2Material and methods

4.3.2.1Subjects

Nineteen FI patients (58.3± 2.8) subjects (mean ± SEM years) were studied, 17 female (14 multiparous). See table 4.1. The symptoms of each individual volunteer were detailed in the table. Ethics was approved (see appendix1) and the studies were carried out at Aalborg Hospital in Denmark. Informed consent was signed by the participants before the procedure. Inclusion criteria were a diagnostic of weakness in EAS and/or IAS. Ano-rectal manometry was performed before the start of each study. All FI participants were diagnosed with weakness in one or two sphincters. The data from the 20 healthy (36.5± 2.5) from previous study (see section 4.2.2.1) were analysed also.

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4.3.2.2 Equipment

The EndoFLIP® equipment already described previously (See section 3.3.2.2) was employed.

4.3.2.3 Study protocol

The protocol for this study is as outlined and used previously in section 3.3.2.3.

4.3.2.4 Data analysis

Data analysis was described previously in chapter 3. Three parameters including: estimated narrow zone length (mm), narrow zone average CSA (mm^2) and bag pressure (mmHg), were calculated at 10ml, 20ml, 30ml and 40ml ramp distension at rate of 40ml/min from 0-40ml. The same parameters were calculated at 20ml, 30ml, and 40ml step distension during resting. Squeezing and coughing manoeuvres. Squeezing duration time (seconds) was calculated at the three step volumes also. Pressure (mmHg) and straining time (seconds) was calculated at 40ml step volume during straining manoeuvre.

Table 4.1: FI patients background information.

patient	Age	Gender	Symptoms	No. of children	Ano-rectal results	Resting pressure	Squeezing pressure
1-xray	49	F	Passive FI x 2 years	2	Low resting pressure , non-exist squeezing pressure	14	37
1	74	F	Combined incontinence x 5 years	3	Very lower resting pressure, Very low squeezing pressure	14	20
2	32	F	Combined passive incontinence x 1.5 years	1	Good resting pressure, non-existing squeezing pressure	47	29
3	69	F	Urge incontinence x 7 years	0	Good resting pressure, non-existence squeezing pressure	59	19
4	62	F	Faecal incontinence x 1 year	2, 1 with forceps	Good Resting pressure , low squeezing pressure	49	21
6	83	M	Passive faecal incontinence x 1year		Very low resting pressure, adequate squeezing pressure	11	101
7	39	F	Urge incontinence x 15 years	3	Good resting pressure, good resting pressure	37	112
8	57	F	Urge combined incontinence x 2 years	2 difficult child birth	Good resting pressure, poor squeeze pressure	21	16
10	66	F	Urge faecal incontinence x 7 years.	4, 1 forceps	Low resting, good squeezing	11	64
11	66	F	Urge passive faecal incontinence x 2 years	1	Adequate resting pressure, very low squeezing	20	15
12	52	F	Combined Faecal incontinence x 6 months	4	Good resting pressure, low squeezing pressure	55	31
13	69	M	Urge FI x 3 years, DM type 2		Adequate to low resting pressure, good squeezing pressure	22	110
14	52	F	Urge FI x 7 months	2	Adequate resting, good squeezing	14	83
15	68	F	Combine FI x 1 year	3, 1 forceps	low resting, low squeezing	14	23
16	46	F	Urge FI x 14 years	0	Adequate resting, adequate squeezing	28	78
17	57	F	Passive FI x 6 years	0	low resting, low squeezing	9	30
18	38	F	Combined incontinence x 6 years	2	Good resting, adequate squeezing	36	85
19	60	F	Urge FI x 3 years	0	low resting, low squeezing	7	75
20	63	F	Passive x 2 years	2	Adequate resting, low squeezing	20	22

4.3.2.5 Statistical analysis

A Mann-Whitney test was used to compare the median narrow zone Length, bag Pressure, and narrow zone average Cross-Sectional Area (CSA) between the males and females, or between cases and controls. Comparisons of the change from rest to squeeze were performed with a Wilcoxon signed rank test. Differences between the three test volumes were performed with Friedman's test for non-independent groups. Comparisons of the changes from rest to squeeze between cases and controls were performed on the difference in the two measurements, for each parameter, with a Mann-Whitney test. EndoFLIP® and ano-rectal parameters were correlated with volume during resting and squeezing manoeuvres using Pearson and Spearman's Rho to establish trends in the data (rho =0.4 & p values 0.05). Statistical significance was defined as $p<0.005$.

4.3.3 Results

4.3.3.1 Distension data set

4.3.3.1.2 Effect of disease

There were significant differences in bag pressure, narrow zone length, and narrow zone average cross-sectional area across the different volumes. This was true for both disease and control individuals (all $p<0.001$). The effect of disease was explored by comparing the parameters between the 20 healthy controls, and 19 individuals with disease, across the four volumes tested, see table 4.2.

Table 4.2: Comparing the pressure, average cross-sectional area (CSA), and length between the FI and controls during ramp distension (0-40) ml at rate of 40ml/min. Medians and interquartile ranges; * Significant differences between volumes, Friedman test $p<0.001$; ** Significant differences between disease and control, Mann-Whitney test.

Parameter	Disease status	Volume			
		10ml	20ml	30ml	40ml
Length(mm)	Disease	45 (40-55)	25 (15-30)	15 (10-15)	10 (10-15)
	Control*	60 (55-65) **	32.5 (25-40) **	20 (15-23.8) **	10 (10-13.8)
Pressure(mmHg)	Disease	2.4 (0.9-5.7)	9.8 (6.6-13.6)	15.4 (13.3-22.7)	24.1 (21.9-33.9)
	Control*	2.7 (-0.1-5.4)	11 (8.8-13.8)	22.6 (16.9-25.8) **	42.6 (35.7-52) **
Average CSA(mm^2)	Disease	26.2 (23.9-28.1)	23.8 (22.6-25.4)	23.0 (21.4-35.0)	58.2 (38.1-85.8)
	Control*	33.5 (31.6-34.7) **	32.2 (28.4-34.1) **	25.6 (22.5-27.6)	22.9 (22.2-27.4) **

Narrow zone Length was significantly lower in the participants with FI than in controls at a 10ml volume (disease mean=45.3, median=45.0; control mean=59.0, median=60.0; $p<0.001$), at the 20ml volume (disease mean=23.7, median=25.0; control mean=33.5, median=32.5; $p=0.002$), and at the 30ml volume (disease mean=14.2, median=15.0; control mean=19.3, median=20.0; $p=0.003$). However there was no significant difference at a 40ml volume (disease mean=11.6, median=10.0; control mean=11.8, median=10.0, $p=0.76$), see figure 4.9.

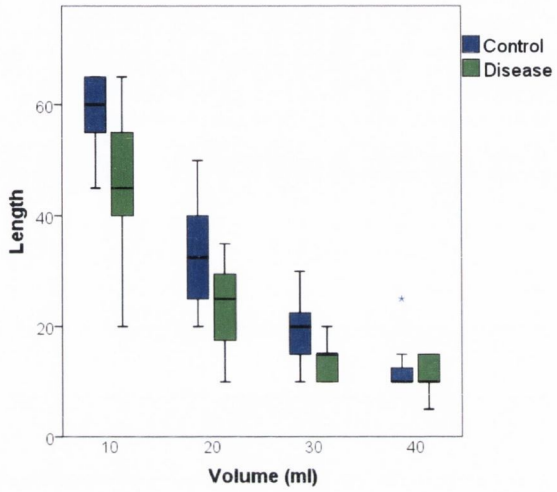

Figure 4.9: Changes in length between control (N=20) and disease (N=19)

Pressure did not show any significant differences between FI and control individuals at the 10ml volume (disease mean=3.37, median=2.4, control mean=2.84, median=2.74; $p=0.55$) or 20ml volume (disease mean=10.6, median=9.79, control mean=11.2, median=11.0; $p=0.57$). However, participants with disease had a lower pressure than controls at the 30ml volume (disease mean=18.0, median=15.4, control mean=23.1, median=22.6; $p=0.031$) and the 40ml volume (disease mean=28.2, median=24.1, control mean=44.1, median=42.6; $p<0.001$), see figure 4.10.

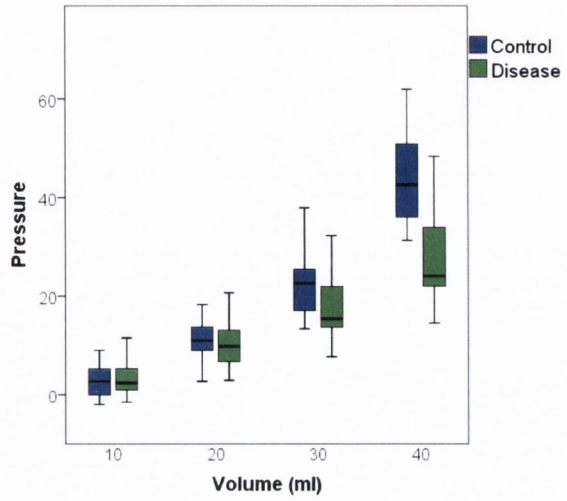

Figure 4.10: Changes in pressure between control (N=20) and disease (N=19)

average CSA was significantly lower in the participants with FI than in controls at a 10ml volume (disease mean=26.0, median=26.2; control mean=33.4, median=33.5; p<0.001), and at the 20ml volume (disease mean=24.2, median=23.8; control mean=30.9, median=32.2; p<0.001), and significantly higher in disease at the 40ml volume (disease mean=64.3, median=58.2; control mean=27.6, median=22.9, p<0.001). However there was no significant difference at the 30ml volume (disease mean=26.8, median=23.0; control mean=26.0, median=25.6; p=0.376), see figure 4.11.

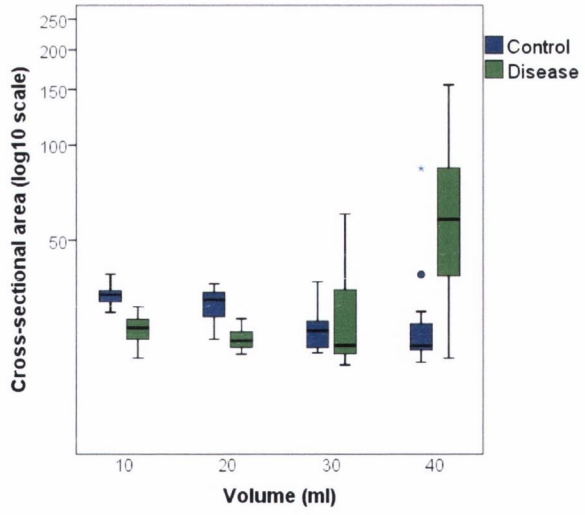

Figure 4.11: changes in cross sectional area between control (N=20) and disease (N=19)

4.3.3.1.1 Narrow zone distensibility

The following plots show the relationships between average cross-sectional area and pressure in the control and in the FI participant distension data set.

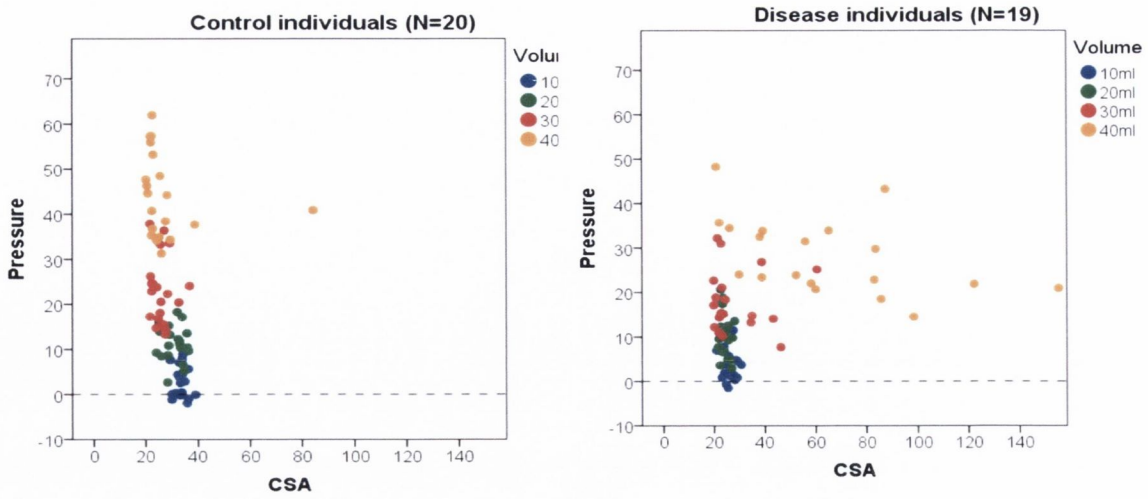

Figure 4.12: Distensibility for control (N=20) VS disease (N=19) at each volume

4.3.3.2 Provocative manoeuvres

4.3.3.2.1Resting status

Comparing the pressure, average cross-sectional area (CSA), and length between the FI patients and healthy controls during resting revealed significant differences in length at 20ml ($p<0.001$) and 30ml ($p<0.001$) but not 40ml ($p=0.626$), and differences in average CSA at 20ml ($p<0.001$), 40ml ($p<0.001$). Only in the 30ml test, there was no difference in average CSA between the groups ($p=0.565$). There were pressure differences between the control and FI groups at rest in the 30ml ($p=0.009$) and 40ml ($p<0.001$) tests, but not the 20ml ($p=0.390$) test (table 4.3).

4.3.3.2.2Squeezing

Comparing the pressure, average cross-sectional area (CSA), and length between the FI and controls during squeezing revealed significant differences in length and the level of CSA by different amount at all test volumes ($p<0.001$). At squeeze pressure, the 30ml ($p=0.028$) and 40ml ($p=0.001$) tests also showed a difference in disease but not the 20ml ($p=0.309$) test (table 4.3).

The length, pressure and average CSA from the Squeeze test were compared with the rest measurements (table 4.3).

Table 4.3: Medians and interquartile ranges from length, pressure and CSA from the Squeeze test were compared with the rest measurements at 20ml, 30ml and 40ml.

Parameter			Volume (ml)		
			20ml	30ml	40ml
Length (mm)	Squeeze test	Control	45.0 (40.0-45.0)	28.0 (23.0- 33.0)	15.0 (15.0- 20.0)
		Disease	25.0 (20.0-25.0)	15.0 (10.0-20.0)	10.0 (10.0- 10.0)
	Rest test	Control	35.0 (30.0-40.0)	20.0 (15.0-22.5)	10.0 (10.0-15.0)
		Disease	25.0 (15.0- 30.0)	15.0 (10.0- 15.0)	10.0 (10.0- 15.0)
Pressure (mmHg)	Squeeze test	Control	21.5 (17.7-32.9)	43.3 (33.7-58.7)	65.8 (57.2-81.7)
		Disease	19.0 (15.0-27.0)	34.0 (21.0-43.0)	45.0 (40.0-59.0)
	Rest test	Control	9.6 (6.9-14.0)	21.2 (15.3-27.1)	37.3 (33.3-41.0)
		Disease	11.0 (8.0-15.0)	15.0 (12.0-19.0)	27.0 (22.0-32.0)
Average CSA (mm^2)	Squeeze test	Control	31.2 (29.5-33.7)	28.9 (25.3-31.3)	25.5 (22.2-27.2)
		Disease	25.1 (22.9-26.8)	21.1 (20.0-23.2)	36.8 (26.8-64.6)
	Rest test	Control	30.0 (28.8-31.5)	24.6 (22.4-27.2)	26.3 (21.4-30.5)
		Disease	23.6 (22.2-24.9)	23.0 (20.8-29.5)	68.0 (39.3-102.9)

The control individuals showed a significance increase between the squeezing and rest length all volumes ($p<0.001$), however, this change showed no significant difference between volumes ($p=0.15$). Significant decrease in length between squeezing and rest in disease was observed at 20ml ($p=0.009$) but not at 30ml ($p=0.320$) or at 40ml ($p=0.260$) volume.

The pressure increased from rest to squeeze at all volumes ($p<0.001$), with the increase becoming larger, the higher the test volume. This pattern was similar for both disease and control participants.

Average CSA significantly changed in from rest to squeeze, in both the control group ($p=0.002$) and the FI group ($p=0.009$), with the 20ml and 40ml volumes showing clear differences, but in opposite directions in the healthy and FI groups. In the disease group, the distribution shifted upward at 30ml, and downward at 40ml.

4.3.3.2.3 Coughing

The proportions of patients ejecting the bag on a cough were examined (table 4.4), for the three volumes. None of the control individuals ejected the balloon, while the FI volunteers did at the 20ml volume in 31.6% of FI ($p=0.008$), at the 30ml volume in 42.1% of FI ($p=0.001$), and at the 40ml volume in 57.9% of FI ($p<0.001$).

Table 4.4: Bag position during cough test for FI volunteers at 20ml, 30ml and 40ml bag volume

Bag Volume	20ml Volume	30ml Volume	40ml Volume
No. of bags stayed in position	13 (68.4%)	11 (57.9%)	8 (42.1%)
No. of bags ejected outside	6 (31.6%)	8 (42.1%)	11 (57.9%)

Comparing the pressure, cross-sectional area (CSA), and length between the FI and controls (table 4.5) during coughing revealed significant differences in length at 20ml ($p<0.001$) and 30ml ($p=0.0031$) but not 40ml ($p=0.334$), and differences in average CSA at 20ml ($p<0.001$), 30ml ($p=0.009$) and 40ml ($p=0.029$), in those individuals who did not eject the bag. There were no differences in Pressure at 20ml ($p=0.109$), at 30ml ($p=0.638$) or at 40ml ($p=0.354$).

The length, pressure and average CSA from the Cough test were compared with the rest measurements (table 4.5).

The control individuals showed differences at 20ml between the cough and rest length ($p<0.001$), pressure ($p<0.001$), and average CSA ($p=0.009$), at 30ml length ($p=0.023$) and pressure ($p<0.001$) but not average CSA ($p=0.136$), and at 40ml only pressure ($p<0.001$) but not length ($p=0.107$) or average CSA ($p=0.079$).

The individuals with FI showed differences at 20ml pressure ($p<0.001$) but not in length ($p=0.337$), or CSA ($p=0.388$). The same pattern was seen at 30ml with significant difference in pressure ($p<0.001$), but not length ($p=0.680$) or average CSA ($p=0.173$), and again at 40ml with pressure significantly different ($p<0.001$), but not length ($p=0.480$) or average CSA ($p=0.176$).

Table 4.5: Medians and interquartile ranges from length, pressure and CSA from the coughing test were compared with the rest measurements at 20ml, 30ml and 40ml.

Parameters			Volume (ml)		
			20	30	40
Length (mm)	Cough test	Control	40.0 (37.5-45.0)	25.0 (20.0-25.0)	12.5 (10.0-17.5)
		Disease	20.0 (15.0-30.0)	15.0 (10.0-20.0)	10.0 (7.5-17.5)
	Rest test	Control	35.0 (30.0-40.0)	20.0 (15.0-22.5)	10.0 (10.0-15.0)
		Disease	25.0 (15.0-30.0)	15.0 (10.0-15.0)	10.0 (10.0-15.0)
Pressure (mmHg)	Cough test	Control	50.6 (34.6-68.8)	77.3 (53.1-86.3)	89.5 (70.1-102.0)
		Disease	73.2 (46.0-92.0)	72.4 (61.0-91.7)	68.0 (57.0-88.0)
	Rest test	Control	9.6 (6.9-14.0)	21.2 (15.3-27.1)	37.3 (33.3-41.0)
		Disease	11.0 (8.0-15.0)	15.0 (12.0-19.0)	27.0 (22.0-32.0)
Average CSA (mm^2)	Cough test	Control	31.2 (28.9-33.1)	26.1 (22.5-28.2)	23.6 (21.7-25.6)
		Disease	22.6 (21.3-24.1)	22.7 (19.8-23.3)	31.2 (23.7-51.4)
	Rest test	Control	30.0 (28.8-31.5)	24.6 (22.4-27.2)	26.3 (21.4-30.5)
		Disease	23.6 (22.2-24.9)	23.0 (20.8-29.5)	68.0 (39.3-102.9)

When the bag was ejected during coughing manoeuvre, only the bag pressure was calculated. The pressure measurements mean (min-max) for the FI subjects who ejected the bag were 70.1(58.7-92.0) mmHg at 20ml, 75.7(61.5-83.9) mmHg at 30ml and 68.0(58.0-84.0) mmHg at 40ml. (see appendices 6&7).

4.3.3.2.4 Straining data set

The only significant difference between the case and control samples was in the duration of strain, with the case individuals having shorter strain duration (table 4.6). The bag volume was 40ml.

Table 4.6: Medians and interquartile ranges of pressures and duration for healthy and FI volunteers during straining at 40ml volume.

Parameter	Control	FI	p-value
Pressure (mmHg)	61.5 (46.0 – 66.1)	45.7 (36.4 – 60.5)	0.194
Duration (seconds)	6.05 (4.60 – 6.85)	3.15 (0.98 – 5.30)	0.008

For controls there were significant changes from rest to strain, in pressure, for both control and disease (table 4.7).

Table 4.7: Medians and interquartile ranges of pressures for healthy and FI volunteers during resting and straining tests at 40ml volume.

pressure	Rest	Strain	p-value
control	37.3 (33.2 – 41.3)	61.5 (46.0 – 66.1)	<0.001
FI	30.2 (22.5 – 37.3)	45.7 (36.4 – 60.5)	0.032

4.3.3.2.6 Correlation with Manometry measurements

Correlations of ano-rectal manometry pressure measurements, and the resting and squeezing pressure measurements were calculated. At rest the modest correlations were observed between the 40ml volume and the manometry pressures (p=0.75, Spearman rho=0.409) while the correlations with the 30ml pressure readings were close to zero (p=0.03, Spearman rho=0.048), and the correlations with the 20ml pressure readings were negative (p=0.004, Spearman rho=-0.123).

After squeeze, the correlations were again strongest between the manometry pressure and the 40ml volume (p=0.87, Spearman rho=0.413), but were more substantial at the 30ml volume (p=0.03, Pearson's r=0.03, Spearman rho=0.324). At the 20ml volume the correlations were small and negative (p=0.018, Spearman rho=-0.178).

The strongest correlations of manometry with cross-sectional area were negative, at rest. With CSA the strength of the correlation increased proportional to volume, from 20ml (p=0.6, Pearson's r=-0.022, Spearman rho=0. 090) to 30ml (p=0.85, Pearson's r=-0.216, Spearman rho=-0.347), to 40ml (*p<0.001, Spearman rho=-0.537*).

On squeeze, the strongest correlation between the manometry measure and CSA was at the 40ml, and again it was a negative correlation (p=0.81, Spearman rho=-0.693). The 30ml volume showed more

modest correlation (p=0.016, Spearman rho=0.082), and the 20ml volume showed a moderate positive correlation (p=0.81, Spearman rho=0.370).

4.3.4 Discussion

The EndoFLIP® procedure was easy to perform and well tolerated by all volunteers. EndoFLIP® allowed us to study the correlation between various anatomical structures that contribute to the closure and distensibility function of the anal canal in exquisite detail. The most important discovery from this study is that the geometry of the lumen and distensibility properties of the anal canal is not uniform during distension as reported by (257).

In this study, distensibility of anal canal was evaluated in a group of healthy and FI volunteers. Narrow zone length, narrow zone average CSA and bag pressure were measured to define the distensibility (see discussion 3.3.4) of the following three parts in healthy and FI volunteers: upper anal canal surrounded by the IAS and PRM, middle part surrounded by IAS and EAS and lower anal canal surrounded by the EAS (see figure 3.17). There was a significant change between healthy and FI volunteers during distension and different provocative manoeuvres at all three parts.

4.3.4.1 Distension

The narrow zone was demarcate along the anal canal during ramp distensions at 10ml, 20ml, 30ml and 40ml in health and FI participants as described previously in chapter three, see figures 3.9&3.10. Narrow zone length and average CSA as well as bag pressure was evaluated at each distended volume.

A significant longer and wider narrow zone in healthy volunteers compared to FI volunteers was observed during distensibility testing at low volumes of less than 20ml. These finding suggest that the middle part seem less distensible in FI than in healthy volunteers, the middle part doesn't seem any less distensible than the upper/lower regions. The same finding was concluded when a bag was distended in the anal canal in a study with three-dimensional ultrasound (US). In this study, the upper and lower ends of the bag distended were found as the least distensible at low volume and US images confirmed that the two ends of the bag are located at the ano-rectal angle or the upper edge of PRM and lower border of the EAS respectively (288).

When the distended volume increased to 30ml, the CSA of healthy volunteers decreased sharply until its value was the same as in FI suggesting that the middle part became less distensible in both groups. This decrease in CSA was accompanied with an increase in pressure in both groups.

At a 40ml distension volume, both narrow zone CSA and pressure increased significantly in FI volunteers. The narrow zone CSA decreased and pressure increased significantly in healthy volunteers. The length of the narrow zone was equal in both groups indicating that the middle part was the least distensible portion in both groups. This concurs with the work of Luft et al (257).

Manometry Pressure recording of the anal canal using side hole manometry and station pull through technique show that the highest pressure during rest is located in the part of the anal canal that is surrounded by the EAS (178).

The relationship between CSA and pressure at low volumes was similar for both healthy and FI groups. See figure 4.13. When the volume increased to 40ml, CSA increased significantly for FI without a significant increase in pressure while the pressure in healthy volunteers increased sharply with no significant increase in CSA.

Sørensen et al investigated the segmental distensibility of anal canal in healthy volunteers and in patients with idiopathic faecal incontinence (IFI) using FLIP technique. They found that the most distinctive difference in distensibility occurred at high distension pressure. This agreed with our data where the two groups had the same behaviour till 30ml and the biggest change was observed at 40ml. Also, it was found that the middle and the distal parts were significantly less resistant to distension in IFI patients compared with healthy controls (278).

The distensibility at higher volumes using EndoFLIP® corresponded well with findings in previous studies which demonstrated that the biomechanical wall properties of the anal canal during anal distension reflect the effects of both the passive visco-elastic component and the active relaxation caused by the reflex mechanism. During distension, the anal canal shortens due to the relaxation of IAS which represents the passive component effect. At the same time the concomitant contraction of the EAS reflect the visco-elastic component effect. Hence it is possible that the two parameters, average CSA and anal pressure, can be independent of each other (232, 289, 290). Further clinical study while the pudendal nerve was paralyzed using an anaesthetic, reveal how the EndoFLIP® measurements can be used to describe the active muscular contraction and visco-elastic properties of the anal canal at the EAS.

4.3.4.2 Squeezing

Narrow zone length and average CSA were calculated at the maximum squeezing pressure for each group. In healthy volunteers, the narrow zone length increased significantly at all volumes when the volunteer performed squeezing. EndoFLIP® images reveal that the two tightened ends tend to get more distinctive at the upper and lower parts of the anal canal in all volumes. At 20ml and 30ml the upper and lower end became less distensible than the middle parts causing the liquid to be trapped in the middle of the EndoFLIP® bag. Consequently, the average CSA increased. At 40ml the middle part became the least distensible so when the volunteer perform squeezing the fluid in the bag was pressed away from the middle part and the CSA reduced significantly as an effect.

The shape of the anal canal during squeezing corresponds well with a previous study of a group of healthy women where the vagina was filled with a probe to increase anal canal pressure and the

volunteers were asked to squeeze. They proposed that these pressure increases were related to contractions of the PRM and EAS respectively. The hypothesis is that the contraction of the two arms of the PRM lifts the anal canal in the anterior direction and compresses it against the vagina, urethra, prostate gland, and back of the pubic bones causing an increase in the anal canal length. (233).

In FI patient group, no increase in pressure was observed at all volumes. No morphological changes were observed along the narrow zone in this group. The only change was the increase in pressure which was still significantly lower than the health participants. The CSA decreased significant at 40ml most likely an effect of the squeezing. However, overall its value remains much higher than in healthy volunteers. The duration of squeezing was the same among different volumes for both health and disease, but it was significantly higher in health all the time.

Fynne and colleagues found that the voluntary contraction of the EAS during squeezing had little effect on anal resistance to distension in health controls when a FLIP bag was distending the anal canal. In contrast, our measurement of the narrow zone length and narrow zone CSA was helpful in evaluating the effect of squeezing on the resting status in health at different volumes and determining the significant difference between health volunteers and FI patients (258).

4.3.4.3 Coughing

Although the bag stayed in position during coughing at all volumes, the increase in length was only observed in at 20ml and 30ml volumes and no morphological changes was observed at 40ml. Currently, EndoFLIP® provides diameter, bag pressure and time data at a rate of 10Hz (10 measures per second). This is quite a low rate for measuring a reflex like the cough and may be a reason that the expected elongation of the narrow zone as an effect of the reflex contraction of the EAS and PRM was not consistent. On other hand, FI volunteers ejected the bag in different percentage and the number of volunteers who ejected the balloons was increasing as the volume increased in the bag. In the FI volunteers who manage to keep the bag in, no change was detected on length or CSA of narrow zone but on pressure. There was no significant difference between the pressure in health and disease at all volumes.

Coughing manoeuvres is related to the reflex in external sphincter and puborectalis muscle and it is about the ability of the canal to contract to keep continence when the abdominal pressure suddenly increases. The importance of this test is that the ability of the volunteers to keep the bag in the anal canal can be used as measure of how bad or good their reflexes are.

4.3.4.4 Correlation of narrow zone CSA and bag pressure measured by EndoFLIP® at rest and during squeezing with pressure derived from ano-rectal manometry at the same manoeuvres

This study sought to initiate the validation of EndoFLIP® in anal sphincters evaluation. The study compared outcomes from EndoFLIP® measures of anal sphincter squeezing were compared to parameters obtained from ano-rectal manometry. The latter parameters were selected for comparison as this evaluation tool considered a gold standard diagnostic tool (see section 1.10.2). Findings from this study indicate that there is a single significant negative correlation between EndoFLIP® resting CSA at 40ml and ano-rectal manometry resting pressure.

Certain methodological issues should be highlighted before concluding from these results. Firstly, the inability to perform EndoFLIP® and ano-rectal manometry simultaneously meant that squeezing outcomes being compared were based on different squeezing. Given the potential variability between squeezing within individuals, the lack of simultaneous examination may have hindered correlation of more parameters. Other factors to consider at this point include the rate of data acquisition across examinations. EndoFLIP® provides ten diameter and bag pressure measures per second (10Hz), while ano-rectal manometry analysis provided eight measures per second (8Hz) (165). This different rate of data acquisition may have impacted on findings. This is only a starting point in the validation of EndoFLIP® in ano-rectal evaluation. Future research will establish the diagnostic accuracy of EndoFLIP®.

4.3.4.5 Straining data

The narrow zone was impossible to analyse during straining, so no specific information was identified about the narrow zone length or CSA. Observations of straining data presented in colour contour plots indicate that a tightening in the narrow zone was accompanied with a gradual movement of the bag outside the anal canal in health. Conversely, most of straining contour plot for FI patients indicated that the bag slipped outside the canal very quickly.

The finding was in accordance with 'vermicular contraction' theory. This theory states that the last faecal portion is dispelled by a process of 'vermicular contraction' induced by the triple loop system of the external sphincter. Thus, while one loop contracts to push the faecal mass, the succeeding loop relaxes to receive it. Such a propulsive mechanism is related not only to the loop arrangement but also to different innervations of each of the two adjacent loops. Top loop contraction together with intermediate loop relaxation, both being differently innervated, allows the faecal mass to proceed downward. Repeated vermicular contractions of the external sphincter cleanse the anal canal of any residual faecal contents (29).

Data analysis of straining should be done with caution. When the balloon moved all the way outside the anal canal and the fluid moved from the distal EndoFLIP® end to the proximal end at the end of straining manoeuvre, the CSA data can give a false impression of tightening. As a result it was very important to define the start and the end of the straining data.

4.3.5Limitations of the current study

The main findings of the study concern the length and the CSA of the anal canal. In this study a young healthy group was compared with elderly FI group. Also the healthy group consists of equal number of male and females; however the FI group consists of 82% of female. Accordingly, the data in tables 4.1 through 4.10 could be affected by the difference in age and gender. Literature demonstrated a high correlation between age and anal sphincter dysfunction. With increasing age, the maximum resting and maximum squeeze pressures of anal sphincters decreases. The reduction in pressures that occurs with aging is greater in women than in men (see section (1.8.2.6). Consequently, the gender and age factors consider as limitation of the interpretation of data in this study. Future large studies of groups of both sexes and different ages will be required to better determine the role FLIP might have in diagnosing and evaluating FI.

There were three main limitations when EndoFLIP® was used to study the anal canal morphological changes: (ii) the 5mm spacing of the electrode pairs limits the spatial resolution of the FLIP there changes within this range may not be detected; (ii) another limiting factor could be that it is unable to detect circumferential curvature variations because it can only measure cross sectional area. This may restrict the usefulness of FLIP in patients with traumatic or obstetric sphincter lesions where muscle trauma or tears at a particular circumferential point is the cause of incontinence; and (iv) lateral and radial accidental movement of the probe during the study could theoretically elicit EAS contraction which may affect the system ability to make consistent findings in a group of similar patients or health controls.

4.3.6 Conclusion

This work suggests that EndoFLIP® may consider as a promise and revolutionise tool for measuring the biomechanical properties of the ano-rectal region. Narrow zone length, narrow zone CSA and bag pressure parameters were able to define and determine a better understanding of the effect of anal canal distension and provocative manoeuvres on the upper, lower and middle parts along the anal canal at different volumes. EndoFLIP® was well tolerated by subjects and the technique of distending the anal canal to measure its ability to resist distension can provide a useful method to study the components of continence in normal subjects and in patients with FI where the anal sphincters structure and motility are affected. Colour contour plots representing EndoFLIP® diameter and pressure data on a time axis provide a novel objective approach to the analysis of anal canal dynamics during coughing and straining. In future, a bigger sample of faecal incontinence patients with low resting and squeezing pressure will proof the results obtained in this study.

Chapter 5: General Discussion and Future Work.

5.1 Discussion

It is well known that the anatomical structure of the continence component is complex. Not only are they composed from different types of muscles, the IAS composed from smooth muscle and both the EAS and PRM composed from striated muscle, but they surround the anal canal in a heterogeneous manner forming three distinctive parts; the upper part which is surrounded by the smooth muscle of the IAS and the striated sling PRM; the middle part is surrounded by the overlapped IAS and EAS and the lower part which is surrounded by the subcutaneous loop of the EAS. Both active contractile and passive viscoelastic biomechanical properties of striated and smooth muscles respectively are acting in a complex fashion along the anal canal to maintain continence and to control defecation. The dysfunction of any of these components can lead to a mild or severe FI.

The aim of this research was to develop and test a new tool for the functional assessment of the continence mechanism in human subjects. The recently developed endoscopic functional lumen imaging probe (EndoFLIP®) for assessing sphincter mechanics in the gastrointestinal tract allows determination of serial cross-sectional area (CSA) during distension. Preliminary data from three pilot studies confirmed that the length and size of EndoFLIP® originally designed to study dynamic wall properties at the gastro-oesophageal junction was optimum design for safe and stable distension in the anal canal. The work here also lead to the development of a new protocol for studying ano-rectal function which involved anal canal distension and subsequent morphological evaluation from the interpretation of selected results.

Testing EndoFLIP® using video-fluoroscopy and endoanal ultrasound guide to a new outcome measures which can define the role of the three principal muscle groups at three levels along the anal canal in health and disease. This new outcome measures demarcate the narrow zone length, narrow zone average CSA and bag pressure.

This new test is well tolerated by all subjects and suggests that the ano-rectal region could be examined and a diagnosis determined much faster in the doctors hands than by previous techniques for many disease states. A key observation from this new technique was that in healthy volunteers the narrow zone tightened towards each end at low bag volumes but at higher volumes the tightened region moved to the middle of the narrow zone. See figures 3.10 & 3.11. This early tightening at the ends during low volume was not as pronounced in FI patients only a shorter and tighter mid narrow zone which opened significantly at higher volumes. In table 4.2, while length of the narrow zone was significantly longer in healthy volunteers than in FI at low volumes of 10ml and 20ml as a result of the early tightening at the two ends of the anal canal.; the average CSA of the healthy volunteers was

significantly larger than the average CSA of FI group at the same volumes. The significant increase in the average CSA in healthy volunteers is a result of relaxation in the middle of the anal canal. On the other hand, the significant decrease in average CSA in healthy volunteers at high volume is a result of the closing of the anal canal as part of the continence mechanism while the significant increase in average CSA in FI is a result of the incapability of preventing leakage along the anal canal. These measurements are a significant finding of this work and can describe the morphological changes along the anal canal. With a little practice could be easily observed by a trained GI physician even upon monitoring the EndoFLIP ® display during ramp distension.

In addition, this new technique was able to describe the distinctive morphological changes and significant elongation in healthy volunteer during the squeezing manoeuvre. The changes in the narrow zone length and narrow zone average CSA during squeezing manoeuvre depends mainly on the FLIP bag volume. The squeezing manoeuvre only affects the two ends of the narrow zone and it changes the morphology of the anal canal depending on its resting status. For FI volunteers, there was no increase in narrow zone length during squeezing. These significant morphological changes can play a vital role in diagnosing the weakness in the muscle that performing squeezing and help the clinician in the investigation of the dysfunction in these muscles.

By representing the CSA and pressure data on a colour contour plot we find an important way of observing the rapid changes in FLIP parameters during a single incident, a rapid event, such as a cough or a strain. This is the first time a specific test was designed to measure the role of the reflex initiated during coughing in continence mechanism. The ability of the anal canal to keep the bag inside can be an evidence of the existence of the contraction reflex and the time needed to expel the bag out during straining strongly indicate the continence status. These tests will add significant value to our knowledge in FI dysfunction.

The correlation between the EndoFLIP output and ano-rectal manometry pressure at rest and squeezing was limited to a negative relationship between resting CSA measured by EndoFLIP at 40ml and resting pressure measured by ano-rectal manometry. This does not undermine the importance of EndoFLIP in diagnosis the dysfunction in the ano-rectal region; it demonstrates that this test has a different approach of measuring continence.

The outcome measurements from this research contribute novel quantitative information that can define the non-uniform biomechanical properties and morphological changes of the anal canal during distension and provocative manoeuvres in health and FI volunteers. The use of the EndoFLIP® technique is still in its infancy but it has potential as a new and better measurement tool and attempts should be made to fully elucidate its clinical implication.

5.2 Future work

5.2.1 Methodological Issues

In future studies, the addition of an adjunct measurement (e.g. surface submental EMG) may be of benefit to ensure strategies such as the squeezing and straining manoeuvres are being executed accurately and consistently. The second methodological issue in this study was the difference in median age across groups, with older subjects in the faecal incontinence groups. As age has been proven to impact on anal sphincters function, this factor needs to be taken into account.

5.2.2 Biofeedback

From our study of squeezing test, we have been able to show the dynamic and morphological changes along the anal canal when squeezing are performed. The next phase of this study will be studying the effect of providing these live images for both physiotherapist and FI patients during biofeedback tests. The FLIP images will help the physiotherapist to explain the biofeedback exercises. We hope that the identification of anal canal profile will help the improvement of biofeedback exercises.

5.2.3 Defecatory Position

The defecatory position that a subject assumes is dictated by a number of factors, including the type of toilet available, physical and mental ability and cultural factors, in western countries sitting on a toilet seat is common, whereas in Africa and Asia squatting is preferred. Using defactography, it has been demonstrated that the ano-rectal angle becomes more obtuse (open) with increasing hip flexion, making evacuation easier. As expected, the evacuation is also easier when sitting compared to lying. Future studies using EndoFLIP® with different position will define if the biomechanical properties of the anal canal are endowed by changes in posture, and that positively influence evacuatory efficiency.

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Appendices

Appendix 1: Letters of ethical approval

THIS NOTEPAPER MUST NOT BE USED FOR PRESCRIPTIONS OR INVOICING PURPOSES

SJH/AMNCH Research Ethics Committee Secretariat
Ursula Ryan Ph: 4142342 email: Ursula.Ryan@amnch.ie
Secretariat Fax 4142371

November 21st. 2012

Dr. Maha Azeez
Tallaght Hospital

THE ADELAIDE & MEATH HOSPITAL, DUBLIN
INCORPORATING
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TALLAGHT, DUBLIN 24, IRELAND
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November 21st. 2012

Re: Faecal incontinence: A population-based prevalence study, impact on quality of life and the development of a novel tool (FLIP) for assessing ano-rectal physiological function.

Please quote this reference in any follow up to this letter: 2012/41/12

Dear Dr. McMahon,

Thank you for your letters dated November 11th 2012 and enclosures in which you request ethical approval of an amendment to the above referenced studies.

The Chairman, on behalf of the Research Ethics Committee, has reviewed this proposed amendment and has given ethical approval.

Yours sincerely

Ms. Ursula Ryan
Secretary,
SJH/AMNCH Research Ethics Committee

335703001

DEN CENTRALE VIDENSKABSETISKE KOMITÉ

The Danish National Committee on Biomedical Research Ethics

Forsøgspersonens rettigheder i et biomedicinsk forskningsprojekt.

Som deltager i et biomedicinsk forskningsprojekt skal du vide at:

- din deltagelse i forskningsprojektet er helt frivillig og kan kun ske efter, at du har fået både skriftlig og mundtlig information om forskningsprojektet og underskrevet samtykkeerklæringen
- du til enhver tid mundtligt, skriftligt eller ved anden klar tilkendegivelse kan trække dit samtykke til deltagelse tilbage og udtræde af forskningsprojektet. Såfremt du trækker dit samtykke tilbage påvirker dette ikke din ret til nuværende eller fremtidig behandling eller andre rettigheder, som du måtte have
- du har ret til at tage et familiemedlem, en ven eller en bekendt med til informationssamtalen
- du har ret til betænkningstid, før du underskriver samtykkeerklæringen
- oplysninger om dine helbredsforhold, øvrige rent private forhold og andre fortrolige oplysninger om dig, som fremkommer i forbindelse med forskningsprojektet, er omfattet af tavshedspligt
- opbevaring af oplysninger om dig, herunder oplysninger i dine blodprøver og væv, sker efter reglerne i lov om behandling af personoplysninger og sundhedsloven
- der er mulighed for at få aktindsigt i forsøgsprotokoller efter offentlighedslovens bestemmelser. Det vil sige, at du kan få adgang til at se alle papirer vedrørende din deltagelse i forsøget, bortset fra de dele, som indeholder forretningshemmeligheder eller fortrolige oplysninger om andre
- der er mulighed for at klage og få erstatning efter reglerne i lov om klage- og erstatningsadgang inden for sundhedsvæsenet

(Dette tillæg udgives af Den Centrale Videnskabsetiske komité og kan vedhæftes den skriftlige information om det biomedicinske forskningsprojekt. Spørgsmål til et projekt skal rettes til den regionale komité, som har godkendt projektet)

Informeret samtykke til deltagelse i et biomedicinsk forskningsprojekt.

Sensoriske forhold i mavetarmkanalen, hud og muskler

Undersøgelser af smertefysiologien hos raske forsøgspersoner og hos patienter med funktionelle og organiske sygdomme i mavetarmkanalen

Erklæring fra forsøgspersonen:

Jeg har fået skriftlig og mundtlig information og jeg ved nok om formål, metode, fordele og ulemper til at sige ja til at deltage.

Jeg ved, at det er frivilligt at deltage, og at jeg altid kan trække mit samtykke tilbage uden at miste mine nuværende eller fremtidige rettigheder til behandling.

Jeg giver samtykke til, at deltage i forskningsprojektet og har fået en kopi af dette samtykkeark samt en kopi af den skriftlige information om projektet til eget brug.

Forsøgspersonens navn: _____

Dato: _____ Underskrift: _____

Erklæring fra den forsøgsansvarlige:

Jeg erklærer, at forsøgspersonen har modtaget mundtlig og skriftlig information om forsøget og har haft mulighed for at stille spørgsmål til mig. Efter min overbevisning er der givet tilstrækkelig information til, at der kan træffes beslutning om deltagelse i forsøget.

Den forsøgsansvarliges navn: _____

Dato: _____ Underskrift: _____

Appendix 2: Patient information leaflet

Title: **Measuring ano-rectal functions in patients with symptomatic anal sphincter disorder using EndoFLIP.**

Faecal incontinence

Faecal incontinence or "soiling oneself" is the involuntary loss of bowel sphincter control and the inability to hold stool until reaching the toilet. It is a common condition but many people who experience it are too embarrassed to talk about it. Multiple factors are involved in causing this condition but it is more common in women who have had multiple pregnancies. It is more common the older you get but can occur at any age.

Introduction

The causes of this condition are poorly understood. We are looking for volunteers for a new test (EndoFLIP) which involves a thin, flexible tube with a small balloon around it. This is gently inserted into the back passage (anal canal) to provide more information on what causes this condition.

This new test may help to explain the cause of faecal incontinence in people who have not received a satisfactory answer from currently available tests. The study may have no direct benefits to any one person but combined results will lead to better understanding and hopefully new treatments for this condition.

The procedure

This test takes approximately 30 minutes to complete.

Before the test the doctor will carry out a general examination and ask some questions about your medical history. The doctor will ask you to sign a consent form after fully explaining the procedure.

A qualified nurse and/or medical doctor will perform the test.

You will be asked to lie on your left side.

1. **A thin flexible plastic tube with a small balloon around it is inserted approx. 6cm into the rectum (back passage).**
2. **The tube is slowly withdrawn while numerous measurements are taken and recorded.**
3. **The small balloon on the end of the tube will be inflated at intervals and you will be asked to a) 'squeeze' the muscles around the anus, b) perform "straining" at certain times.**

You may experience an urge to have a bowel movement at certain times, but this feeling passes quickly.

There are no serious adverse event associated with this procedure.

How will it feel?

It will be mildly uncomfortable. Only fully qualified medical professionals who are used to performing this procedure will be in the room with you at any time and we will endeavour to make you as comfortable as possible.

Exclusion from participation:

The following people are excluded from this test:

If you are pregnant

If you have had previous rectal or anal disease or surgery of the back passage.

If you are unsure if any of these apply to you please contact the number below. If you are experiencing pain around this area on passing stool please let us know and we will postpone the test until this has resolved.

Voluntary Participation:

You have volunteered to participate in this study. You may withdraw at any time, including on the day of the procedure. If you decide not to participate, or if you withdraw, you will not be penalised and it will not affect your treatment at all. We are very grateful to volunteers who help us with this study to try and improve our knowledge and treatments of this condition.

If you require more information or have questions please contact Bernadette McGovern (RGN) *01 4144173, 01 4143015* Maha Alqudah (Researcher) *0851205091*

Appendix 3: Protocol: A study of the use of the FLIP probe in the assessment of ano-rectal function in normal volunteers

Equipment

Functional lumen imaging probe (FLIP).

Physical measuring system Endo FLIP (Crospon, Galway, Ireland) capable of plotting cross sectional areas in real time, and all data will stored on a memory stick.

Accessories

Lubricating gel

100 ml syringe

Towels and washcloths

Subjects

Ideally 10 males and 10 females under 40 years old have no history of diagnostic and/or treatment of ano-rectal dysfunction or surgery for same.

The procedure is as follow:

Before study

Obtain volunteer medical history: symptom (constipation, urinary or faecal incontinence), allergies, past treatments (anal surgery).

Written consent is signed

No special preparation of the bowel is needed

No sedation is given to the patient.

Explain the procedure to the volunteer in order to increase volunteer cooperation and comfort level, create environment patient relaxed and not speaking, no disturbances

Insertion of the FLIP:

Volunteer are positioned on their left side, with hips and knee flexed.

Place a blue pad under the left hip to collect leakage.

Insert well-lubricated FLIP catheter into the anal canal.

To allocate the FLIP in its right position, inflate the FLIP balloon to small volume (0-10ml) so two measurement will be inside the rectum and outside the anal canal profile.

Allow the patient to become accustomed to the catheter before starting the procedure. Wait at least 5-10 minute.

Obtain a recording with a stable baseline in the anal canal. This is important since all of the subsequent measurements will be referred to it. Note if there are any ultra-slow waves and spontaneous contraction and relaxations. If the patient moves or talks, etc., note it on the tracing as an artefact. The resting pressure of the anal canal can be measured at the start of the procedure or later in the procedure when the patient is thought to be more relaxed.

Observe the profile of the anal canal at different volumes during ramp distension from 0 to 40 ml

Deflate the FLIP balloon.

The maximal voluntary squeeze and duration. The push/strain manoeuvre. Rest/relax manoeuvre. Cough manoeuvre

Inflate the FLIP balloon with 10ml volume of saline, while the rectum balloon still empty.

The volunteer is asked to squeeze as hard as possible for 10-20s, observe the anal canal profile. The external anal sphincter should normally stay contracted for at least 3-5s. Less than 3s is considered abnormal. Relax for 30 sec

The volunteer is asked to push/ strain as if trying to defecate. The external sphincter should normally relax during this manoeuvre.

The volunteer is asked to rest without squeeze or strain for 20-30s, observe the anal canal profile

Repeat the procedure with 20ml, 30ml and 40ml with more than 1 min between each manoeuvre

Deflate the FLIP balloon.

Removing the ano-FLIP

Make sure the FLIP balloon is deflated

Ask the patient to relax

Remove the FLIP slowly.

The data sheet attached should be completed according to the following procedure.

Patient ID: Date: / /

Action	Starting time	Ending time	Notes
Probe allocation			
Anal distension 1			
Anal distension 2			
Anal distension 3			
At 20ml vol.			
Resting			
Squeezing			
coughing			
Straining			
At 30ml vol.			
Resting			
Squeezing			

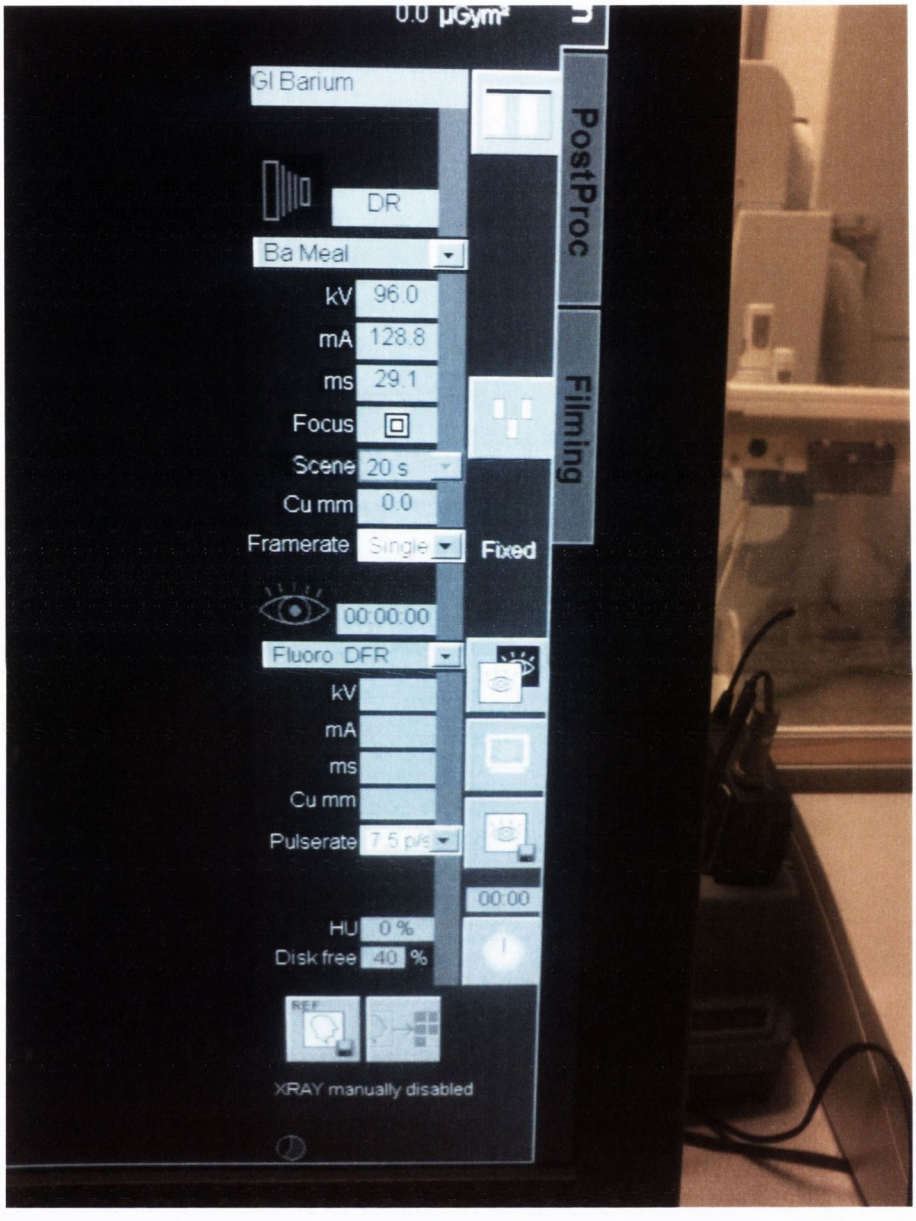
coughing			
Straining			
At 40ml vol.			
Resting			
Squeezing			
coughing			
Straining			
Remove probe			

Appendix 4: Radiant Solutions

1.Urographin (Bayer, Dublin, Ireland)

2. Niopam (Merck, Dublin, Ireland)

Appendix 5: Video-fluoroscopy parameters



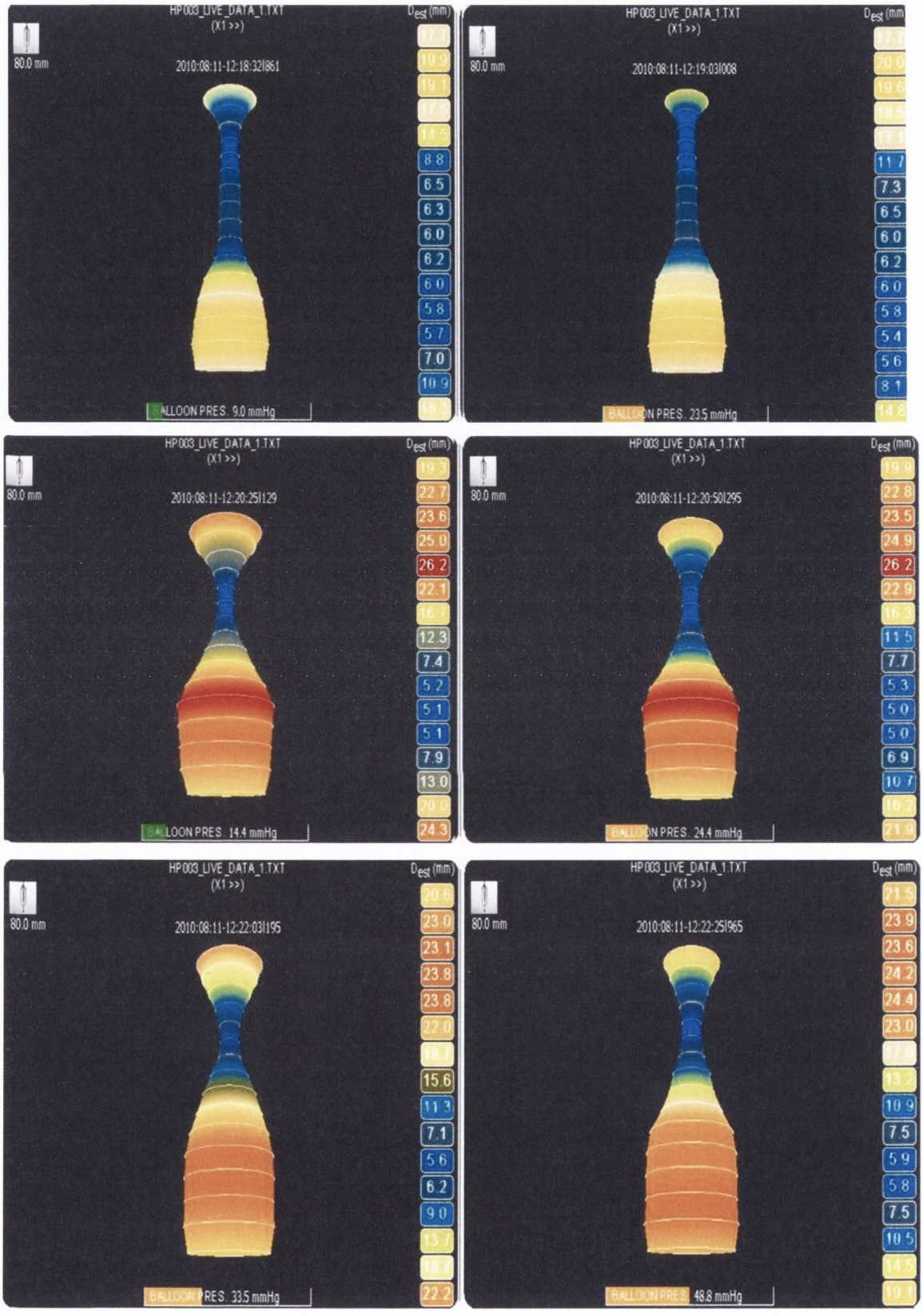
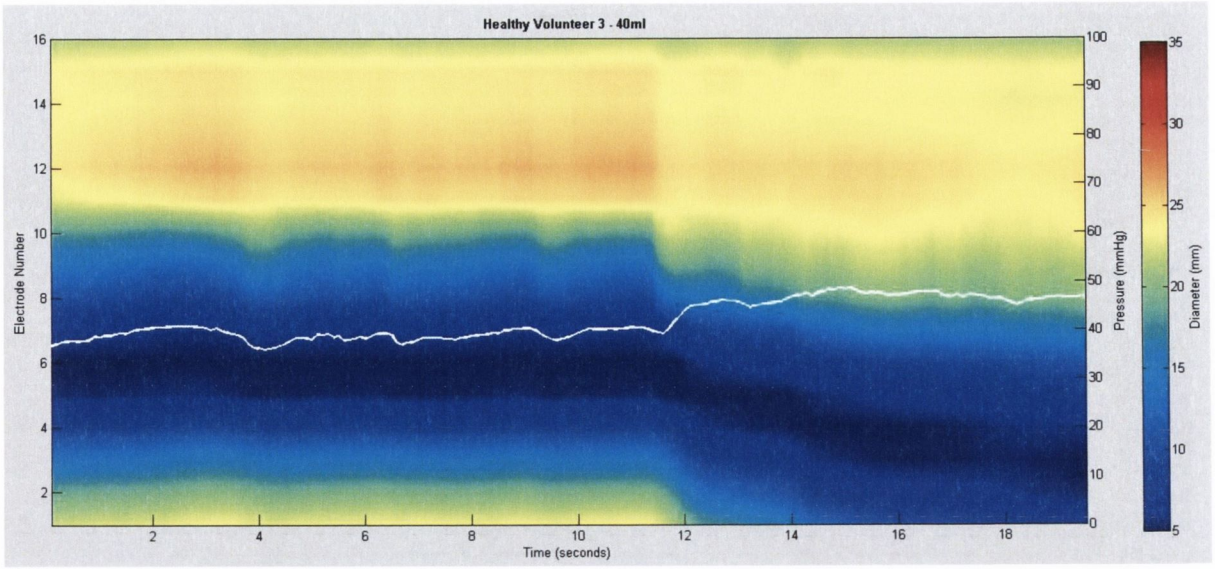
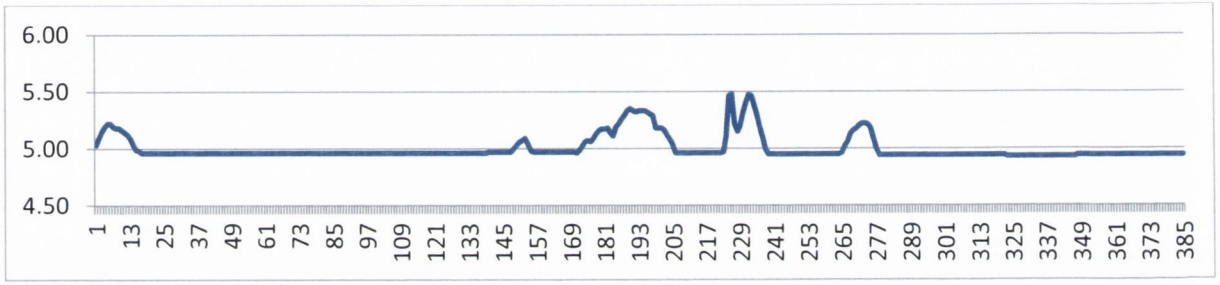
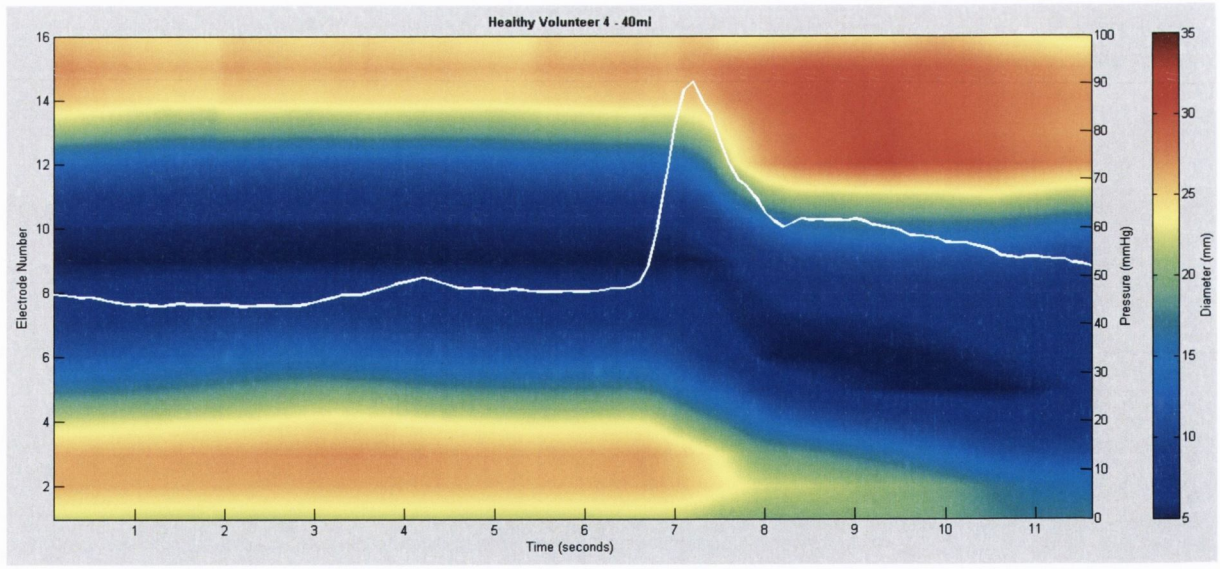
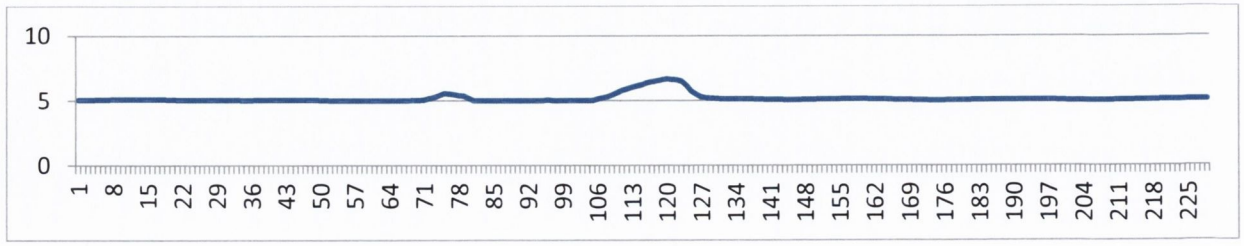


Figure 8.5: still image from three healthy volunteers at rest and during coughing. The cylinder represents the average CSA of the anal canal and the arrow represents the narrow zone length at each step volume


Healthy Volunteer 3 - 40ml
Electrode Number
Time (seconds)
Pressure (mmHg)
Diameter (mm)


6.00
5.50
5.00
4.50


Healthy Volunteer 4 - 40ml
Electrode Number
Time (seconds)
Pressure (mmHg)
Diameter (mm)


10
5
0

Appendix 6: Healthy contour plot cough data

Link:

https://drive.google.com/?usp=folder&authuser=0#folders/0B5dCwvvVNRbBSDFISnVSdW55SjQ

Appendix 7: Non-healthy contour plot cough data

Link:

https://drive.google.com/?usp=folder&authuser=0#folders/0B5dCwvvVNRbBOUJ0YWNjU01DQ00

Publications and Presentations

Published in peer-reviewed papers to date:

1. Alqudah, M. M., Gregersen, H., Drewes, A. M., & McMahon, B. P. (2012). Evaluation of anal sphincter resistance and distensibility in healthy controls using EndoFLIP©. Neurogastroenterology & Motility. Volume 24, Issue 12, pages e591–e599.

2. Maha Alqudah, Hans Gregersen, Asbjorn M. Drewes, Barry P. McMahon (2012). Using Distensibility Technique to Establish the Effect of Straining on Ano-rectal Function in Males and Females Healthy Subjects. Volume 142, Issue 5, Supplement 1, May 2012, Pages S-19. 2012 DDW Abstract Supplement to Gastroenterology

Poster Presentations to date:

1. Maha Alqudah, Colm Costello, Dr. Barry McMahon, A new method to describe the biomechanical action of the ano-rectal region in the human body, Nov 2009, Irish Society of Gastroenterology (ISG).

2. Maha Alqudah, Anne Brokjaer, Prof. Hans Gregersen and Dr. Barry McMahon. A novel method of measuring ano-rectal function using distensibility testing, Aug 2010, Neurogastroenterology and Motility Joint International meeting (NMJ)

3. Alqudah, Maha; Gregersen, Hans; Drewes, Asbjørn M.; McMahon, Barry P. Evaluation of anal sphincter resistance and distensibility in health using Endoflip. May 2012, Digestive Disease Week (DDW), San Diego, USA.

4. Maha Alqudah; Wheelan, Maria, McNamara, Deirdre, Neary, Paul; McMahon, Barry. Novel assessment of anal sphincter distensibility using Endoflip. Nov 2013, Irish Society of Gastroenterology (ISG)

Oral Presentations:

1. Maha Alqudah, Colm Costello, Prof. Hans Gregersen, Dr. Barry McMahon, Clinical evaluation of the ano-rectal functional lumen imaging probe, Feb 2009, Innovations in Medical Devices (IMED) Conference).

2. Maha Alqudah, Dr. Emmanuel Eguare, Prof. Hans Gregersen, Dr. Barry McMahon, A new method to define the biomechanical action of the ano-rectal region in the human body, March 2010, Innovations in Medical Devices (IMED) Conference.

3. Maha Alqudah, Asbion, Gregersen, McMahon (2.11). Application of functional lumen imaging distensibility testing to asses ano-rectal function. European Medical Physics and Engineering Conference (EMPEC). Dublin, Ireland.

4. Alqudah, Maha; Gregersen, Hans Drewes, Asbjørn M.; McMahon, Barry P. Using distensibility technique to establish the effect of straining on ano-rectal function in healthy subjects. May 2012, Digestive Disease Week (DDW), San Diego, USA.