DEVELOPMENT OF NOVEL METHODS OF ASSESSMENT IN OESOPHAGEAL AND GASTRIC FUNCTION

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Abstract

The objective of this thesis was to develop new methodologies to assess upper gastro-intestinal function in health and disease. Several different technologies were studied in a range of upper gastro-intestinal diseases and adapted to try and provide more meaningful insights. The thesis has three main sections.

In the first section, High Resolution Oesophageal Manometry (HRM) was used to assess unexplained upper gastro-intestinal symptoms in a group of patients referred to a tertiary centre. 46 patients were diagnosed with rumination syndrome following HRM. A retrospective review was completed of these patients case notes and HRM data. The predominant aim of this section was to identify if common mechanisms exist within rumination and its variations and to establish if the variety of presenting symptoms is due to different underlying problems or a common behavioural response to a variety of stimuli, with symptoms being dependent on the circumstance the behaviour exists in. This would support a generic biofeedback technique being useful regardless of presenting complaint.

Comparing the variety of symptoms, exhibited behaviour and manometric findings, a new classification system for rumination was then developed;

1. Primary or "classical" rumination

    a. Increase in abdominal strain with corresponding rise in intra-gastric pressure and return of gastric contents to the mouth

2. Secondary or reflux-related rumination
    a. Reflux event causes the patient to respond with increase in intra-abdominal muscle strain and subsequent rumination
3. Supra-gastric belching independent of meals.
    a. Rise in intra-gastric pressure whilst a closed gastro-oesophageal junction, therefore producing rapid belching of air from the oesophagus without any return of gastric contents

Generic biofeedback therapy was used (regardless of presenting symptoms) to control the abnormal behavioural response to symptoms. 20/46 patients reported full resolution of their symptoms and a further 13 / 46 reported improvement in their symptoms with this, while underlying mechanisms were targeted e.g. reflux with proton pump inhibitors, pain in functional dyspepsia.

In the second main section of this thesis, gastro-oesophageal reflux disease (GORD) is considered. GORD is currently diagnosed by 24 hour pH studies. These are often difficult for patients to tolerate and require time off medication. A more attractive method

would be for diagnosis to occur at the same time as gastroscopy. A novel instrument is the EndoFLIP® device. This measures cross-sectional area (CSA) and distensibility at the gastro-oesophageal junction (GOJ) via a long catheter with a balloon at the end that straddles the GOJ. It has been hypothesised that these measurements will be increased in those with GORD, as the GOJ is more distensible, allowing more retrograde movement of gastric contents. The aim of this section of the thesis was to establish if GOJ CSA and distensibility differentiate between healthy volunteers (HV) and GORD patients based on i) symptoms and ii) prolonged oesophageal acid exposure.

21 HV and 18 patients with GORD (based on symptoms) had EndoFLIP® measurements and wireless pH studies to assess this. 14% of HV and 50% GORD patients had pathological acid exposure. CSA and distensibility were both significantly higher in the HV's compared to GORD patients. However, there was an inverse correlation between CSA and body mass index (BMI) which was significantly higher in the patient population. This may explain differences seen due to corresponding higher intra-abdominal pressure in those individuals with a high BMI, sub-sequentially affecting the CSA and distensibility. The complex structure of the GOJ and multiple factors involved in the pathogenesis of GORD present difficulties in using EndoFLIP® to diagnose GORD. It may

find applications in other areas, such as serial measurements in single patients.

In the final section of this thesis, gastric emptying is the focus and its pathogenesis in functional dyspepsia (FD). Current gastric emptying studies only find abnormalities in approximately 40% of patients with FD. Gamma scintigraphy is used in routine clinical practice for gastric emptying studies. Magnetic resonance imaging (MRI) is emerging as a modality in gastric emptying assessment and potentially provides additional information.

This thesis hypothesised that standard gastric emptying studies may not be measuring the parameters reflective of underlying pathophysiology in FD. Also, most have a relatively small meal size that may be too small to trigger dysfunction. MRI may provide additional insights as can assess gastric contents and surrounding structure (unlike GS). To investigate these a 400ml test meal was utilised and gastric emptying parameters i) gastric contents volume at time 0 (GCV0, representative of early emptying), ii) gastric emptying rate at the time taken for half the meal volume to empty (GE rate @T50, representative of later emptying) and the more traditional measurement iii) time taken for half the gastric contents to empty (T50) in bopth GS and MRI studies. The hypothesis of this study is that early emptying is more rapid in FD due to impaired accommodation (therefore a lower GCV0) leading to a

slower later emptying (therefore a lower GE rate @ T50). Following validation studies in a large healthy population (n=53), GS and magnetic resonance imaging (MRI) studies with a test meal of 400ml were used in 8 FD patients and 24 matched HV (from the pool of HV) . FD had a significantly lower BMI. Early emptying (represented by gastric contents volume after ingestion of meal (GCV0)) was significantly lower in GS for FD patients but higher in MRI. Time for half the meal to empty (T50) and gastric emptying rate at T50 (GE rate @T50) were similar. The difference between the two modalities was thought to be due to increased secretion production in the patients, which is measureable in MRI but not in GS. A further study with a solid component of 12 non-nutrient agar beads in addition to the liquid component was completed. 24 HV's, 17 FD patients and 11 gastro-oesophageal reflux disease (GORD) patients were studied. FD patients and GORD patients had rapid early gastric emptying in comparison to HV in gamma scintigraphy (represented by GCV0) but higher GCV0 in MRI (significantly so between HV and GORD), suggesting increased secretion production is present in both conditions. These findings do support impaired fundal accommodation within the FD population but that other factors, such as secretion production and the rate of this in comparison to gastric emptying are important in the later stages of emptying. Further work is ongoing within the MRI department to quantify and measure the emptying of these secretions.

This thesis explores how existing and new technologies can be applied to clinical conditions to identify possible pathophysiology and potential targets for treatment. Only by these ongoing efforts can we endeavour to improve the care we deliver to our patients.

Publications from thesis

Papers

Rumination variations: aetiology and classification of abnormal behavioural responses to digestive symptoms based on high-resolution manometry studies.

Tucker E, Knowles K, Wright J and Fox MR. *Aliment Pharmacol Ther*. 2013. Jan;37(2): 263-74

Measurement of Esophago-Gastric Junction Cross-Sectional Area and Distensibility by EndoFLIP® (Endolumenal Functional Lumen Imaging Probe) for the Diagnosis of Gastro-Esophageal Reflux Disease (GERD).

Tucker E, Sweis R, Anggiansah A, Wong T, Telakis E, Knowles K, Wright J, Fox Mr. *Neurogastroenterol Motil*. 2013. Nov;(25)11:904-10

Abstracts

Gastric Volume Responses and Emptying After a Large Liquid Nutrient Meal in Functional Dyspepsia and Health Assessed by Non-Invasive Gastric Scintigraphy (GS) and Magnetic Resonance Imaging (MRI): A Pilot Study to Identify Candidate Biomarkers.

Tucker E, Parker H, Hoad C, Hudders N, Perkins AC, Blackshaw P, Marciani L, Costigan C, Fox MR. *Gastroenterology*, Vol. 142, Issue 5, S-194.

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Declaration

I declare that this thesis is the result of my own work. This thesis has not, in this or any other form, been presented to this or any other university in support of an application for another degree.
Dr Kevin Knowles and Dr Jeff Wright helped collect manometric recordings for high resolution manometric data alongside myself. Dr Mark provided expert opinion on manometric findings. The rumination classification, case note review, follow up data and classifications were all completed by myself.
Dr Rami Sweis recruited patients and recorded patient EndoFLIP® data, completed endoscopy and performed wireless pH recording. All healthy volunteer recruitment and procedures were completed by myself, with the aid of Emmanouil Telakis and Dr Kevin Knowles and Dr Jeff Wright helped with wireless pH data collection. Analysis of all the patient and healthy volunteer data was completed by myself. Dr Philip Kaye analysed histology.
Within the gastric emptying study healthy volunteers were recruited by myself and Dr Helen Parker. Patients were recruited by myself or Dr Mark Fox. Gamma scintigraphy raw data collection was completed by Dr Helen Parker and Elaine Blackshaw. MRI data was collected by Carolyn Costigan and Eleanor Cox. MRI program design and fitting program were designed by Dr Caroline L Hoad. Fitting of MRI data was completed by myself, Dr Helen Parker and Miss Nicola Hudders and Dr Caroline Hoad.
Analysis of results was completed by myself with input from Dr Mark Fox.

Abbreviations

^{111}In – indium 111

3D – 3- dimensional

5-HT – 5-hydroxytrytamine

5MBq -mega Becquerel

^{99m}Tc – technetium 99m

B-HCG – β human chorionic gonadotropin

AC – alternating current

ARSAC - Administration of Radioactive Substances Advisory Committee

b-SSFP - balanced steady-state free precession

bFFE (balanced fast field echo)

BMI – body mass index

CCK – cholecystokinin

cGMP - cyclic guanosine-3',5'-monophopshate

CI – confidence interval

CSA – cross-sectional area

CT – computed tomography

D – diameter

DIS – dilated intracellular spaces

DTPA- diethylene-triamine-pentaacetate

EndoFLIP® - endoluminal functional imaging probe

EPS – epigastric pain syndrome

EQ – EuroQuol

EUS – endoscopic ultrasound

FD- functional dyspepsia

FORS – fiber-optic recording system

FOV- field of view

$GABA_B$ - Gamma-Aminobutyric $acid_B$

GCV – gastric contents volume

GCV- - gastric contents volume at time 0

Gd-DOTA - gadolinium and 1,4,7,10-tetraazacyclododecane1,4,7,10-tetraacetic acid

GE – gastric emptying

GE rate @ T50 = gastric emptying rate at half gastric emptying time

GI- gastro-intestinal

GMP – good manufacturing practice

GOJ – gastro-oesophageal junction

GORD – gtasro-oesophageal reflux diease

GP – general practitioner

GS-gamma scintigraphy

HADS – hospital anxiety and depression score

HRM – high resolution oesophageal manometry

HV – healthy volunteer

I - current

IBP – intra-bag pressure

IBS – irritable bowel syndrome

IEN –intra-epithelial neutrophils

IQR – inter quartile range

ITLC - Instant Thin Layer Chromatography

LA- Los Angeles

LOS – lower oesophageal sphincter

Min - minute

MAA - Microaggregated albulim

Ml – milliliter

Mm - millimetre

MRI- magnetic resonance imaging

mSv – milliSievert

MTV –maximum tolerated volume

NDT – nutrient drink test

NHS – National Health Service

NO – nitric oxide

NUH – Nottingham University Hospitals

OGJ – oesophago-gastric junction

PHQ – perceived health questionnaire

PPI - Proton pump inhibitor

QMC Queens Medical Centre

ROI – region of interest

SAP – symptom association probability

SD - Standard deviation

SENSE -SENSitivity Encoding

SI – symptom index

SPECT – single photon emission computed tomography

T50 – emptying at half gastric emptying time

TCA - Tri-cyclic antidepressant

TE- echo time

TGV – total gastric volume

TLOSR – transient lower oesophageal sphincter relaxation

TR - repetition time

UK – United Kingdom

UOS – upper oesophageal sphincter

USS- ultrasound scan

V-voltage

VAS – visual analogue score

VIP - vasoactive intestinal peptide

Z – impedance

Chapter One – Overview of gastro-oesophageal function in health and disease and methods for assessment

1.1 Introduction

Gastro-oesophageal disorders are one of the major groups of disorders presenting to gastroenterologists, with dyspepsia accounting for 25% of presentations to out-patient services(1). Currently, the majority of tests within the gastro-intestinal tract (GI) use starved, static examinations, such as gastroscopy, for investigation of symptoms. This will assess for any structural or mucosal disorder but does little to look for any abnormality of function. Current standard methods of investigation, such as barostat measurements and 24 ambulatory pH studies are invasive and are not necessarily representative of normal physiology as either interfere with this or restrict individuals normal behaviour. Therefore, the development of non or minimally invasive tests for the assessment of GI dysfunction is attractive. This report reviews the current methods of investigation of GI function, with a focus on functional dyspepsia (FD) and gastro-oesophageal reflux disease (GORD). Although these conditions are separate entities, increasingly it is recognised that there is a great degree of overlap between GORD and dyspeptic symptoms (2). A significant number of patients with heartburn (the classic symptom in GORD) will have negative 24 hour pH studies for reflux (3). Conversely many

patients with epigastric pain have pathologic oesophageal acid exposure (4). This suggests the possibility of similar underlying pathophysiologies within the two conditions and a target for investigation.

1.2 Gastro-oesophageal reflux disease and functional dyspepsia

Gastro-oesophageal reflux disease (GORD) is a common condition in the community, with reported prevalence in the Western World between 10-20%(5). GORD is defined as the reflux of gastric contents into the oesophagus and causes symptoms (e.g. retro-sternal burning and regurgitation) and/or complications (e.g. Barrett Oesophagus) (6). Many individuals will experience these symptoms within their life but frequency of symptoms to 2 or more times a week is regarded as having an impact on quality of life by patients (6, 7). As well as these symptoms of GORD many patients complain of other upper abdominal symptoms including nausea, post-prandial fullness and bloating. These are classified as dyspeptic symptoms and patients with these and with no concurrent diagnosis, such as GORD, are diagnosed as having functional dyspepsia (FD). Currently 24 hour ambulatory oesophageal pH monitoring is used as gold standard for the testing of GORD, (by establishing whether patients symptoms are attributable to prolonged oesophageal acid exposure time and a relationship between symptom episodes and reflux events) (8)

whilst functional dyspepsia (FD), as with many function gastro-intestinal (GI) disorders as predominantly a diagnosis of exclusion i.e. tests for organic disease are negative and symptoms are attributed to FD.

1.3 Functional dyspepsia

1.3.1 Background and definition

Functional dyspepsia is defined as bothersome postprandial fullness, epigastric pain, early satiation and epigastric burning in the absence of structural disease, by the ROME III criteria (9). This is further sub-divided into post-prandial distress syndrome and epigastric pain syndrome, depending on which of the listed symptoms are most prominent.

Table 1. ROME III criteria for Functional Dyspepsia

B1. Diagnostic Criteria* for Functional Dyspepsia Must include 1. One or more of: a. Bothersome postprandial fullness b. Early satiation c. Epigastric pain d. Epigastric burning AND 2. No evidence of structural disease (including at upper endoscopy) that is likely to explain the symptoms *Criteria fulfilled for the last 3 months with symptom onset at least 6 months before diagnosis
B1a. Diagnostic Criteria* for Postprandial Distress Syndrome Must include one or both of the following: 1. Bothersome postprandial fullness, occurring after ordinary sized meals, at least several times per week 2. Early satiation that prevents finishing a regular meal, at least several times per week *Criteria fulfilled for the last 3 months with symptom onset at least 6 months before diagnosis Supportive criteria 1. Upper abdominal bloating or postprandial nausea or excessive belching can be present 2. EPS may coexist
B1b. Diagnostic Criteria* for Epigastric Pain Syndrome Must include all of the following: 1. Pain or burning localized to the epigastrium of at least moderate severity at least once per week

2. The pain is intermittent

3. Not generalized or localized to other abdominal or chest regions

4. Not relieved by defecation or passage of flatus

5. Not fulfilling criteria for gallbladder and sphincter of Oddi disorders

*Criteria fulfilled for the last 3 months with symptom onset at least 6 months before diagnosis

Supportive criteria

1. The pain may be of a burning quality but without a retrosternal component

2. The pain is commonly induced or relieved by ingestion of a meal but may occur while fasting

3. Postprandial distress syndrome may coexist

Although symptoms have been well described, the underlying causation has not been clearly identified. Associations have been made with various pathophysiological mechanisms but this partly limited by the heterogeneous nature of functional dyspeptic patients. Attempts at therapeutic interventions have only been partially successful due to this and the lack of identifiable therapeutic targets. Although there is rarely any mortality associated with FD, patients with the condition describe a negative effect on their health related quality of life, as reported in population studies (10). It also causes patients to attend both primary and secondary care physicians, resulting in a socio-economic burden (1). Therefore methods to assess the cause of symptoms and potential identify new areas of development for drug therapies are attractive to improve quality of life but also

attempt to reduce economic costs. Many mechanisms have been studied as the potential cause of symptoms in functional dyspepsia and these are outlined below.

1.3.2 Normal gastric physiology

In normal physiology the proximal stomach (fundus and upper part of body) acts as a reservoir for food and accommodates it here initially after ingestion, via relaxation of the stomach wall. This is mediated by receptive relaxation (fall in gastric tone), initiated by swallowing and promoted by gastric stretch by vagally mediated release of nitric oxide (11, 12). These changes allow the stomach to accommodate the food without a large, corresponding rise in intra-gastric pressure. The reservoir action also allows pepsin (a proteolytic enzyme) and hydrochloric acid to mix with the food to begin the digestive process. Additionally, gastric tone is modulated by nutrient feedback and other factors that are important to regulate digestion (adaptive relaxation) which is partly dependent on nutrient qualities of the meal ingested. The mechanisms by which these responses are managed represent a highly sensitive response to the calorie load and composition of the meal. Neuro-hormonal mediators including cholecystokinin (CCK) and other peptide hormones (13)act directly and via the vagal nerve (14) to regulate the delivery of nutrients to the small bowel.

This adaptive mechanism also acts by modulating gastric tone and the opening of the pyloric sphincter: the so-called ileal brake (15).

The distal part of the stomach (antrum and distal section of body) acts rather differently to the proximal part. It exhibits regular slow wave depolarisation activity at approximately 3 cycles per minute. This is steady, sequential depolarisation of cells initiated by the interstitial cells of Cajal, a type of smooth-muscle cell located in the greater curve of the stomach (16) that act as the pacemaker of the stomach (17). As food is moved distally by a tonic contraction from the fundus, the distal part of the stomach responds with rhythmic contractions. These allow mixing of the food and grinding into small particles (trituration) to permit passage into the duodenum through the pylorus(18). Food particles need to be approximately 2-3 mm in diameter before they are small enough to pass through the pylorus in the post-prandial fed state (19). Once the stomach has emptied, in the fasted state, a regular set of contractions, known as the migrating motor complex occurs. This is split into four phases. Phase I is a quiescent phase, with very little activity. Phase II is short, irregular contractions that rarely result in bolus transport. Phase III are regular, high amplitude contractions that move any remaining contents through the stomach, although over a relatively short period of time. Phase III contractions continue down the length of the small intestine and

sweep remaining contents through the intestine. Phase IV contractions are less regular and of lower amplitude than phase III and represent the motility returning to phase I. The whole cycles lasts on average 120 minutes but can be highly variable (12). This set of contractions are often termed “housekeeping” activity within the gut (20) and are present in the fasted state.

Gastric emptying is different for liquids and solids. Liquids enter the stomach and move quickly from the proximal to distal stomach and pass through the pylorus into the duodenum (21). Here, feedback from the duodenum will affect subsequent gastric emptying. Food with a nutrient value, when compared to non-nutrient saline, causes a subsequent delay of overall gastric emptying proportional to the calorie load (21). Solid emptying is slower than liquid because of an initial lag phase(22). This delay in gastric emptying is present because solids are initially retained in the proximal stomach and must be triturated prior to passage through the pylorus.

Once nutrients have moved into the duodenum, increasing duodenal distention reduces distal stomach motility to reduce content moving through the pylorus (23, 24). This negative feedback mechanism is under neuro-humoral control. Glucose within the duodenum of rats has been shown reduce proximal gastric motility via 5-HT3 receptors on afferent vagal nerve fibres

and it is thought that enterochromaffin cells lying within the duodenal mucosa release 5-hydroxytrytamine (5-HT) to instigate this (25). Distal stomach motility is also reduced by the presence of duodenal lipids. These causes the release of CCK (26, 27) and its effects on afferent vagal nerve fibres(28). This is sometimes referred to as the "duodenal" break with similar mechanistic properties as the ileal brake described above.

The duodenum also experiences regular depolarisation of cells. This is at an increased rate in comparison to the distal stomach, with a rate of 11-12 cycles per minute(29).

Once food is in the duodenum (as gastric chyme), activity of pancreaticobiliary secretions allow the chyme to be broken down into particles that can be absorbed via the epithelia barrier(30).

1.3.3 Pathophysiology in functional dyspepsia

Many different mechanisms have been suggested as potential abnormalities in functional dyspepsia. The principle hypotheses are represented in the schematic below.

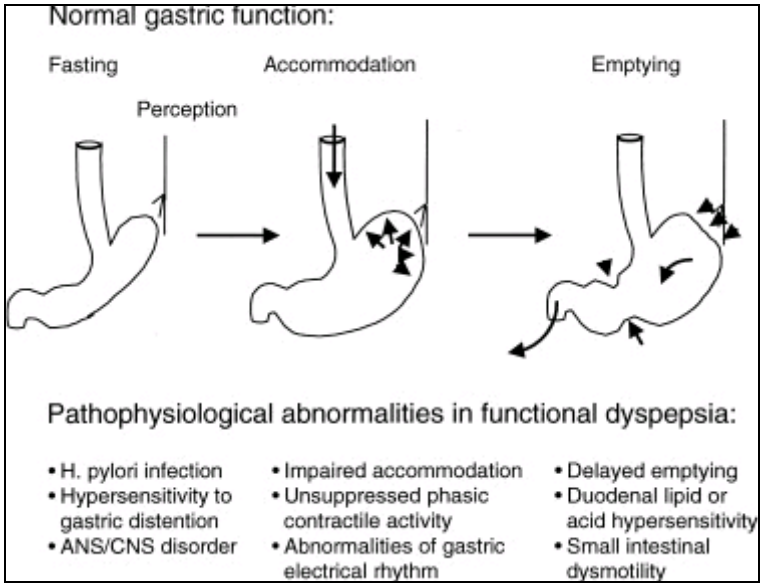


Figure 1. Normal gastric function – reproduced from Pathophysiology and Treatment of Functional dyspepsia, Tack et al. Gastroenterology, Vol 127:4:1239-1255.2004

1.3.3.1 Delayed gastric emptying in FD

Delayed gastric emptying has long been documented in functional dyspepsia (31). However, correlating severity of symptoms and gastric emptying rates has not been straight forward. Most studies report rates of delayed gastric emptying between 20-50% (32, 33). Therefore it is not uniformly present throughout the FD population, although some studies found that those with delayed gastric emptying are more likely to complain of post-prandial fullness and vomiting (31) this is generally limited to those with very severe delay (x3-4 normal) and has not been consistently shown throughout the literature. A large, multi-centre trial of 551 FD patients in 2001 showed that the presence of post-prandial

distress symptoms and their severity were not predictive of delayed gastric emptying, although female sex was (34). 24% of FD patients in this trial had delayed gastric emptying. Thus, although delayed gastric emptying does appear to play a role in FD it clearly is involved in a complex manner and as part of other processes. Another study in 2009 compared 11 FD patients to 23 healthy volunteers. Gastric emptying was measured with 13C breath test at t50 but also over time %dose per hour curves. This showed that 3/11 FD patients had increased gastric emptying in the early post-prandial phase and 4/11 had increased gastric emptying in the mid-post-prandial phase, when compared to healthy volunteers. However, t50 measurements were no different between the two groups (35). This supports the previously described findings that simple overall gastric emptying rate does not explain the cause of symptoms in FD and suggests that impaired accommodation plays an important role.

1.3.3.2 Impaired accommodation in FD

Normal physiology has been detailed above. Previous studies have shown that impaired proximal accommodation is present in FD (36), with reports of up to 40% of FD patients affected (37) and is associated with symptoms of early satiety and weight loss (38). However, other studies have shown no real difference in the intra-gastric meal distribution between FD patients and healthy controls

(39). These results demonstrate the heterogenicity of FD patients and the complex nature of cause of pathology. A small study has shown that increased early post-prandial fundal contractility (<30mininutes) is increased in a small subset of 15% FD patients and was also associated with bloating. However, differences, although significant, are small and bloating was reported into 82% of patient cohort, making cause and effect unclear (40). Some of the variations in findings may be due to methods used for interpretation, as both non-invasive and barostat methods are used for assessment. Mundt *et al* found that the present of a barostat bag (commonly used for assessment of gastric accommodation and hypersensitivity) results in larger antral areas and increased distal meal distribution(41).

1.3.3.3 Hypersensitivity

It has been suggested that FD patients are more sensitive to the normal physiological stimuli and changes within the stomach. A barostat is frequently used to monitor symptom response to gastric distension, and shown to be reproducible (42). A balloon located in the proximal or distal stomach (or both in double balloon barostats) can be inflated to set volumes and/or pressures and response monitored. Using this technique a variety of parameters can be measured. A study in 2001 found that pressure measurements of absolute over minimal distending pressure (equal

to baseline intra-gastric pressure) were most likely to differentiate between FD patients and healthy volunteers, with 37% of FD patients displaying gastric hypersensitivity and that these patients were more likely to complain of post-prandial pain, belching and weight loss (37). These findings were consolidated by a further study by the same group (43).

1.3.3.4 Helicobacter pylori

Helicobacter pylori is associated with the development of duodenal and gastric ulcers. Many groups have looked for a link between FD and this bacteria, with varying results (44). A systematic review performed in 2000 found no reliable association(45).

1.3.3.5 Central Nervous System dysfunction/psychological factors

It has long been known that FD impacts on quality of life. A recent Swedish study group have compared FD patients and controls with health related quality of life questionnaire, using the validated short-form 36 questionnaire(46). They found that quality of life was reduced in their FD population (10). Questions arise about whether the symptoms, such as anxiety and depression are because of the FD or if they are part of the FD spectrum and heterogenicity. A meta-analysis from 2003 found that functional dyspepsia was associated with anxiety and depression (47). A long term follow up study of FD patients in a tertiary referral centre (mean follow up period 68 months) found that depressive and

anxiety symptoms were more likely to be associated with persistent dyspeptic symptoms than abnormal gastric sensorimotor testing at initial visit (48). Also, up to 44% of women with functional GI disorders report physical and/or sexual abuse (49) and this has also been reported in the functional dyspeptic population (50). Mechanisms have been debated about the possible central processes that could be responsible for these findings. The majority of work has been completed in functional GI disorders as a whole, often with a focus on irritable bowel syndrome (IBS). Autonomic nervous system dysfunction has been suggested. It has been found that IBS patients displayed increased peripheral sympathetic function by laser Doppler flowmetry, in comparison to healthy controls (51). The stress response has also been implicated. Hypothalamic-pituitary-adrenal axis hyper- and hypofunction has been reported but such conflicting results make interpretation of this difficult at present (52, 53).

Studies have been completed looking at gastric response to stress. A study of healthy volunteers assessed sensorimotor function response in the stomach with experimentally induced anxiety (54). 14 healthy volunteers underwent gastric barostat studies and 18 healthy volunteers underwent a nutrient drink test (NDT), whilst experiencing emotionally fearful or emotionally neutral facial recognition or recollection of events. Reduced gastric compliance

was found in the anxious state in the barostat group, reflecting impaired gastric accommodation. Also, higher symptom scores for satiety, fullness and bloating were documented in the anxious state in the nutrient drink test. However, although gastric response to stress is implicated in the functional dyspepsia, it is difficult to assess whether the dyspeptic symptoms have caused the anxiety or vice versa.

1.3.3.6 Altered duodenal response to lipids/acid and dysmotility

Duodenal abnormalities have been thought to be involved in FD. Duodenal distension normally reduces antral motility via negative feedback mechanisms(55). FD patients have been found to have a reduced motor response to a direct duodenal acid infusion. 59% of patients reported nausea with the acid infusion, in comparison with none of the control group(55). An initial study showed no significant differences in symptoms between the patient and control group were found with the infusion of lipid into the duodenum. However, it is worth noting only a low volume of lipid was used (5ml). Another group infused a much higher volume of lipid into the duodenum and scored symptoms whilst increasing gastric distension in healthy controls and FD patients (56). Symptoms occurred sooner in the FD patients with lipid infusion than in the fasted state. These findings suggest that abnormalities

within chemoreceptors in the duodenum of FD patients may account for some of their symptoms.

FD pathophysiology is heterogeneous and although abnormal findings can be identified, they are not consistent through the whole population. This represents significant challenges in identify underlying mechanisms but also directing treatment strategies. The development of further techniques and technologies to elicit the causes of symptoms in subsets of functional dyspeptic patients is required.

1.3.4 Other factors that can affect gastric motility and symptoms of functional dyspepsia and upper gastro-intestinal disease

1.3.4.1 Exercise

Exercise has been associated with upper gastro-intestinal symptoms. One study showed that up to 90% athletes have reported fullness, regurgitation, belching and chest pain (57). We know that gastro-oesophageal reflux disease is associated with transient lower oesophageal sphincter relaxation (58). A study of ten healthy volunteers found that episodes of reflux during exercise were associated with transient lower oesophageal sphincter relaxation (59). However, a review of 100 patients with confirmed reflux disease found that differing levels of everyday physical activity were not associated with increased reflux symptoms (60).

Gastric emptying has been found to be mildly accelerated or not affected by light exercise (61, 62). However, strenuous physical exertion, when VO2 maximum is around 70% (i.e. 70% of the maximum volume of oxygen that can be utilized in one minute during maximal or exhaustive exercise) has been associated with slower gastric emptying (63, 64) for both liquids and solids (65).

Overall, mild to moderate physical activity does not has significant effects on gastric emptying, but more strenuous exertion can (66). For the majority of patients with functional dyspepsia, they are unlikely to reach the exertional level of exercise required to have significant effects on gastric motility.

1.3.4.2 Alcohol

Alcohol is frequently consumed with a meal. Varying reports have been described with its effects on gastric emptying and upper gastro-intestinal symptoms. Several studies have found that alcohol slows gastric emptying (67-69) while a study comparing low and normal alcoholic concentration wine, had no differing effect on gastric emptying which could imply that it is the calorie content of the alcohol and not the alcohol itself that causes the delay in gastric emptying (70). However, a recent study comparing the effects of drinking black tea or Schnapps when eating cheese fondue found that increasing alcohol concentrations were associated with a very rapid delay in gastric emptying more

consistent with direct than indirect effects (71). Patients do sometimes report symptoms worsening with alcohol, but study results have been inconclusive (72).

1.4 Gastro-oesophageal Reflux Disease – pathophysiology and current methods of diagnosis

1.4.1 Definition

GORD is a condition characterized by the occurrence of symptoms or mucosal damage related to the retrograde movement of gastric contents from the stomach into the oesophagus. Reflux occurs in normal, healthy individuals but excessive reflux or excessive sensitivity to reflux can cause symptoms of heartburn, indigestion and regurgitation. The repeated exposure of the oesophageal mucosa to acid stomach contents can lead to the development of oesophagitis or Barrett's oesophagus – the development of columnar metaplastic epithelium within the oesophagus, replacing the normal squamous lining(73). This increases the risk of oesophageal adenocarcinoma therefore is of significant impact (74). Extra-oesophageal symptoms of GORD, such as cough and hoarse voice can also occur.

1.4.2 Normal gastro-oesophageal structure and physiology

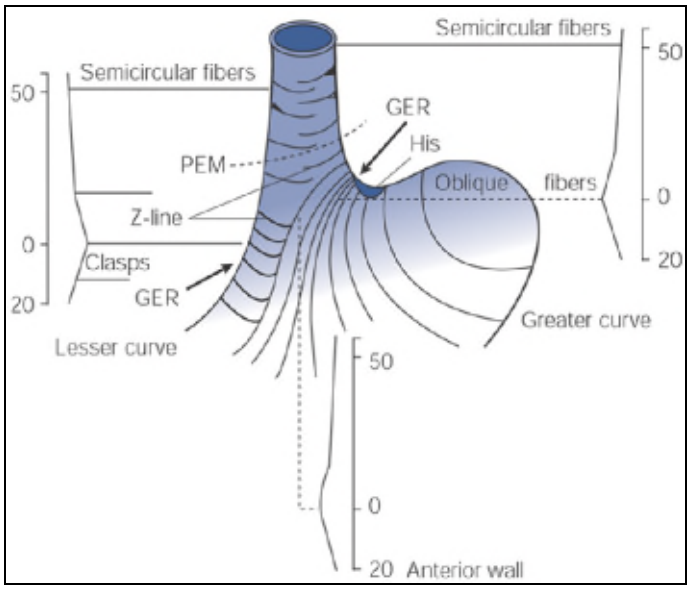


Figure 2. The normal structure of the lower oesophageal sphincter – reproduced from GI motility online (May 2006) doi:10.1038/gimo21

The lower oesophageal sphincter (along with the crural diaphragm) acts as an anti-reflux barrier via the mechanism of a high pressure zone, to prevent retrograde movement of gastric contents from the stomach. Reflux occurs when lower oesophageal sphincter pressure is lower than that of gastric pressure. Rather than being a discrete symmetrical ring of muscle, it is made up of a semicircular clasp (transverse) and gastric sling (oblique) fibres. The clasp fibres sit transversely across the area in between the oesophagus and stomach and open anteriorly and posteriorly. The sling fibres reach from the angle of His and greater curvature, in the direction of the antrum, parallel to the lesser curvature of the stomach. On closure

of the lower oesophageal sphincter the sling fibres pull the greater curve down and towards the midline and the clasp fibres pull the lesser curve transversely across (75). The clasp fibres have a high degree of intrinsic tone which is relaxed by the release of nitric oxide. The sling fibres respond to cholingeric excitation and relax when this is absent.

Recent work has looked at the angle of insertion of the oesophagus into the stomach, using MRI and HRM in GORD patients and HV(76). This study found that the angle if insertion was greater in the GORD patient population, suggesting this is a factor in reflux disease.

Contractions of the crural diaphragm also add to the so called high-pressure zone at the lower oesophageal sphincter, to facilitate closure and prevent reflux especially during cough and physical exertion that increases abdominal pressure.

1.4.3 Mechanisms of GORD

1.4.3.1 Transient lower oesophageal sphincter relaxation

Transient lower oesophageal sphincter relaxations (TLOSR) are the relaxation of the lower oesophageal sphincter without a preceding swallow(77). These normally occur to allow air to escape from the stomach and are mediated by a vaso-vagal reflex due to gastric distension. These occur most frequently after a meal (78, 79) and along with air, often allow stomach contents to reflux into the

oesophagus. When first discovered it was thought that these events were increased in GORD patients and the cause of reflux but subsequent work has shown that numbers are similar in healthy controls and GORD patients (80). This suggests increased frequency is not the pathology of reflux disease but the increased incidence of reflux within the TLOSR in GORD patients.

At other times not associated with a TLOSR, reflux can occur when intra-gastric pressure is greater than that of the lower oesophageal sphincter.

1.4.3.2 Gastric acid

Gastric acid causes oesophagitis after repeated/prolonged contact with oesophageal mucosa. It had previously been suggested that patients with GORD may produce larger amounts of gastric acid. This has not been found to be the case (81). Mechanisms which allow prolonged contact of the gastric acid with the oesophageal mucosa may play a role, and are described below.

1.4.3.3 Oesophageal dysmotility

GORD is associated with oesophageal dysmotility. A recent high resolution oesophageal manometry study has shown that hypotensive oesophageal swallows in reflux patients were associated with longer oesophageal acid exposure time. Interestingly, water swallows did not consistently demonstrate this dysmotility in comparison to solid swallows. The authors proposed

that very little motility is required for water transport through the oesophagus due to the effects of gravity but that solid swallows provide a more significant challenge and are therefore more likely to elicit abnormalities in the physiology. Hypotensive oesophageal motility was not however associated with increased reflux events, indicating that dysmotility is a cause of poor oesophageal clearance, prolonging the reflux events rather than allowing an increased number (82).

1.4.3.4 Structural abnormalities - Hiatus hernia

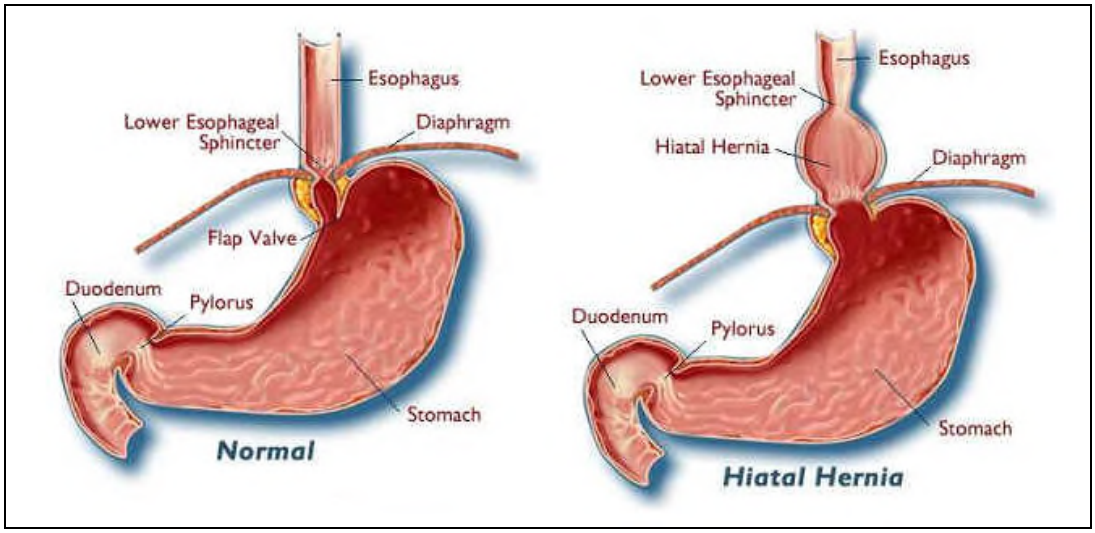


Figure 3. Anatomy of a hiatus hernia. Reproduced from http://www.kmcpa.com/gastroenterology/education/hiatal_hernia.ph

The presence of a hiatus hernia affects reflux. Van Herwaarden *et al* compared GORD patients with and without hiatus hernia, with oesophageal manometry and 24 hour pH studies. They found those with a hiatus hernia had prolonged acid exposure and increased reflux events. TLOSR were similar in both groups but hiatus hernia patients were more likely to reflux when LOS pressure was low and with normal relaxation of the LOS following a swallow (83). Possible mechanisms underlying this have been identified by Pandolfino *et al* who identified that the gastro-oesophageal junction (GOJ) of GORD patients with hiatus hernia opened wider than GORD patients without hiatus hernia and normal controls (84). They also found that distension pressures greater than

atmospheric pressure (but less than gastric) provoked GOJ opening in the hiatus hernia group only. Patients with hiatus hernia also have poorer clearance, with acid/reflux material accumulating in the hiatal sac and subsequently refluxing into the oesophagus(85).

1.4.3.5 Acid pocket

The acid pocket is a concept recently revisited in current research. Intra-gastric pH rises after meal (due to meal related buffering) but the pH of the proximal stomach remains remarkably low. This is referred to as the acid pocket (86). It is thought to be particularly important in the post-prandial reflux and be a potential specific target for the relief of reflux-related symptoms.

1.5 How do we measure Gastro-intestinal function?

1.5.1 Current assessment of methods of upper GI function?

There are many possible options when investigating the GI tract. Methods that can assess function as well as structure are detailed below.

1.5.1.1 Gastroscopy

Any patient presenting with symptoms such as weight loss, recurrent vomiting or dyspepsia will undergo gastroscopy to exclude structural disease. The majority of functional dyspepsia patients and those with reflux symptoms that do not respond to acid suppression medications will undergo this early in the disease process.

1.5.1.2 Pepsin testing

Pepsin is a proteolytic enzyme produced by the chief cells in the stomach mucosa. It is produced as a precursor, pepsinogen, and is converted to pepsin in the presence of an acidic pH. It is damaging to laryngeal cells and disrupts intercellular junctions and thought to be one of the major causes of symptoms of extra-oesophageal reflux symptoms, classically cough and hoarse voice, via aerosoled reflux. Tests for the presence of pepsin have been developed as a potential marker of reflux disease, as its presence in salvia can only be explained by reflux from the stomach(87). It

is yet to be seen if this will become standard practice for the investigation of GORD.

1.5.1.3 24 hour pH studies

If heartburn is a predominant feature or significant oesophagitis seen on gastroscopy then ambulatory 24 hour pH studies are recommended. A pH catheter is inserted trans-nasally, through the oesophagus and the GOJ, into the stomach. This records pH in an oesophageal sensor 5cm above GOJ and gastric pH sensor, 2cm below the GOJ. The patient then records any symptoms on electronic device and these can then be correlated to the pH recording and any drops in oesophageal pH below 4, indicating reflux into the oesophagus. The addition of impedance can add additional information. Impedance measures the electrical resistance of a substance. Multiple impedance sensors can be added to a pH probe and record retrograde and anterograde flow of substances (88). Substances with higher impedance (with a lower number of ions) such as air will produce a different impedance trace to low impedance substance, such as gastric acid.

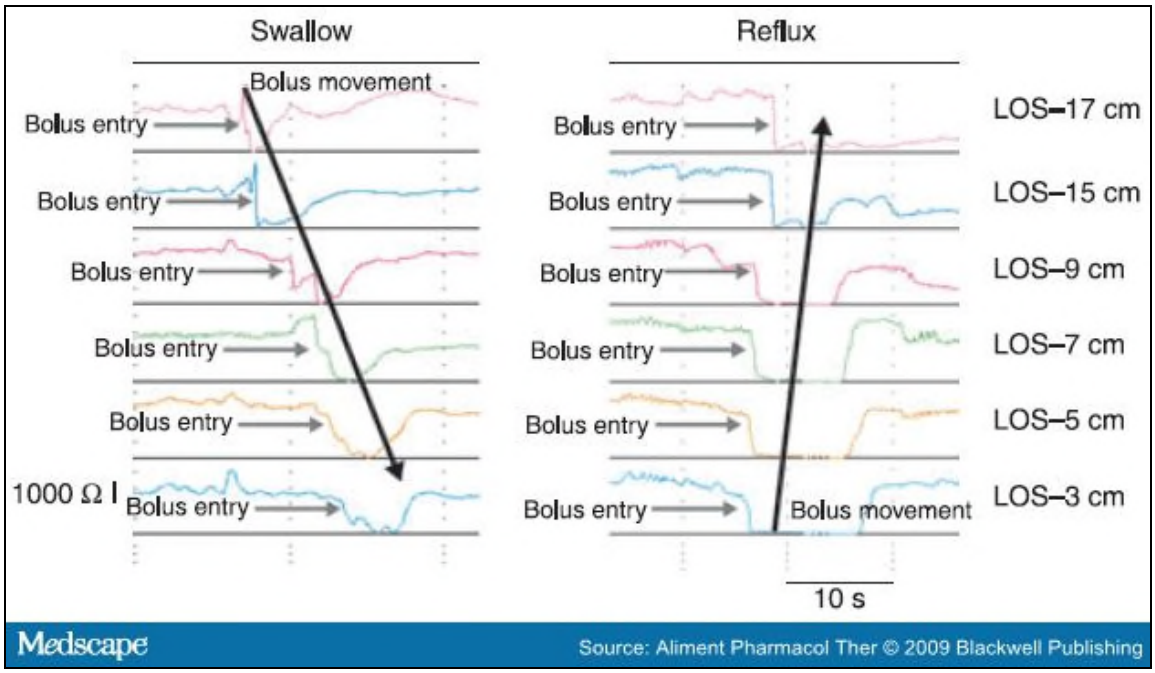


Figure 4. Impedance trace showing swallow on the left and reflux of liquid on the right (due to drop in impedance). Reproduced from Intra-oesophageal Impedance Monitoring for the Bolus Transit and GORD. Conchillo and Smout. APT. 2009;29(1):3-14.

Therefore, impedance can document reflux events that are non-acid and weakly acid, gas and liquid, providing further evidence of whether refluxing material (acid or not) is the cause of symptoms. Although this is the current gold standard for GORD testing, the invasive nature of the test and variability of symptoms on a day-to-day period provides many problems. Patients often adapt their behaviour and diet with the presence of the pH catheter with subsequent results not being representative of their "normal" symptoms.

1.5.1.4 Wireless pH studies

A group of patients do not tolerate nasal intubation for catheter based studies or tolerate it badly such that their eating and activity is reduced to a degree that impacts on the frequency and severity of reflux. An alternative is a wireless capsule which is attached 6 cm above the Z line and transmits pH data to a radio receiver worn by the patient. The advantage of these is prolonged measurement time of up to 96 hours, reducing the potential for variability encountered in 24 hour studies (89). Patients are also more likely to perform normal tasks and data therefore more representative of "real life" is obtained. A study looking at patients with a negative 24 hour pH recording and persistent symptoms consistent with GORD showed that in 14/38 patients wireless pH recording demonstrated abnormal oesophageal acid exposure time, when considering average exposure time over the 96 hour period (90). This can have significant impact on clinical management as 12 of these patients went on to have anti-reflux surgery on the basis of these findings and subsequent improvement in symptoms.

Wireless pH studies are not without disadvantages. The cost is significantly higher than a 24 hour catheter study, endoscopy is often used to confirm position, the capsule can detach early and no impedance values can be measured with this technique. In addition, there is debate over the best measurement to use. Worst

day of symptoms, average over recording period are both discussed in the above paper with cases made for both. Further studies with comparison in negative and positive catheter studies with wireless technology is needed provide answers to these issues (study cited only used patients who'd had negative catheter based studies). Currently, within the United Kingdom it is used when catheter studies have failed in a small number of hospitals.

1.5.1.5 High resolution oesophageal manometry with impedance

Oesophageal manometry records pressure measurements within the oesophagus. A catheter is inserted as in a pH study. Multiple sensors are spaced along the length of the catheter and pressure measurements recorded. Conventional manometry used a low number of sensors (approximately 3-5 cm apart) and produces line plots of pressure. A relatively new technology, high resolution manometry (HRM) uses 36 sensors, less than 1 cm apart to record pressure. The HRM then produces spatio-temporal plots which provide far more information about the structure and function of the oesophagus (91). Impedance sensors can also be added to the catheter to provide information about bolus transport. The pharynx, oesophagus and lower oesophageal sphincter can all be studied in detail.

Gastro-oesophageal reflux can be identified with HRM. A transient lower oesophageal relaxation is seen, followed by the retrograde

movement of gastric contents in to the oesophagus(with impedance). A clearance swallow will then follow.

An advantage of HRM is that symptoms can be elicited during the study e.g. during a test meal. The observer can than directly identify the mechanism of symptoms by concurrent measurement of oesophageal activity. This can be especially useful in treatment resistant/unexplained symptoms, such as proton pump inhibitor (PPI) resistant reflux or unexplained vomiting/regurgitation.

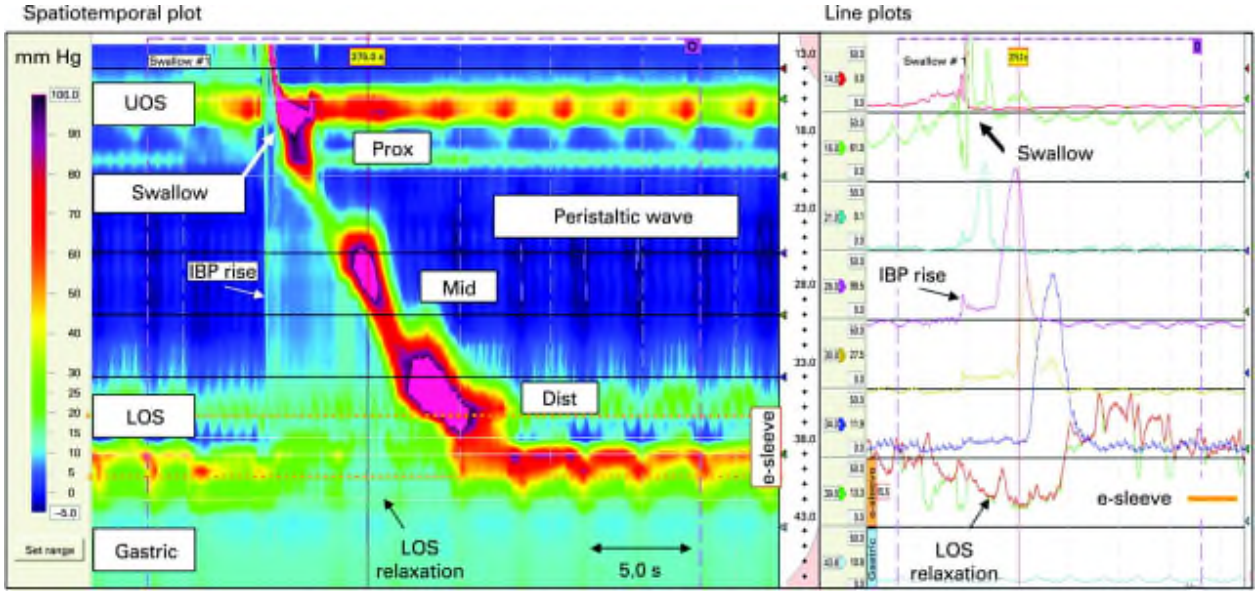


Figure 5. Representation of normal swallow – as represented by HRM spatio-temporal plot and conventional manometry line plot. Reproduced from Fox and Bredenoord. Gut 2008;57 :405-423

Manometry is not that useful within the stomach. To record pressure measurements the organ needs to come into contact with the catheter. As the stomach is a large cavity and the average HRM

catheter approximately 4mm in diameter, there is rarely the contact needed to provide pressure measurements.

1.5.1.6 EndoFLIP®

Another way of approaching the diagnosis of GORD is to assess whether any other physiological investigation could establish the diagnosis, without the need for pH testing. A novel probe has been suggested for this using impedance planimetry. The Endoluminal Functional Lumen Imaging Probe (EndoFLIP®, Cropson, Galway, Ireland) is a probe with a balloon at the end, with 16 paired electrodes situated inside, at constant distances. The balloon is filled with a conductive medium with a known conductivity at a constant temperature. An AC current is then passed through the medium. Using the principles of Ohm's law, the diameter of the site the balloon is inflated in can be calculated.

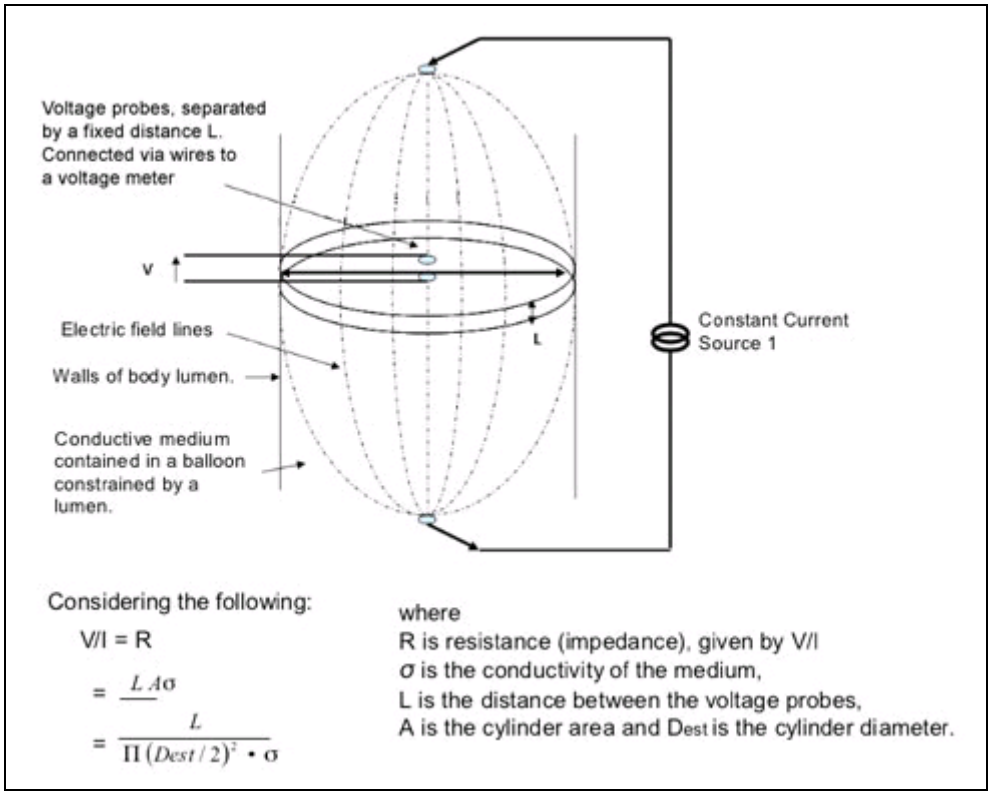


Figure 6. EndoFLIP® technology. Reproduced from http://www.cropson.com/

Impedance (Z) = voltage (V)/current(I)

Z = distance between electrodes (L) / CSA . σ

(CSA=cross-sectional area, σ = conductivity coefficient)

Therefore;

V/I=L / CSA . σ

CSA = ∏r2 = ∏(D/2)2

Therefore;

V/I=L/((∏(D/2)2 . σ)

Voltage is measured across the electrodes, current and distance between electrodes is fixed and conductivity is known at a certain temperature. Therefore the diameter of the area of the balloon is inflated in can be estimated from the voltage. With a cylinder and paired electrodes throughout, diameter can be estimated throughout the length of the balloon, and subsequently cross sectional area (92). The distensibility of the area can then be studied as this is equal to cross-sectional area divided by the intra-bag pressure.

This probe is passed through the mouth and straddled across the GOJ. The balloon is inflated sequential volumes and diameter, cross-sectional area and distensibility recorded. Lower oesophageal sphincter incompetence is implicated in GORD, especially in the context of hiatus hernia(93). It has therefore been suggested that this could be important (without hiatus hernia) in the pathophysiology of reflux disease. If distensibility could be measured at the time of endoscopy, it could potentially identify GORD patients without the need for pH monitoring, making it an attractive option. A study based on symptoms has shown that increased GOJ distensibility and cross-sectional area are associated with symptoms of GORD, in 20 healthy volunteers and 20 patients with symptoms consistent with GORD (94). However, only 2/20 patients had confirmed prolonged oesophageal acid exposure

meaning the diagnosis of GORD was based on symptoms alone in the vast majority. For endoFLIP® to be useful in the diagnosis of reflux disease, the GOJ distensibility and CSA need to be compared to symptomatic patients and those with confirmed GORD on pH studies. Only then can its value as a diagnostic aid be confirmed.

An alternative use for the EndoFLIP® probe could potentially be in known GORD patients being assessed for anti-reflux surgery. If a patient's GOJ was very distensible with EndoFLIP® this could predict who would be most likely to benefit from anti-reflux surgery. However, no prospective trial has been completed using the EndoFLIP® in this way to date.

1.5.1.7 Barostat studies

The barostat has been used to measure gastric accommodation and sensitivity to gastric distension in many studies of functional dyspepsia (38, 95) and shown to be reproducible(42). Studies are normally done fasted and a double lumen tube with balloon on the end (normal possible volume 1000-1200ml) is inflated in the proximal stomach to either set pressure (isobaric) or set volume (isovolume). When isobasric measurements are performed the barostat is first calibrated for intra-gastric pressure. This has been defined as the pressure required to unfold the balloon, normally to about 30ml, and termed the minimal distending pressure (96). The barostat balloon is then inflated to set pressures with subsequent

measurements of intra-balloon volume recorded. This reflects gastric tonic relaxation and allows measurements of gastric compliance and, if sensation scores are assessed, sensitivity. Isovolumetric measurements involve pre-selected volumes inserted into balloon and subsequent intra-balloon pressures recorded during interventions (e.g. a meal), reflecting accommodation. Differences in patient and volunteer groups have been described earlier in pathophysiology section.

Although barostat is currently regarded as the gold standard, it is invasive, unpleasant for the patient and the presence of the balloon itself can interfere with normal physiology(41).

1.5.1.8 Gamma scintigraphy

Gamma scintigraphy is currently used in clinical practice to assess gastric emptying. Liquids and/or solids are radiolabeled with radionuclide. These are then ingested and a gamma camera monitors emitted gamma rays as the radionuclide decays. As the radiolabeled substrate moves through the stomach, a 2D image is produced from the gamma camera. Within the stomach this can inform on meal distribution within the proximal and distal stomach, gastric emptying rate and small bowel transit time. It is a non-invasive test and performed in the physiological, upright position (unlike some other non-invasive methods). It does require a relatively low dose of radiation exposure.

It has been used in FD to demonstrate abnormal gastric emptying and impaired gastric accommodation in terms of the differences in meal distribution within the FD population and their relevance to symptoms(14). However these effects have never been repeated in an unselected population or validated in comparison to other tests. Also currently, gastric emptying study results have little correlation to symptoms or have any effect on guiding treatment. Reasons for this may be many. Is the correct test meal being utilised or the correct gastric emptying parameters being measured?

1.5.1.9 Single Photon Emission Computed Tomography (SPECT)

SPECT studies involve the intra-venous administration of a gamma emitting radioisotope which is taken up by parietal and mucin-producing cells of the gastric mucosa (97) with a gamma camera. 3D images of the stomach are produced (via analytical software) and information about gastric volume and volume response to a meal can be obtained. A recent review by Breen *et al* of 433 previous study participants undergoing SPECT (volunteers and patients) demonstrated comparable inter-individual coefficients of variation of fasting and post-prandial gastric volumes and comparable intra-individual coefficients in those who had had repeat studies when compared to other modalities such as gamma scintigraphy for gastric emptying. (98)

The advantages of SPECT are that it is non-invasive and can provide detailed 3D images in both the fasting and fed state. It does however require the participant to be supine, expose the individual to a relatively high radiation dose, is expensive and requires specialist centre and equipment.

1.5.1.10 Magnetic Resonance Imaging (MRI)

MRI is commonly used in clinical practice for diagnostic imaging. The object to be studied is placed in a magnetic field. Hydrogen nuclei will align with this magnetic field (due to protons) and create a directional magnetic field. A radiofrequency pulse is then applied to the directional magnetic field, causing this to move away, via exciting the protons within the nucleus. The radiofrequency pulse is removed and the nuclei realign themselves with the original magnetic field. As they return to this position, they emit their own electromagnetic field, which is recorded by a coil (signal detection) and used to reconstruct a 3D image of the object being studied (99).

Functional upper GI MRI can gather many different parameters. Gastric emptying, accommodation, secretions and intra-gastric distribution can all be measured. A recent study compared inter-observer reproducibility with gastric MRI and found it satisfactory with greatest agreement at larger gastric volumes (100).

MRI is an attractive source of imaging as it is non-ionising, non-invasive and provides detailed images of the internal organs. However, it is expensive, not readily available in all centres (used as a research tool at present), requires the patients to be supine and is not available to patients who have contraindication to going into a magnetic field e.g. cardiac pacemaker.

1.5.1.11 Breath tests for gastric emptying

Breath test appeal for measurement of gastric emptying as they are quick, cheap, easy to use and can be repeated in one subject many times. An isotope is added to a meal which is them converted within the duodenum to a measurable substance.

The ^{13}C-octanoic breath test is commonly used. The isotope is added to a solid meal, this remains stable until the duodenum where it is then absorbed and oxidised by the liver (transported via portal venous system) to ^{13}CO2 (101). ^{13}C-acetic acid breath test has also been used, with the similar principles. This labelled carbon dioxide can then be measured in the breath by mass spectrometry. The arrival of ^{13}C in the duodenum and subsequent detectable levels in the breath, is rapid, indicating any variation in time is primarily because of gastric emptying into the duodenum, rather than other steps such as transport to the liver and oxidisation (102). However this is contradicted by the fact that only about 20% of the ^{13}C dose is recovered over the course of the study. Also

there is no agreement about the most appropriate method for analysis with important differences documented for different analytical techniques. Further the test provides information only about gastric emptying without detail as to the dynamics of this process or information about meal distribution.

1.5.1.12 Gastric Ultrasound

Gastric ultrasound predominantly provides information in gastric volume, either total or partial. Both 2D and more recently 3D, ultrasound have been used for this purpose. It is desirable as is non-invasive and non-ionising. It is however, still highly specialist and operator dependent.

3D ultrasound scanning (USS) was compared to the current gold standard of barostat for gastric volume. 3D USS performed best with proximal stomach volume, in comparison to barostat, with an r value of 0.55 (103). Correlation was less for total gastric volume and non-significant for distal gastric volume. Comments from the authors were that air pockets were the greatest problem in gastric USS, making assessment with this technique more problematic. Gastric USS exists currently as a research tool with variability in recorded values and is limits by technological factors.

Doppler ultrasonography has also been used to study trans-pyloric flow to estimate gastric emptying (104).

1.5.1.13 Nutrient drink tests

The techniques described already provide information about anatomy and physiology. However, most are expensive, invasive or require exposure to radiation. As one of the predominant symptoms in FD is that of post-prandial distress, drink tests (water and nutrient) have been suggested as an easy and accessible way of assessing symptoms. Early satiety has been associated with abnormal fundal accommodation and therefore an indirect way of measuring accommodation (38). A drink test involves the patient drinking water or nutrient at a set rate, whilst scoring dyspeptic symptoms until a maximum tolerated volume is reached (when symptoms prevent further ingestion). However, there are many different options for nutrient drink tests. Drinking rate, nutrient or non-nutrient substrate, best outcome measure are examples. A Dutch group compared nutrient drinking and water drinking tests in FD patients, those with mild dyspeptic symptoms and healthy volunteers with the results of barostat studies(105). Although the FD group drank less water and nutrient than the two control groups, it was not associated with one particular symptom and did not predict an abnormal barostat study. It was noted that the rate of drinking was 100ml/min, a relatively high rate of ingestion. A different study by Tack *et al* showed that a slow drinking test (15ml/min) with calorific nutrient showed a weak but significant correlation in functional dyspeptic patients with maximum tolerated

volume and impaired accommodation in concurrent barostat studies(106). A possible explanation suggested for this was that rapid drink tests don't allow for gastric accommodation, which can take up to fifteen minutes to have full effect (38) and that non-nutrient drink tests don’t evoke the inhibition of gastric emptying from via negative feedback from lipid within the duodenum.

These results suggest a slow rate, calorific drink test is the most appropriate to use as within a functional dyspepsia population. However the drink test alone provides no direct information about the mechanism of symptoms which could be related to impaired accommodation or heightened sensitivity or some other abnormality of gastrointestinal function.

2 Chapter Two: Rumination Variations: aetiology and classification of abnormal behavioural responses to digestive symptoms based on high-resolution manometry studies

2.1 Introduction

2.1.1 Definition

Rumination, as classified by the ROME III criteria (107) is the voluntary, albeit unconscious, contraction of the abdominal muscles forcing return of food to the mouth, followed by re-chewing, swallowing or spitting.(108) Although these events are often described as "vomiting", no violent retching is involved and stomach contents are usually returned to the mouth as a series of small volume events rather than one large volume expulsion. They are not necessarily preceded by nausea, as is normal in vomiting.

Associated symptoms, including repetitive belching is also a consequence of abnormal behaviour, either due to excessive swallowing of air (i.e. aerophagia), or suction of air into the oesophagus during forced inspiration (i.e. supra-gastric belching) (109-111). This is distinguished from gastric or "normal" belching in that there is no associated relaxation of the lower oesophageal sphincter or release of air from the stomach. Rumination and supra-gastric belching are similar due to both being behavioural in origin.

2.1.2 Epidemiology

Rumination was previously diagnosed predominantly in the paediatric and learning disabled populations, but it is increasingly recognised in an adult patients with normal intelligence (112-114). Although rumination itself is rarely associated with significant mortality, it can be associated with major morbidity, such as social embarrassment and weight loss (115, 116)

2.1.3 Approaches for diagnosis

The diagnosis of rumination and belching disorders can be made from clinical history (Table 2); however there is often a delay due to lack of awareness of these conditions by physicians (112). As a result, those affected may see many doctors and undergo multiple, invasive investigations before a definitive diagnosis of rumination is made (112)

Table 2. Definition or rumination syndrome and aerophagia- symptoms must have been present for at least 6 months with 3 months prior affected

Rumination Syndrome in Adults
Diagnostic criteria. Must include both of the following:
1. Persistent or recurrent regurgitation of recently ingested food into the mouth with subsequent spitting or remastication and swallowing 2. Regurgitation is not preceded by retching
Supportive criteria
1. Regurgitation events are usually not preceded by nausea 2. Cessation of the process when the regurgitated material becomes acidic 3. Regurgitant contains recognizable food with a pleasant taste
Aerophagia
1. Troublesome repetitive belching at least several times a week 2. Air swallowing that is objectively observed or measured
Gastric belching
1. Venting of air from the stomach, with increase in intra-luminal impedance from distal to proximal oesophagus
Supra-gastric belching
1. Anterograde movement of gas followed by followed by rapid expulsion (Rapid increase in impedance from proximal to distal, with rapid retrograde return to baseline)

Objective diagnosis can be based on the close temporal association of typical symptoms with evidence of abnormal behaviour on physiologic studies. Recent advances, such as High Resolution Manometry (HRM), facilitate the detection and diagnosis of dysmotility and dysfunction during and after meals.(82, 91, 110, 117-119) Combination with impedance provides independent confirmation that these pressure events are associated with retrograde movement of food, fluid or gas through the oesophagus

(118). This work has raised awareness of these conditions; however the aetiology and classification of abnormal behavioural responses to digestive symptoms events have not been well defined. Specifically, the symptoms that provoke abnormal behaviour and the clinical utility of advanced physiologic measurement in describing this response remain uncertain, as diagnosis has predominantly been based on manometric findings alone.

2.1.4 New classifications of rumination

This study proposed that rumination and many cases of repeated belching are not distinct conditions but are caused by a common behavioural response to abdominal pain or other, unpleasant digestive symptoms (.

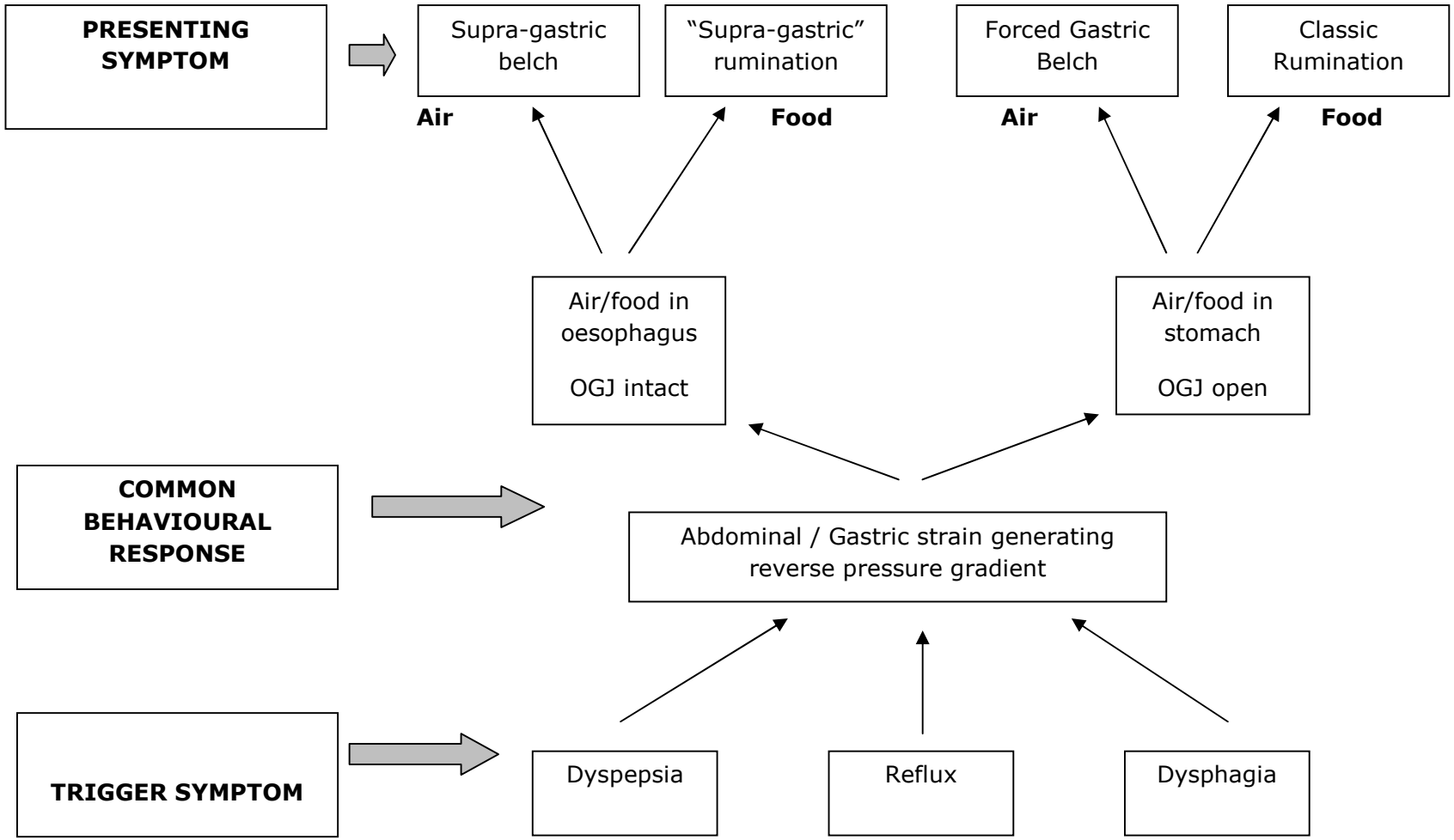


Figure 7. Flowchart of trigger symptom, behavioural response and resulting presenting symptom in rumination and its variations

This view is supported by several observations. Both rumination and repeated belching have been associated with chronic abdominal pain and the presence of psychiatric disorders (113, 120). Cases of regurgitation and belching have been associated with abdomino-gastric strain (3) and both conditions have been shown to respond to cognitive behavioural interventions (121).

This hypothesis was tested. If a common behavioural response is the cause of both conditions then (i) the presenting complaint (i.e. return of food to the mouth or belching) should be produced by similar behavioural responses to a variety of digestive symptoms (e.g. epigastric pain, bloating, reflux), (ii) a generic behavioural intervention should provide effective management whether the presenting complaint is "vomiting / regurgitation" or "belching" and (iii) effective treatment can be directed either at the symptoms that trigger the behaviour or at the abnormal behaviour itself. A retrospective review of consecutive cases with a diagnosis of rumination and repetitive belching made on HRM based on published diagnostic criteria (82, 91, 110, 117-119) to assess whether these predictions were supported by clinical observation. Based on the results a new classification system for these behavioural disorders is proposed based not on the presence of repetitive regurgitation or belching or manometry (e.g. "R wave") alone, but rather on the underlying mechanism of disease.

In summary, the main aims of this study are:

- To assess if the variety of symptoms exhibited by patients with rumination are produced by a similar mechanism, identified through HRM
- To assess if a simple and generic biofeedback mechanism will help all patients despite a variety of different symptoms

2.2 Methods

2.2.1 Patients

A retrospective case note review and evaluation of HRM data of consecutive patients with a diagnosis of rumination or other belching / regurgitation disorders based on HRM studies performed at Nottingham University Hospital NHS Trust, Queens Medical Centre (QMC) site between August 2009 and October 2011. Patients were identified from the specialist upper gastro-intestinal clinic at QMC referred for HRM or who were referred directly for HRM from other hospital trusts. As retrospective work with no intervention and data presented anonymously, no ethical submission was necessary.

2.2.2 High Resolution Manometry

HRM with (impedance when available) were performed in the upright, seated position by a 36 channel solid-state catheter (Manoscan 360, Given Imaging, Yoqeam, Israel). Following a

standardized protocol (122), baseline measurements of upper and oesophago-gastric junction (OGJ) pressure were followed by 10 swallows of 5ml water and 200ml water taken by rapid, repeated swallows. A solid test meal of cheese and onion pasty (Ginsters of Cornwall, Tavistock Road, Callington, Cornwall, per pasty; 521 kcal, 33.1g of fat, 38.9 g carbohydrate, 13.3g protein) was then ingested followed by a further 200ml drink and 10 minute postprandial observation period. The cheese and onion pasty was chosen as symptoms are commonly reported after fatty food in upper GI discomfort (especially functional dyspepsia and reflux disease (72, 123) and was a meal that was easily reproducible. Patients were instructed to report any swallowing problems, dyspeptic or reflux symptoms and these were documented in the electronic record, as were any events such as the return of food to the mouth or belching. Only those symptoms / observations documented within 10 seconds of the manometric event were considered to be causally related.

Proprietary software (ManoView v1.2, Given Imaging, Yoqeam, Israel) analysed water swallows and the Chicago classification system defined oesophageal dysmotility,(124) modified for use in the upright position and with solids.(122) Rumination was defined as a rise in intra-gastric pressure (abdomino-gastric strain) of at least 20mmHg above baseline associated with a retrograde

pressure gradient and return of gastric contents without retching to the mouth up to 10 seconds after the strain event.(110) This is commonly referred to as they R wave. Reflux regurgitation and gastric belching were defined as the passage of liquid or air from the stomach respectively with most such events occurring during a transient OGJ relaxation without evidence of abdominal strain on HRM (119). Supra-gastric belching was defined as a patient report or direct observation of belching in the presence of an intact OGJ.(109) These events were subdivided into supra-gastric belching after (i) air swallowing with subsequent expulsion related to abdominal strain as above, and (ii) intake of air through the open upper oesophageal sphincter by negative intra-thoracic pressure with rapid expulsion.(109, 111) Impedance confirmed retrograde flow of gastric or oesophageal contents (liquid, gas) during these events.(110) Cough was distinguished by the presence of rapid pressurisation through the stomach and oesophagus with contraction of the lower and upper oesophageal sphincters. Retching was distinguished from voluntary behaviour by the presence of a massive increase in abdominal and thoracic pressure with coordinated and prolonged relaxation of the oesophageal sphincters to facilitate rapid expulsion of luminal contents.

Ambulatory reflux studies were performed off acid suppression; however this was not performed in most patients with rumination on HRM since abnormal behaviour confounds the results. This is due to multiple repeated events of gastric contents return with rumination, which are often seen as weakly acidic events on pH studies. When performed a 2 pH sensor, 6 paired impedance sensor system (Sandhill Scientific Instruments) was used according to standard techniques (125). A pH-impedance probe is inserted through one nostril after anaesthetising with local anaesthetic spray. The proximal pH sensor is placed 5 cm above the upper border of the lower oesophageal sphincter after identification of the LOS by HRM. A band of high pressure is seen at the level of the distal oesophagus representing the LOS on HRM (126). The distal pH sensor lies 2 cm below GOJ. The pH-impedance probe has the 6 impedance sensors at 3, 5, 7, 9, 15, and 17 cm above the LOS. The catheter was secured and the patient was instructed to eat normally and maintain normal activity for the next 24hr while documenting symptoms on the electronic log, and providing a food diary. Analysis was completed by automatic software (Sandhill) with pH measurements related to retrograde bolus movement, with a 50% drop in impedance from baseline, and exclusion of meal periods. Patients recorded symptoms (up to 3) and these were recorded as associated with either acidic, weakly acidic, weakly alkali events. Traces were then checked by manually by the study

team. Symptom index and symptom association probability were also recorded as percentage values. Routinely, all tests were done off all anti-acid medication and analysis as published protocols (8).

2.2.3 Therapy and Follow-up

All patients received a 20 minute behavioural intervention by a clinician (Dr Emily Tucker or Dr Mark Fox) immediately after HRM investigation. This included a description of the rumination events, cause of symptoms and explanation of the rationale for behavioural therapy.

The patient assumed a semi-recumbent position on the examination couch within the HRM laboratory. Following a demonstration of the different breathing techniques of "chest wall / thoracic" and "diaphragmatic / abdominal" breathing by the clinician, the patient and the investigator each placed a hand on their own abdomen. Behavioural instruction was focused on deep muscle relaxation and diaphragmatic breathing (generic techniques applied in many conditions (127)). In this case, diaphragmatic breathing with relaxation of the abdominal wall prevents the patient from contracting the abdominal wall muscles to force gastric contents back in response to any symptoms. True "biofeedback" with the HRM catheter in place was not performed in most cases since the presence of the catheter is very uncomfortable and impairs patient compliance. Once the patient

was able to adopt diaphragmatic breathing on command, the behavioural control was challenged with a drink of water or a bread roll to induce postprandial symptoms. This challenge made it obvious that regurgitation / belching did not occur as long as diaphragmatic breathing was maintained.

Following the diagnosis patients referred to the consultant gastroenterologist (Dr Mark Fox) received further treatment in clinic as required. If these brief interventions were not effective, then further physiotherapy by a trained practitioner was requested. Leaflets describing the condition and the rationale of therapy were provided to the patient, primary care physician and physiotherapist to promote understanding of the condition and the rationale of therapy.

Additional treatments directed at reducing the dyspeptic or reflux symptoms that trigger abnormal behavioural response were recommended, depending on the individual patients symptom set. Follow-up of success of behavioural intervention was performed by review of the notes and electronic patient record at a minimum 3 months after initial diagnosis and therapy, with initial diagnosis being the date of HRM study as this was an objective time point, even if rumination had been suspected at initial clinical review.

Summary of study process:

HRM performed with biofeedback as normal clinical protocol on clinical basis → HRM database reviewed for those with diagnosis of rumination → case notes then reviewed for symptoms → patients grouped depending on symptoms → HRM data then reviewed to identify patterns within patient groups → response to biofeedback/treatment assessed → common categories identified

2.3 Results

2.3.1 Patients

46 patient notes and HRM data were reviewed.

Of the 46 patients (34 (74%) female; age range 18-68 years), 1 patient had mild learning difficulties but all others were of normal intelligence.

A variety of presenting complaints and associated symptoms were reported (

Table 3). 25 referrals were from Nottingham University Hospitals and 21 from other hospital trusts. The majority were referred for chronic unexplained "repetitive vomiting", 11 with a working diagnosis of treatment resistant reflux and 4 with presumed motility disorder. Only 8 (17%) had clinically suspected rumination.

Table 3. Presenting complaint and associated symptoms

Primary Complaint	N=46
Volume Reflux / Regurgitation / "Vomiting"	32 (70%)
Belch with meals	8 (17%)
Belch independent of meals	6 (13%)
Associated symptoms (may overlap)	
"Treatment resistant reflux" (heartburn, chest pain, acid regurgitation)	11 (24%)
Post-prandial dyspepsia (early satiety, nausea, bloating, epigastric pain on eating)	13 (29%))
Dysphagia	5 (11%)
Belching	12 (26%)

Symptoms had been present for median 23 (inter-quartile range 12-39) months prior to diagnosis. Of 38/46 (83%) patients with adequate records, 5 (11%) had symptoms for >60 months prior to diagnosis and only 2 (4%) had a diagnosis within 6 months. Further 5 (11%) patients required enteral nutrition (2 naso-jejunal tubes, 2 surgical jejunostomy and 1 percutaneous gastrostomy). No case received parenteral nutrition.

A number of patients described an acute event that precipitated the onset of symptoms, or worsened pre-existing symptoms. 11/46 (24%) described an acute medical illness prior to the development of symptoms. 5 had an acute episode of gastroenteritis with vomiting +/- diarrhoea, one had an episode of biliary colic, one

pancreatitis, one following surgery for a perforated duodenal ulcer, one following surgery for a choledocal cyst and two patients following respiratory infections.

Two cited acute psychosocial stress as a precursor with one patient having occasional symptoms only but with dramatic worsening following a burglary at his home. Another individual was in UK as an asylum seeker with unclear immigration status. As well as rumination, he displayed multiple symptoms consistent with somatisation disorder.

2.3.2 Previous investigations and procedures

All patients had undergone diagnostic studies prior to referral. Of 37/46 (80%) patients with adequate records, 34 had undergone upper gastrointestinal endoscopy (many had multiple procedures), 12 barium swallow (some with follow through), 6 abdominal ultrasound, 3 computed tomography of the chest and abdomen, 8 gastric emptying studies and 3 oesophageal transit studies. None of these tests had demonstrated findings that explained patient symptoms. Additionally, 13/46 (28%) had undergone conventional manometry with water swallows only. These excluded major dysmotility in all cases; but rumination or other behavioural abnormality was not reported. Ambulatory pH or pH-impedance studies showed pathologic acid reflux and/or a significant symptom association diagnostic of GORD in 8/13 (62%).

2.3.3 Previous therapy

All patients had been prescribed medications; however these rarely improved symptoms. Proton pump inhibitors (PPI) had no effect on rumination or belching in 27/46 (59%) patients. A partial response was present in 16 (35%). Only 2 had never received PPI. A variety of anti-emetics were prescribed to 31/46 (67%) patients (metoclopramide, domperidone and prochloperazine). Although this improved nausea in some and occasionally reduced the frequency of "vomiting", these medications never suppressed the problem completely. Other medications included tricyclic anti-depressants, anti-spasmodics and opiates. Additionally, 1 patient had received cognitive behavioural therapy to help cope with functional digestive symptoms.

Endoscopic therapy had been performed in 2 patients, 1 received botulinum toxin injection to the distal oesophagus and lower oesophageal sphincter for presumed spasm and 1 had botulinum toxin injection to the pylorus for presumed gastroparesis. A gastric neuromodulator was implanted in one patient. No patient reported any benefit from these procedures.

Anti-reflux surgery had been performed in 5 patients. In 2 cases symptoms appeared *de novo* several years after fundoplication. In 3 cases symptoms were either similar or identical to those present

before fundoplication but were not identified as abnormal behavioural responses until after the procedure.

2.3.4 Previous psychological diagnoses

2 patients had a previous diagnosis of obsessive compulsive disorder, with one also having co-existent non-epileptic attack disorder and depression.

2.3.5 HRM findings

Standard HRM procedures with 5ml water swallows revealed normal oesophageal motility in 34/46 (74%) patients, hypotensive dysmotility in 10/46 (22%) and hypertensive dysmotility in 2/46 (4%). During the 200ml water swallow oesophageal motility was suppressed fully and there was no evidence of impaired OGJ function. With solid swallows 7/10 patients with hypotensive motility improved to normal limits (122).

Rapid, repetitive belching was observed independent of oral intake in 6/46 (13%) patients. Digestive symptoms were reported during the study *prior to the onset of abnormal behaviour* by 33/46 (72%) patients (33/40 (83%) of those with postprandial symptoms). 25/46 (54%) individuals reported postprandial distress compatible with functional dyspepsia, 7 (15%) typical reflux symptoms, and 1 dysphagia. No digestive symptoms were reported during the study by 7 (15%) patients.

An abnormal behavioural response diagnostic of rumination or supra-gastric belching was considered positive if (i) observed events were typical of the symptoms that led to referral and (ii) associated with rumination behaviour (abdomino-gastric strain / R waves) on several occasions (at least twice, but usually many occasions). These events occurred during or immediately after the water swallows in 22/46 (48%), 7 (15%) after the first 200ml water swallow and the remaining 17 (37%) after the test meal.

Table 4 relates patient symptoms to objective HRM findings during the test meal. The majority of patients (35/46 (76%)) showed manometric findings of "classical" rumination with a sharp increase in abdomino-gastric pressure ("R-wave") before return of gastric contents to the mouth (Figure 8. Rumination demonstrated by combined HRM with impedance.).

Table 4. Comparison of patient's symptoms and objective behaviour during HRM test meal study.

	Objective Mechanism / Abnormal Behaviour during HRM study		
Predominant Observation of Patient	Rumination gastric content	Rumination oesophageal contents	SG belching rapid, repeated
Regurgitation of food after meal	32*		
Regurgitation of food and air during / after meals	4	4	
Belching independent of food			6

*includes all 5 reflux ruminators who may return gastric air as well as food

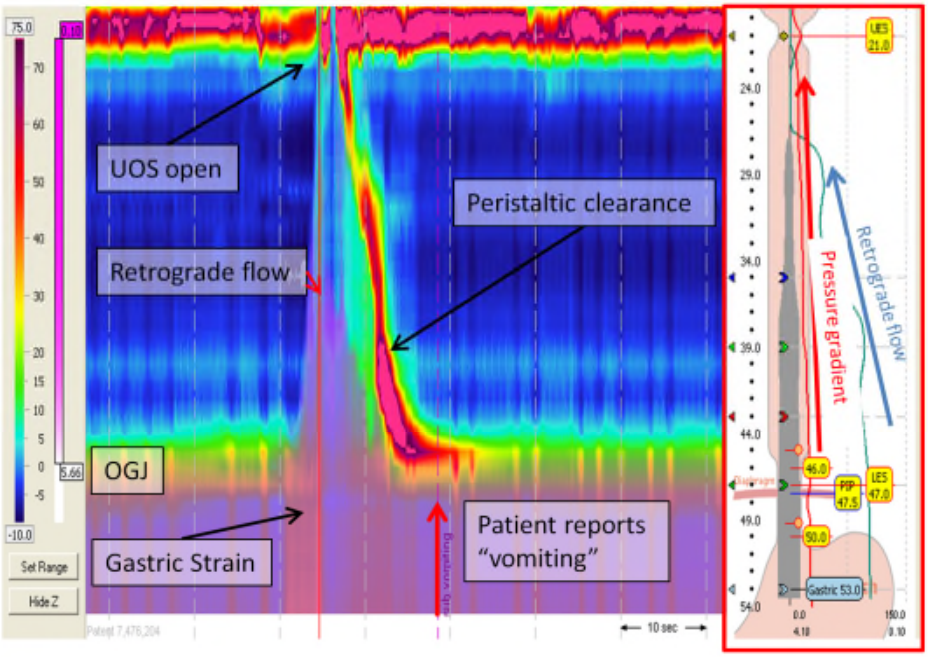


Figure 8. Rumination demonstrated by combined HRM with impedance.

Figure 8 shows how gastric strain overcomes the OGJ barrier and the retrograde pressure gradient drives retrograde flow of gastric contents through the oesophagus and upper oesophageal sphincter. The patient reports “vomiting” and swallows producing effective clearance by primary peristalsis.

In the “classical rumination manometry cases” (n=35) 31 patients the increase in abdominal pressure exceeded the resting OGJ pressure. In other cases (4/35) the abdominal contraction appeared to trigger a “transient OGJ relaxation” on each occasion. In the latter, the abdomino-gastric strain prior to OGJ relaxation differentiated these events from normal reflux events. Both variants were observed in certain individuals. In the remaining 11 (24%) patients, who didn’t display classical rumination manometric patterns, and on occasion in those with “classical” findings, a number of other “rumination variations” were observed. These are summarised in Table 5. Rumination “variations” from the classical mechanism / behavioural abnormality and illustrated in Figure 9, Figure 10 and Figure 11. More than one behavioural abnormality was occasionally in some cases. Figure 12 demonstrates how retching and cough differ from the behavioural abnormalities that characterize rumination and belching disorders.

Table 5. Rumination "variations" from the classical mechanism / behavioural abnormality

Rumination Variation	Patient details	Mechanism
Cough 1/46 (2%)	Previous anti-reflux surgery as a child	Cough used with gastric strain to create pressure required to cause retrograde gastric contents movement across previous fundoplication
Gas trapping 1/46 (2%)		Unable to co-ordinate relaxing UES with gastric strain, resulting in gas trapping in oesophagus and forced retching by inserting fingers to back of throat to relieve "trapped air"
Reflux rumination 5/46 (11%) Figure 11		Gastric strain occurs immediately following a transient lower oesophageal sphincter relaxation with common cavity pressure
Supra-gastric rumination 4/46 (9%) Figure 9		Air swallowed and subsequent gastric strain then used to return contents from oesophagus, prior to passing into stomach. At meal time, food returned in similar manner
Supra-gastric belching 6/46 (13%) Figure 10	Includes 2 post-fundoplication patients	Air suctioned into the oesophagus through the open UOS by negative intra-thoracic pressure (forced inspiration) and then immediately released

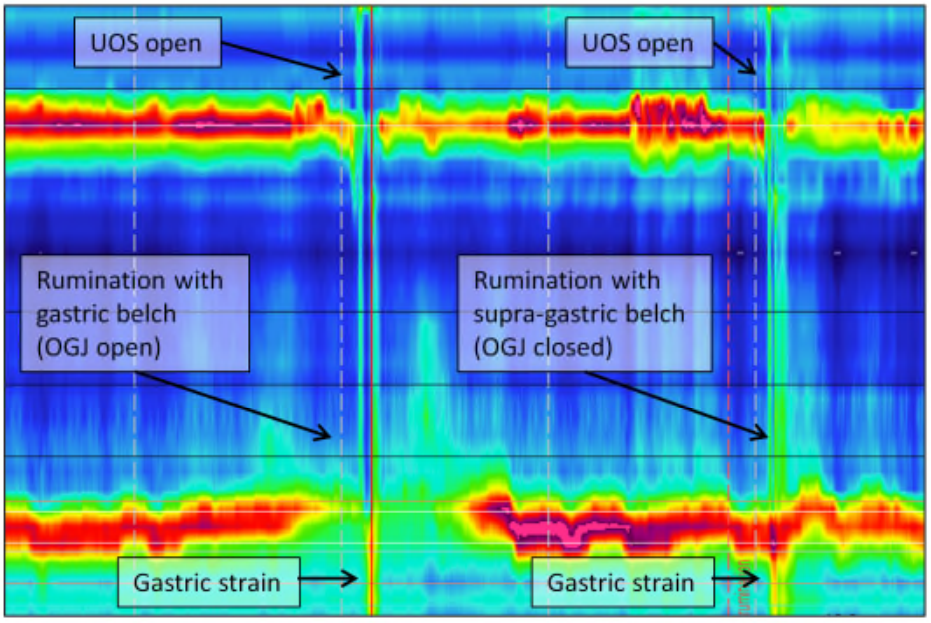


Figure 9. Rumination variations in the same patient.

Figure 9 shows the first gastric strain (left) is associated with OGJ relaxation and results in forceful expulsion of gastric contents (typical rumination). The next gastric strain occurs with closed OGJ and results in the expulsion of only oesophageal contents (supra-gastric rumination).

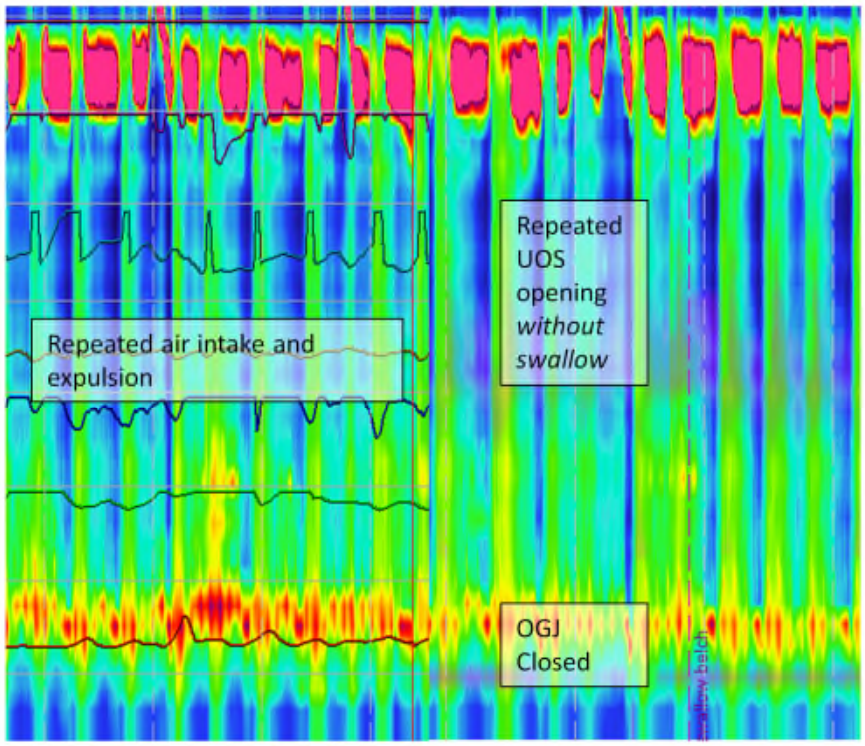


Figure 10. Supra-gastric belching in a representative patient during combined HRM and impedance

In Figure 10 the OGJ is closed while the UOS opens repeatedly as air is sucked in and expelled rapidly (see impedance trace superimposed on left hand side of image)

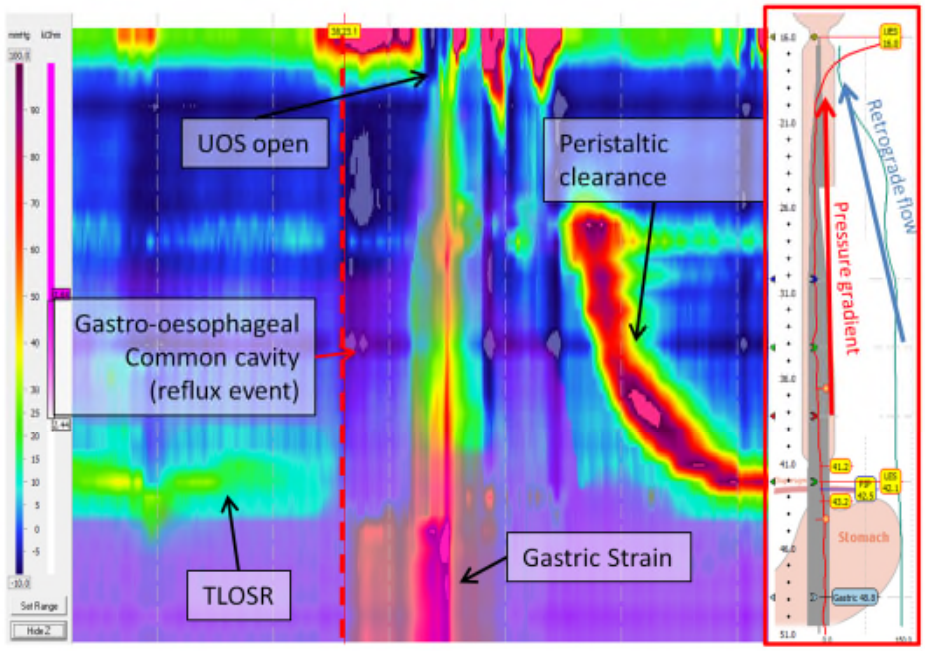


Figure 11. Reflux rumination

In Figure 11 transient Lower Oesophageal Sphincter Relaxation (TLOSR) with gastro-oesophageal common cavity (i.e. reflux) is rapidly followed by gastric strain with UOS relaxation (i.e. rumination) resulting in more forceful expulsion of gastric contents.

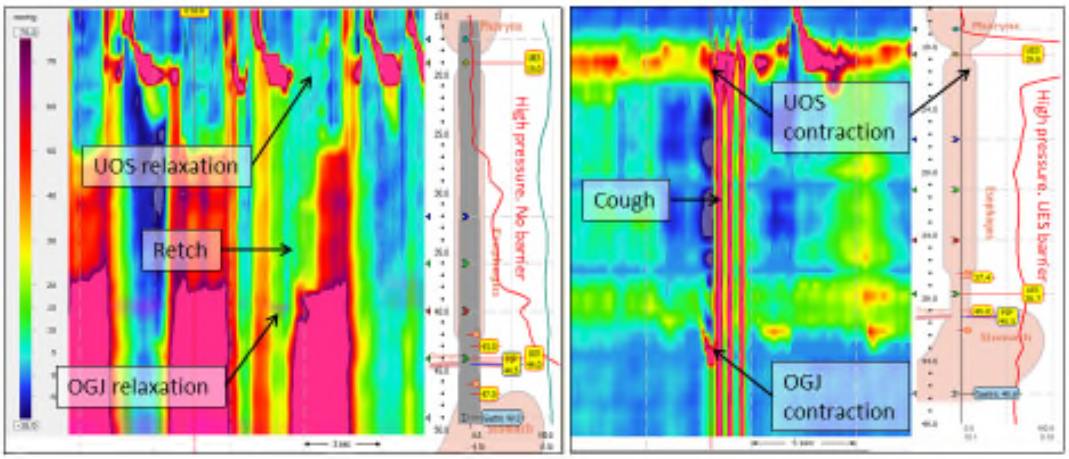


Figure 12. Retching (left) - Coughing (right)

Figure 12 demonstrates two variations on rumination.

Retching. Note prolonged OGJ and UOS relaxation coordinated with a massive increase in gastric pressure. This produces a large retrograde pressure gradient and allows rapid expulsion of large volumes of gastric contents.

Coughing. Note rapid increase in gastric and oesophageal pressure coordinated with UOS contraction to protect the airway. There is no retrograde pressure gradient

2.3.6 Treatment outcome

All patients received a brief behavioural intervention including abdominal breathing exercises at the time of diagnosis. 23 patients received further instruction at follow-up. Outcome assessment was based on patient reported improvement in symptoms as no scoring system has been developed to evaluate treatment response in these conditions. After median 5 (3-11) months follow up, complete improvement in rumination and / or belching was reported in 20/46 (43%) patients, including 3/6 with supra-gastric belching. Partial improvement was reported by 13 (28%) patients. 2 patients reported no improvement and 11 patients had no follow up within NUH Hospitals. No patient was given an alternative diagnosis. However, in 3 cases, further investigation led to specific therapy directed at the underlying cause of the symptoms that provoked abnormal behaviour.

- 46 year old women. Presented with intermittent dysphagia, vomiting with abdominal pain with background of pancreatitis. HRM showed rumination. Endoscopic Ultrasound (EUS) showed pancreatic pseudocyst with herniation through diaphragmatic hiatus. A coeliac plexus block via EUS was performed for abdominal pain. Resolution of dysphagia and rumination occurred on drainage of pseudocyst

- 59 year old women with abdominal pain, weight loss and vomiting with previous abdominal surgery. HRM showed rumination. A laparoscopy planned for insertion of surgical jejunostomy to support nutrition. Adhesions removed at the time of laparoscopy / jejunostomy insertion. Rumination and pain resolved after removal of adhesions

- 18 years old female. Presented with heartburn and large volume regurgitation. HRM showed hypotensive dysmotility and rumination following typical reflux events. pH-studies confirmed correlation of symptoms with acid reflux events, (not only at meal times). Fundoplication was performed following successful biofeedback. Resolution of reflux and rumination (trigger for rumination removed) and behaviour addressed

2.4 Discussion

This study provides evidence that rumination and many cases of repetitive belching are not distinct conditions but represent common behavioural responses to a variety of digestive symptoms. Only rapid, supra-gastric belching independent of oral intake represents a distinct abnormal behaviour. The findings also

demonstrate the clinical utility of advanced physiologic measurement during a test meal to describe mechanism of disease and establish diagnosis in patients with chronic, unexplained symptoms. In addition, encouraging data is presented that even one, brief behavioural intervention can produce lasting clinical benefit in many cases.

2.4.1 Results summary

The clinical presentation, investigation, treatment and outcome of 46 adult patients in with rumination and other belching / regurgitation disorders identified by HRM were reviewed. This patient group was typical of those in previous studies.(110, 112, 116, 118). The majority were female with a long history of functional gastrointestinal symptoms. As reported in children (116), a proportion of patients reported onset of symptoms following acute infection (interestingly not always gastrointestinal), surgery or psychosocial stress; however the presenting symptoms of these individuals was otherwise no different than the group as a whole. In almost all cases extensive investigation, sometimes including conventional manometry, had failed to establish a diagnosis and a variety of empirical treatments had failed to improve symptoms. Referral letters noted a variety of symptoms but rarely included rumination syndrome in the differential diagnosis and never mentioned supra-gastric belching.

Since the study investigators also provide therapy for these conditions in our region, this finding is almost certainly due to low awareness and not because other physicians are making the diagnosis based on clinical presentation alone.

2.4.2 Manometric criteria in ruminations

Physiologic measurement with concurrent documentation of symptoms and clinical events during a test meal provided objective evidence of behavioural disorders. HRM with impedance is considered to be an accurate test;(82, 91, 110, 117-119) however we suspect false negative results in a small number of patients with typical symptoms that had a normal "stationary manometry" but findings on ambulatory pH-impedance that may represent "rumination" (e.g. repeated, symptomatic non-acid reflux after meals) or "supra-gastric belching" (e.g. aerophagia followed by expulsion). These individuals were excluded as our analysis focused on the mechanism of disease and required a definitive description of the physiologic events. False positive results are also possible; however further investigation did not change the diagnosis for any individual during median 5 month follow-up. During HRM studies, the majority of patients (33/46 (72%)) spontaneously reported dyspeptic symptoms before diagnostic pressure events and the return of gastric or oesophageal contents to the mouth. The handful that reported no postprandial symptoms

prior to the onset of rumination tended to have a long history (often since childhood). In these cases, the abnormal behaviour may be so well established that even the normal sensation of fullness after a meal could trigger this response. About half the patients had diagnostic results after 10 water swallows; however the yield was doubled by the inclusion of free drinking (200ml water) and a test meal. The close temporal association of abdominal symptoms before the appearance of rumination or belching confirms the behavioural aetiology of these conditions. Moreover it provides patients with a clear explanation of the cause of symptoms that many found extremely helpful in coming to terms with the diagnosis and engaging with behavioural treatment.

2.4.3 Symptoms and behavioural response

Consistent with the study hypothesis, the symptoms that preceded the onset of rumination and supra-gastric belching were varied; however the range of behavioural responses was very limited. Almost all the rumination and belching events were preceded by voluntary, albeit unconscious, contraction of the abdominal wall. In the majority with dyspeptic symptoms after the meal this behaviour resulted in typical rumination of gastric contents; however, the same response could force out oesophageal contents if it occurred during eating. In other patients abdomino-gastric strain occurred exclusively in response to typical reflux events and,

since lower oesophageal sphincter relaxation reduced the resistance to retrograde flow, such individuals tended to eject large volumes of gastric contents. These observations with combined HRM impedance technology build on those of Rommel *et al* (110) and show that the timing of abdominal strain in relation to drinking and eating can determine the clinical presentation. Specifically, what was present in the lumen at the time the abdominal muscles contracted determined whether air, liquid or food returns to the mouth. Thus, the same mechanism can result in "gastric rumination", "reflux rumination", "supra-gastric rumination" (i.e. return of oesophageal contents) and, in cases of aerophagia, "supra-gastric belching" (

Table 4).

2.4.4 Supra-gastric belching

The exception was rapid, repetitive belching that occurs independent of meals as described by Bredenoord *et al* (111). This cannot be produced by aerophagia and / or straining. Rather, it is achieved by suction of air into the oesophagus through an open

UOS during forced inspiration with immediate release on expiration. In our experience this behaviour can produce such a rapid succession of belches that it caused breathlessness and distress reminiscent of panic attacks.

2.4.5 Treatment options in rumination

Once the diagnosis is established behavioural treatment is the mainstay of treatment.(128, 129) It was shown that even a single, brief intervention can suppress rumination and belching. However, if the study hypothesis is correct and abnormal behaviour is a response to digestive symptoms, then it should be possible to direct therapy either at those symptoms or at the response itself. The majority of patients with rumination and supra-gastric belching had functional dyspepsia. In addition to behavioural management these patients were prescribed low-dose antidepressants that reduce gastric hypersensitivity (130) and visceral pain in this condition.(131, 132) Control of rumination and / or belching could be achieved quickly with behavioural management; however, initially, some individuals struggled to maintain control because of on-going dyspeptic symptoms. This became easier as the abdominal pain settled on the medication (typically over 4-8 weeks). These observations are consistent with the study hypothesis; however, in a small number of cases, investigations revealed specific, treatable causes of symptoms.

In one patient, abdominal pain was the result of a pancreatic pseudocyst and drainage resulted in immediate relief. In another, adhesiolysis around the proximal jejunum during insertion of a feeding tube released occult obstruction with immediate improvement in both dyspepsia and rumination such that the jejunostomy never had to be used. In another patient rumination occurred exclusively in response to "typical" reflux events and fundoplication provided excellent control of her symptoms. The immediate effect of specific treatment in these instructive cases supports the view that relief of unpleasant digestive symptoms can be sufficient to suppress also abnormal behaviours related to these symptoms.

2.4.6 Associations with rumination syndrome

Rumination is most often associated with dyspepsia; however abnormal behavioural responses associated with reflux disease may also be quite common.(133, 134) Direct observations by HRM with impedance can document whether abdomino-gastric strain is forcing gastric acid into the oesophagus or whether spontaneous reflux events trigger the abnormal behavioural response. If rumination is the cause of, rather than a response to, acid reflux then pH-studies will often produce false positive results (see above). In these cases fundoplication may physically prevent rumination, but dyspeptic symptoms are likely to increase and

patient behaviour will adapt to the new circumstances. Conversely, if reflux is the trigger for rumination then reflux suppression with the Gamma-Aminobutyric acid$_B$ (GABA$_B$) receptor agonist baclofen (135) or anti-reflux surgery (136) may be effective options.

2.4.7 New treatments in rumination syndrome

A recent study has utilised baclofen in 16 patients with rumination +/- belching (135). A HRM with impedance was completed at the start and at the end of a week long period of open label baclofen, (10mg three times a day). Patients recorded symptoms of regurgitation or belching throughout each recording period. This did significantly reduce rumination events. Interestingly, increased lower oesophageal sphincter tone was associated with a reduction in flow events, but the reduction in TLOSR was not. This may suggest that some of the effects of baclofen are central, sedating effects causing changes in the patient's behaviour, rather a mechanistic change.

2.4.8 Anti-reflux surgery in rumination syndrome

If anti-reflux surgery is considered then patient selection is critical. In our case series, 5 patients had rumination syndrome diagnosed after anti-reflux surgery. In two cases this behaviour commenced *de novo* years after surgery following an acute physical or psychological stress. However, in the three other cases, although

fundoplication suppressed rumination, dyspeptic symptoms persisted and abnormal behaviour either overcome the wrap or resulted in supra-gastric rumination and belching.

2.4.9 New proposed classification in rumination syndrome

On the basis of these observations a classification of rumination and other regurgitation / belching disorders can be proposed:

(i) "primary" or "classic" rumination *with or without belching* during / after a meal

(ii) "secondary" or "reflux-associated" rumination

(iii) supra-gastric belching independent of meals

Previous attempts at classification have been descriptive, based on the presence and timing of abdominal strain and association with retrograde flow of liquid and gas (110). In contrast, this system identifies three groups with distinct mechanisms of disease that may respond to specific management. Classic rumination is most often triggered by dyspeptic symptoms. In this condition, abdomino-gastric strain results in the return of food or belching (depending on what is in the lumen), from the stomach or oesophagus (depending on the timing of contraction). Reflux rumination has a similar mechanism but is triggered by reflux events. Both may respond to behavioural therapy directed at abdominal wall relaxation; however the events that trigger this

behaviour are different and may respond to specific therapy. In contrast rapid, repetitive supra-gastric belching is produced by a distinct behavioural abnormality and may require specific therapy focused on the upper oesophageal sphincter.(137)

2.4.10 Study limitations

This study has the limitations of most case reviews, discussed below.

2.4.11 Follow up data

Clinical data and follow-up was not always complete, especially in out of area referrals, and medical treatment was not provided in a systematic manner. In particular, although all patients received at least one session of behavioural instruction, only a minority received physiotherapy in the community. This is due to healthcare providers and staff being unfamiliar with the diagnosis. The behavioural instruction requires no specialist knowledge, simply the principles of diaphragmatic breathing applied at the time of symptom onset. As a result of this this study almost certainly underestimates the potential benefits of this approach. However, even in cases where behavioural therapy was not effective and individuals continued to have recurrent rumination or belching a definitive diagnosis was helpful as it helped to avoid further investigation and inappropriate treatment.

2.4.12 Psychological factors

2 patients within our cohort had stressful life events associated with the development of rumination syndrome. 1 had significant worsening of symptoms following a burglary and another was an immigrant with unclear residency status. In the largest previously published case series of 147 children and adolescents with rumination, an acute onset of symptoms following a stressful life event was described in 15 (10.2%) (116). It would seem sensible for any therapy to address the rumination should focus on biofeedback techniques but should also include methods to deal with the original stressor.

2.4.13 Patient response to diagnosis

Patient response to the diagnosis was not recorded in the HRM data or patient notes. However, anecdotally as one of the clinical staff delivering the diagnosis and behavioural therapy, the majority of patients and their family members present were accepting of the diagnosis. They felt that a behavioural element could be a causative factor. The important fact to deliver was that although the behavioural component was voluntary it is sub-conscious.

2.5 Conclusion

These findings support the hypothesis that rumination and many of its variations represent common behavioural responses to digestive symptoms after meals and that a simple, generic behavioural

intervention can provide effective management whether the presenting complaint is “vomiting / regurgitation” or “belching”. Further, this study demonstrates the clinical utility of HRM studies during a test meal in a group of patients with medically unexplained, treatment resistant symptoms. Advanced physiologic measurement identifies three groups with distinct mechanisms of disease that are likely to respond to specific management. Moreover the vivid, visual demonstration of oesophageal function provided by HRM can help patients (and their doctors) understand the cause of their symptoms; enhance patient acceptance of the diagnosis and the effectiveness of behavioural therapy.

3 Chapter 3 – Assessment of oesophago-gastric junction and novel assessments for gastro-oesophageal reflux disease

3.1 Introduction

3.1.1 GORD – definition and treatment

Gastro-Oesophageal Reflux Disease (GORD) is a common condition that typically presents with heartburn and acid regurgitation (5). Extra-oesophageal features such as chest pain and cough can also be associated (138). Proton pump inhibitors are recommended as first line empirical management after lifestyle adaption(139). When symptoms do not respond to acid suppression, and especially if volume regurgitation is prominent, further investigation is required to confirm the diagnosis and guide further management including anti-reflux surgery.(140)

3.1.2 GORD - diagnosis

Upper gastrointestinal endoscopy is often performed in patients with persistent reflux symptoms to identify mucosal disease related to chronic acid exposure (e.g. oesophagitis, Barrett's oesophagus), peptic ulcer disease and malignancy. Those on long term acid suppression often exhibit no mucosal disease and ambulatory pH monitoring is required to provide objective evidence of pathologic

oesophageal acid exposure and/or symptom-reflux association (8, 140). However ambulatory studies require time off medication prior to the investigation and during it (normally a total of 7-8 days), involve invasive procedures, require compliance with instructions and additional hospital visits. Most patients also find catheter based pH studies uncomfortable and tolerate them poorly (141).

3.1.3 Novel methods for diagnosis of GORD

New approaches which can provide a reliable diagnosis of reflux disease at the index endoscopy and/or without the need for catheter based pH studies would be attractive to patients and physicians and potentially reduce costs to the health care system.

3.1.3.1 Oesophageal histology

Histology has been proposed as a surrogate marker for disease severity. In the absence of macroscopic oesophagitis, various microscopic changes have been reported in the mucosa of GORD patients (142). However, similar to macroscopic oesophagitis, these microscopic changes often resolve on PPI therapy and the clinical utility of using histology to diagnose GORD in patients on acid suppression has not been confirmed (143).

This has been addressed by certain groups, with efforts being made for histology to be utilised in the diagnosis of GORD. Zentilin *et al* have developed a reflux score to try and use histology as a

diagnostic marker for reflux disease. 6 criteria were used, each with an associated score and compared between controls and reflux patients with some success – listed in Table 6

Table 6. Histological parameters

Histological parameter	Scoring system		
Basal cell thickness	0 = none	1 = mild	2 = marked
Papillary length	0 = none	1 = mild	2 = marked
Dilation of intercellular spaces	0 = none	1 = mild	2 = marked on basis of size
Intra-epithelial eosinophils	0=absent	1=1 eosinophil	2=>1 eosinophil
Intra-epithelial neutrophils	0=absent		2=present
Necrosis/erosion	0=absent		2=present

3 biopsies were taken (Z line, 2 cm above and 4cm above), but results found to be comparable with just two biopsy results (Z line and 2cm above). A score of more than 2 in one single parameter distinguished healthy controls from reflux patients (established on the basis of pH studies, symptoms and endoscopy findings) with a reflux score above 2 in 100/ (84%) reflux patients and 3/20 (15%) healthy controls, with significant difference. The negative predictive value and positive predictive value were 46% and 97%

respectively (142). The specificity was highest for intra-epithelial neutrophils and erosions at 100%.

This work has further been elaborated on by colleagues in St Thomas's Hospital. Sweis *et al* (144) compared histology with prolonged pH monitoring in reflux patients. 57 patients were separated into reflux (n=37) with prolonged oesophageal acid exposure and/or positive symptom index (>50%) or functional heartburn (n=20) with both parameters negative. All histological parameters were similar in both groups except for intra-epithelial neutrophils (IEN) This group found a combined Zentilin reflux score of ≥7 and increased IEN still had relatively low sensitivity but improved specificity; for IEN at Z line sensitivity 30%, specificity 92.6% then 2cm above Z line sensitivity 20% and specificity 100%. A Zentilin reflux score ≥7 had sensitivity 40.5% and specificity 95% at Z line and sensitivity 18.9% and specificity 100% proximally. Histology may be used to diagnose GORD and potentially replace pH studies in certain cases.

3.1.3.2 EndoFLIP®

An alternative and novel approach is to interrogate the anatomy and function of the Oesophago-Gastric Junction (OGJ) as pathology within this region is the most important cause of GORD (140). The Endoluminal Functional Lumen Imaging Probe (EndoFLIP®, Crospon, Galway, Ireland) is a novel technology that applies

impedance planimetry to measure cross-sectional area (CSA) and pressure at the OGJ, therefore allowing distensibility of the "reflux barrier" to be measured.

The EndoFLIP® assembly is 240 cm long with a 3-mm outer diameter. The distal end of the probe is 14cm long comprised of a catheter with 16 paired impedance electrodes and pressure sensor enclosed within an infinitely compliant bag able to fill a volume of 40ml. The catheter is passed through the mouth until the impedance electrodes straddle the OGJ at its mid-point. The bag is filled with a conductive medium to set volumes (20ml and 30ml). An AC current is passed through the medium and impedance is recorded. This allows the diameter and cross-sectional area (CSA) of the bag to be measured, along with intra-bag pressure.

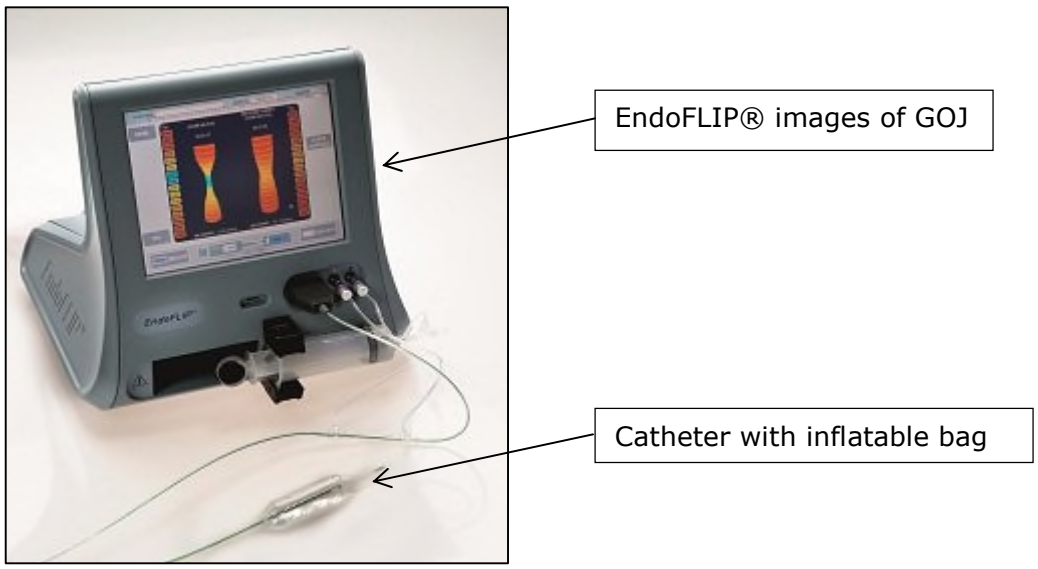


Figure 13. EndoFLIP® images at GOJ and catheter – reproduced from Cropson, Galway, Ireland.

Both increased cross-sectional area and decreased OGJ opening pressure have been documented by barostat studies in GORD patients, being most marked in those with hiatus hernia (84). Consistent with these findings, initial trials with EndoFLIP® in reflux patients reported increased OGJ distensibility in 20 reflux patients with evidence of oesophagitis (LA grade A-C) on endoscopy compared to 20 asymptomatic healthy controls (94). However there was a wide variability in the results with important crossover of values between health and disease. Moreover, only 4/20 of the patient group had pH studies and only 2 of these had a GORD diagnosis based on prolonged oesophageal acid exposure.

3.1.3.3 Wireless pH monitoring

The wireless Bravo® capsule uses is a capsule suctioned to the oesophagus to monitor oesophageal pH for up to 96 hours. This has the advantage of allowing prolonged measurements, allowing a more "normal day" in the patient in comparison to a nasal catheter and also has better patient tolerability(145). It also potentially reduces the problem of day-to-day variability of symptoms (146) as a longer measurement. In one study it was used to identify patients with prolonged oesophageal acid exposure who had had previously had a negative catheter based study(90). The disadvantages of Bravo® compared to conventional pH-studies

include increased costs and the need for endoscopy to guide placement of the capsule in the oesophagus and unavailability of any impedance measurements.

3.1.4 Aim of the study

The aim of this study was to assess the feasibility OGJ CSA and distensibility measured by the EndoFLIP® device for the diagnosis of GORD. Can EndoFLIP® could differentiate between participant groups based on clinical (patient vs. healthy volunteer) and/or physiologic (normal vs. pathological acid exposure ambulatory pH-studies) parameters. For EndoFLIP® to be useful in this manner, patients with high OGJ distensibility will have pathological levels of oesophageal acid exposure on prolonged, wireless pH testing. If proven, this would remove the need for ambulatory pH-studies to confirm the diagnosis. As well as the opportunity to establish the diagnosis of GORD at the time of endoscopy, measurement of OGJ distensibility with EndoFLIP® may identify a subgroup of patients with a highly distensible OGJ that report 'volume regurgitation' more often than patients with normal OGJ distensibility. This is clinically relevant because regurgitation associated with persistent weakly acid reflux is the predominant cause of persistent symptoms in patients on double-dose proton pump inhibitors (147), and regurgitation is less likely to respond to acid

suppression than heartburn or chest pain (148, 149). In these GORD patients, surgery may be the most effective treatment option. Prolonged, wireless pH monitoring would be used alongside distensibility measurements.

In summary, the aims of this study are:

- Is measurement of OGJ CSA and distensibilty feasible in GORD patients and HV with the EndoFLIP® device?
- Will the differences in OGJ CSA and distensibility differentiate between GORD patients and HV based on symptoms?
- Will the differences in OGJ CSA and distensibility differentiate between GORD patients and HV based on prolonged oesophageal acid exposure?
- Will increased OGJ distensibility be associated with volume regurgitation within the GORD patient group?

3.2 Methods

Healthy volunteers were recruited and studied were completed at the National Institute of Health Research Biomedical Research Unit, University of Nottingham and were recruited by poster around QMC hospital. Patients were recruited from referrals for pH measurements and studies were completed at Guys and St Thomas's NHS Foundation Trust. Identical procedures were adhered to in both centres. Approval was through National Research Ethics Committee (Ref: 09/H0802/104) for patient

studies and the University of Nottingham Ethics committee for the healthy volunteer studies. Patient recruitment and EndoFLIP® measurement were completed by Dr Rami Sweis at Guys and St Thomas's but all analysis of data from both patients and healthy volunteers was completed by Dr Emily Tucker.

3.2.1 Screening visit

At the screening visit upper gastrointestinal symptoms were documented. Demographic information including height and weight were recorded together with any past medical history of disease and current use of medication. Healthy volunteers had to have no symptoms of reflux disease and be on no regular medications and have no history of gastro-intestinal disease or surgery.

Patients had typical symptoms of reflux disease (heartburn, regurgitation) with at least partial response to acid suppression therapy, no previous upper gastro-intestinal surgery and no evidence of oesophageal dysmotility on oesophageal manometry (these were performed on a clinical basis and had to be completed before recruitment into this study). Dysphagia could be co-existent (often reported by GORD patients (150)) but could not be the predominant symptom. Participants with co-morbidity requiring medical management were excluded. Pregnancy was excluded by urine β-human chorionic gonadotropin (β-HCG) testing prior to all invasive procedures. All anti-acid and pro-kinetic medications were

stopped for 7 days prior to study day and for the duration of the study.

All participants completed written consent.

Healthy volunteers with endoscopic evidence of GORD were excluded from the categorical analysis (i.e. large hiatus hernia, Barrett oesophagus LA grade B-D oesophagitis).

3.2.2 Gastroscopy

On the study day participants were starved from midnight. The participant was placed in the left lateral position. Sedation and analgesia were provided to all participants using midazolam (2-10mg) and pethidine (25-50mg). Pharyngeal anaesthesia was not used due to the risk of aspiration with the Bravo capsule should it not adhere correctly to the oesophagus. A protective mouth guard was inserted to protect teeth. An endoscope (Olympus, KeyMed House, Stock Road, Southend-on-Sea, SS2 5QH) was then inserted through the mouth. Complete visualisation from the oesophagus to the second part of the duodenum was obtained before any study procedures. Z line distance was recorded and any oesophagitis or hiatus hernia documented. Oesophageal biopsies were then taken from the Z line at the 3 o’clock and 9 o’clock position and in the same pattern from 2cm above Z line.

3.2.3 Endoluminal Functional Lumen Imaging Probe - EndoFLIP®

This was completed following biopsies as the oesophageal mucosa could potentially be damaged by the inflation of the EndoFLIP® bag, preventing accurate analysis of the biopsies.

Data analysis was performed as described by Kwiatek *et al* (94). The median of each of 16 CSA and intra-bag pressure measurements along the length of the EndoFLIP® device was taken over a 30 second recording at each of the distension volumes. It was assumed that the OGJ position represented the segment with the smallest CSA (94). The minimum CSA with intra-bag pressure was used to calculate OGJ distensibility (CSA/intra-bag pressure).

3.2.4 Prolonged, wireless pH measurements

Following the oesophageal biopsies and EndoFLIP® measurements, the pH capsule was attached. The Bravo capsule (Bravo®, Given Imaging, Yoquem, Israel) was first calibrated in pH 7.01 buffer for ten minutes. After this time the Bravo receiver was activated and wireless connection between receiver and capsule ensured. The receiver calibrates to pH 7.01. Once complete the capsule was rinsed in sterile water and placed in pH buffer 1.07. The same calibration process was completed except that only 30 seconds soaking is required. Once this is complete the capsule was rinsed

again in sterile water and placed back into the pH 7.01 buffer. The receiver was then checked to ensure the correct pH is recorded (7.01).

The capsule was inserted trans-orally on an introducer. A wireless pH capsule was placed 6cm proximal to the Z-line after 1 minute suction according to manufacturer instructions and as previously described (90, 146). A small section of oesophageal mucosa was sucked into the capsule and a locking pin then released that fired through the mucosa to hold the capsule in place. The capsule was then released from the introducer and the introducer removed from the mouth. The wireless receiver was activated to start recording pH at the end of the procedure. Participants documented reflux symptoms, position (upright, supine) and all oral intake on the Bravo data logger and these were noted also in a paper diary. Data from the wireless recorder was downloaded after 48 hours, the position and function of the Bravo capsule was checked by measuring pH and ensuring data recording. The batteries were replaced for a further 48 hours monitoring with the intention of completing up to 96 hours of monitoring (or as long as capsule stayed attached).

3.2.5 Data analysis of pH monitoring

Data analysis documented the number of reflux events, total oesophageal acid exposure time (% time oesophageal pH dropped

below 4); an average 5.3% acid exposure over the study was defined as the upper limit of normal (146). Meal periods were excluded from analysis. Symptom association was measured using a 2 minute window. Symptom index (SI) is the percentage of symptoms associated with reflux episodes (diagnostic cut off >50%). Symptom Associated Probability (SAP) is a statistical function which calculates the probability that the observed association between reflux and symptoms is not by chance (diagnostic cut off >95%)(151).

3.2.6 Histology

Oesophageal biopsies were orientated and embedded in paraffin and were assessed by single pathologist, who was aware these were all healthy volunteers but blinded to the gastroscopy and pH study results.

3.2.7 Statistical analysis

With parametric and nonparametric data, unpaired t test and the Mann Whitney test were used for comparison respectively. Association between demographic and physiologic variables with CSA and distensibility was calculated using univariate and multivariate linear regression.

In summary, the process of data collection

- Patients awaiting pH studies recruited

- Healthy volunteers recruited
- Gastroscopy performed
- Oesophageal biopsies (HV only)
- EndoFLIP® measurements taken
- Bravo capsule attached
- Wireless pH measurements taken
- Results compared between HV and patients

3.3 Results

3.3.1 Participant groups and demographics

21 healthy volunteers (16 females, 5 male, Age 22 – 46 years) with mean body mass index (BMI) mean 24 kg/m^2 (range 19-32 kg/m^2) and 22 patients were recruited. 2 patients were excluded due to Schatzki ring on endoscopy, 1 due to early drop off of Bravo capsule and 1 due to negative bag pressure on EndoFLIP®. The consort diagram below shows recruitment for trial.

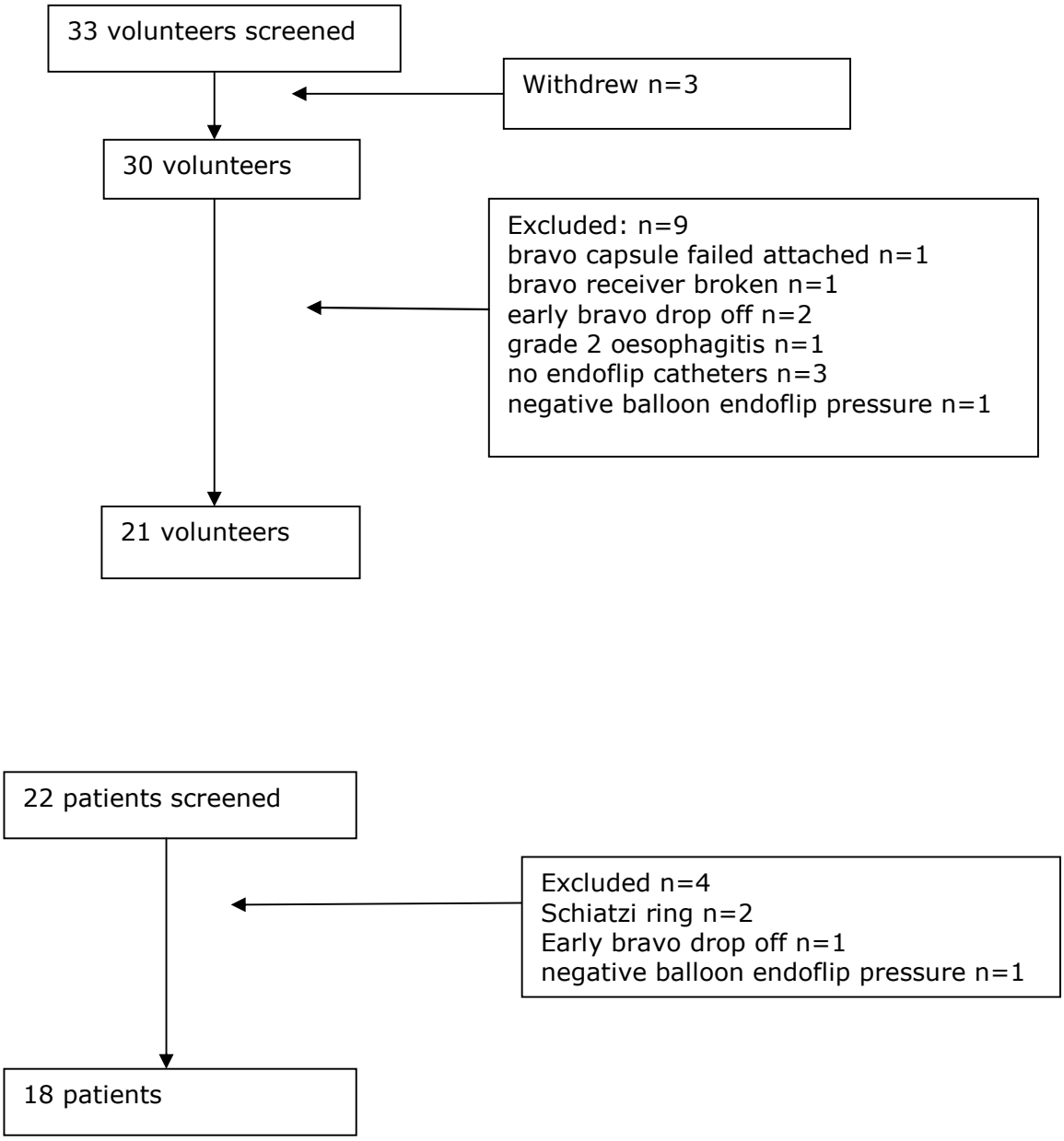


Figure 14. Consort diagram for EndoFLIP® trial

Thus 21 healthy volunteers and 18 patients (13 female, 5 male, age range 27 – 78 years) with mean BMI 33.9 kg/m2 (range 21-57 kg/m^2) were included in final analysis. Patients were older than healthy volunteers ($p<0.0001$). Mean BMI was also higher than that of healthy volunteers ($p=0.001$).

3.3.2 Endoscopy findings

No healthy volunteer was taking acid suppressant medications. 7 healthy volunteers had grade A oesophagitis and 1 volunteer grade B oesophagitis on white light endoscopy.

All patients had been taking proton pump inhibitors until 7 days before the procedure. 7 patients had persistent grade A oesophagitis (5 of whom also had a hiatus hernia) and 3 further patients had hiatus hernia but with no oesophagitis.

3.3.3 Bravo© oesophageal acid exposure

Occasional mid-chest discomfort was reported by 2 healthy volunteers on eating due to the presence of the wireless pH capsule, but this did not prevent them from eating or interfere with daily function. In total, 21 healthy volunteers and 18 patients had at least 48-hours of wireless pH recording and EndoFLIP® measurements.

Average oesophageal acid exposure time was greater in the patient group, although this did not reach significance compared to HVs (5.2 (IQR 2.3-7.7) % vs. 2.0 (1.2-5.1) %; p=0.088, Figure 15).

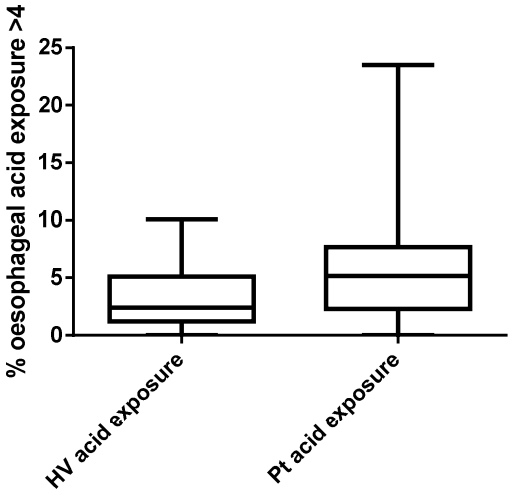
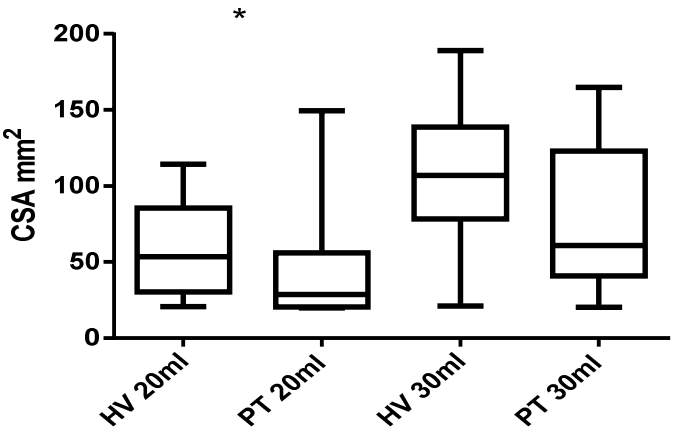


Figure 15. Comparison of Healthy Volunteer and patient group for percentage of time oesophageal acid exposure >pH 4

Categorical analysis found 3/21 (14%) healthy volunteers and 9/18 (50%) patients had oesophageal acid exposure above 5.3%. No healthy volunteers had reflux symptoms during the study. The only symptom recorded by any healthy volunteers was mid-chest discomfort as discussed earlier. 7 patients had positive symptom index above 50% and 10 patients had a SAP >95% for at least one symptom. 9 patients complained of volume regurgitation and 5 had a positive SI and SAP for this symptom. 6/9 patients with regurgitation also had pathological acid exposure.

3.3.4 EndoFLIP® OGJ Cross-sectional area and distensibility

OGJ cross-sectional area was greater in HV than patients at 20ml (p=0.018) and trended towards being greater at 30ml (p=0.0580) as displayed in Figure 16.



Participant group and endoFLIP® balloon volume (HV=Healthy Volunteer, PT=patient) * denotes significant difference

Figure 16. Cross-sectional area of OGJ in healthy volunteer and patient groups at 20ml and 30ml endoFLIP® balloon volume

3.3.5 OGJ Distensibility

Figure 17 shows the distensibility was lower in the GORD patient group than healthy controls, at both 20ml and 30ml bag inflation volume (p=0.001 and p=0.020 respectively).

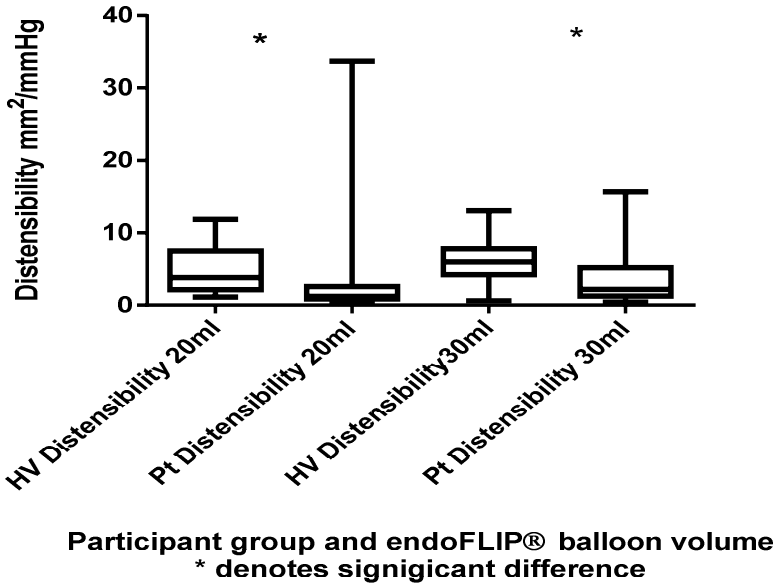


Figure 17. Distensibility of OGJ in healthy volunteer and patient groups at 20ml and 30ml endoFLIP® balloon volume

3.3.6 Oesophageal acid exposure and EndoFLIP®

When participants were separated into those with and without pathological oesophageal acid exposure (>5.3%), there was no significant difference between the two groups in terms of OGJ cross-sectional area, shown in Figure 18 or distensibility, as demonstrated in Figure 19.

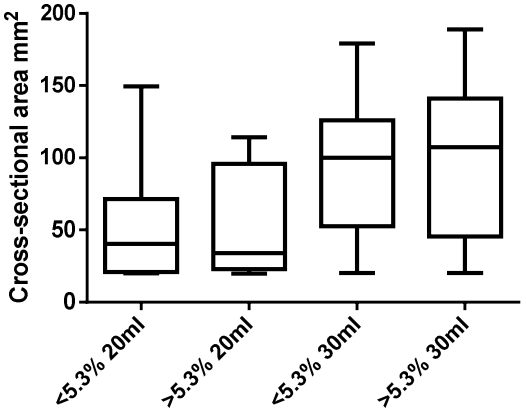


Figure 18. Comparison of participants with oesophageal acid exposure time over 5.3% with cross-sectional area of OGJ

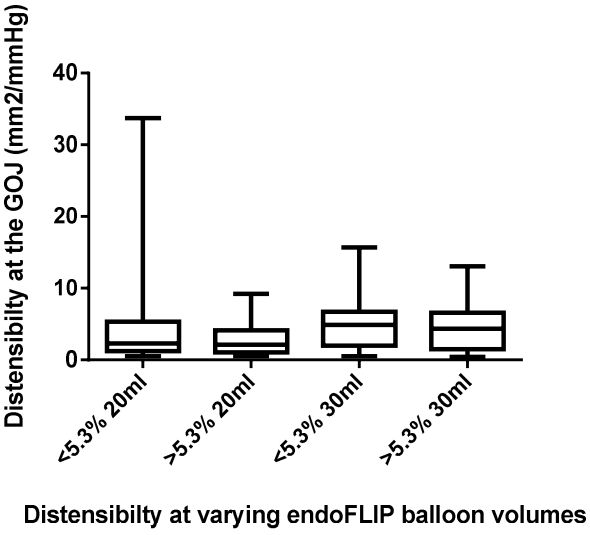


Figure 19. Comparison of distensibility of OGJ with varying oesophageal acid exposure time

6 patients had pathological oesophageal acid exposure *and* regurgitation as one of their prominent symptoms. Also in this

predefined sub-group OGJ CSA and distensibility were not significantly different to healthy volunteers or participants with pathological acid exposure.

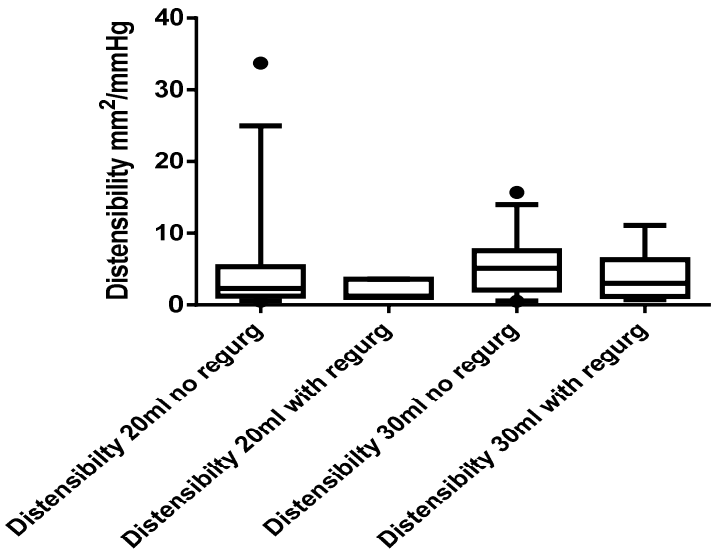


Figure 20. Comparison of distensibility in participants with and without regurgitation

3.3.7 Association of EndoFLIP® results with demographic variables

Due to the unexpected findings of the primary analysis, a post-hoc analysis was performed to assess whether demographic factors explained the lack of association between EndoFLIP® results and acid exposure / patient group. On univariate linear regression there was no correlation of OGJ CSA or distensibility with sex. Similarly there was no consistent correlation between age and OGJ CSA or

distensibility, only a weak correlation between age and CSA at 30ml bag volume (R^2=0.1218, p=0.030,Figure 21).

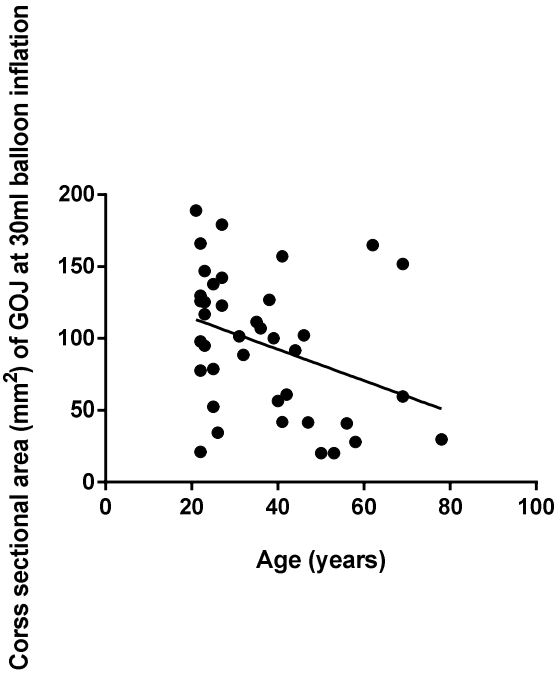
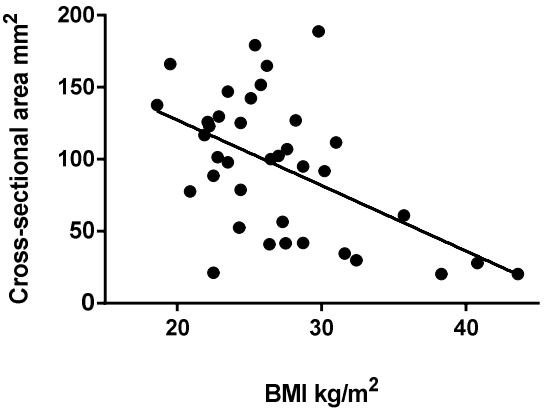


Figure 21. Cross-sectional area of GOJ against age

In contrast, a significant link between EndoFLIP® results and obesity was present. Univariate regression showed an inverse correlation between BMI and OGJ CSA decreasing with increasing BMI (R^2=0.2758, p=0.001). This is shown in Figure 22.



(2 patients with BMI greater than 50 kg/m^2 were exlcuded from this analysis)

Figure 22. Correlation of BMI with cross-sectional area at 30ml endoFLIP® balloon volume

An inverse correlation was present also between BMI and distensibility (R^2=0.2005, p=0.005).

3.3.8 Histology

No oesophageal biopsies were taken from the patient group. This was due to the constraints on time on during the endoscopy, with the EndoFLIP® measurements adding significant length to the overall procedure time. The healthy volunteer biopsies were still examined as there were some positive pH results within the healthy volunteer population.

21 volunteers were included in final analysis (see previous consort diagram, Figure 14). 3 were excluded from histological analysis as

there was no squamous epithelium in the GOJ samples and their corresponding proximal biopsies were also excluded.

Table 7. Prevalence of individual histological lesions with score of 1 or 2 in controls

	GOJ biopsy N=18	2cm above GOJ biopsy N=18
Basal cell thickness	14/18 78%	11/18 61%
Papillary length	9/18 50%	6/18 33%
Dilated intercellular space	13/18 72%	15/18 83%
Intra-epithelia eosinophils	7/18 39%	8/18 44%
Intra-epithelial neutrophils	1/18 6%	0/18 0%
Necrosis/erosions	0/18 0%	0/18 0%

11/18 had a score less than 7, the score used in the comparison of histology and wireless pH monitoring.

The healthy volunteers were then separated into 4 groups, depending on endoscopy and pH study results. Positive pH was based on oesophageal acid exposure time >5.3% and positive endoscopy on any oesophagitis (LA grade A-D) present.

- Group 1: positive endoscopy, positive pH study
- Group2: negative endoscopy, positive pH study
- Group 3: negative endoscopy, negative pH study
- Group 4: positive endoscopy, negative pH study

Table 8. Comparison of endoscopy, pH and histology results

Group	**1**	**2**	**3**	**4**
No of cases (total n=18)	2	1	12	3
Basal cell thickness	1	0	10	3
DIS	1		8	3
Papillary length			6	3
Intra-epithelia eosinophil			5	
Intra-epithelial neutrophils			1	
Necrosis				

Both the Zentilin (142) paper and Sweis (144) had increased histological findings at the GOJ, seen within this healthy volunteer cohort. However, neither noted any intra-epithelial neutrophils within pH negative groups while we have one control with neutrophils present despite no symptoms, gastroscopic changes

and negative pH study. This suggests that these can still occur within a healthy population, although rarely.

3.4 Discussion

The primary aim of this diagnostic feasibility study was to assess whether measurements of OGJ cross-sectional area (CSA) and distensibility by the EndoFLIP® device had the potential to identify GORD patients with pathological oesophageal acid exposure on pH monitoring. The results demonstrate that this technology cannot be used to diagnose GORD in isolation. Further analysis indicates that this failure may be due to an important interaction between BMI and the biophysical properties of the OGJ.

3.4.1 EndoFLIP® results

The result that both OGJ CSA and distensibility were greater in the healthy volunteers than patients referred for investigation of typical reflux symptoms was unexpected. Even when participants were re-assigned into groups based on the presence or absence of pathological oesophageal acid exposure time there were no differences between CSA and OGJ distensibility in health and disease. EndoFLIP® measurements were not increased in those with severe reflux symptoms, (including volume regurgitation).

3.4.2 Results compared to previous literature

These results are not consistent with those of Kwiatek *et al*. which reported significantly higher OGJ distensibility in GORD patients

compared to healthy controls (94). That study used reflux symptoms and endoscopic findings to identify GORD patients rather than definitive evidence from ambulatory pH studies. It is impossible to know whether the severity of disease was comparable; however, endoscopic findings were similar in both studies and it is unlikely that the GORD patients recruited in the previous study had much more severe disease. It is more likely that these inconsistent findings are due to wide variability within the study populations. In both studies, EndoFLIP® measurements of OGJ distensibility ranged between 1 and >30mm^2/mmHg with median values in single figures at both 20 and 30ml distension volumes. This variability impacts on the power of any study to show a significant difference between groups; however, together, these findings indicate that, even if OGJ distensibility is higher in GORD patients, establishing a set of "normal values" for EndoFLIP® measurements would be difficult.

3.4.3 Technical factors with EndoFLIP® measurement

It is well-established that the OGJ reflux barrier in GORD patients is weak and distensible compared to healthy controls (84). However, there are several reasons why EndoFLIP® measurement of OGJ distensibility may not reflect this and does not predict the presence or absence of reflux symptoms or the severity of acid reflux.

- Patient and control groups were selected on the basis of symptoms and not objective diagnosis based on endoscopy or pH-studies; it is inevitable that some HVs will have GORD on testing and some patients will not. Supporting this, a study of 1000 randomly selected responders to a postal questionnaire who then underwent gastroscopy found oesophagitis in those without symptoms. 155 individuals had erosive oesophagitis of whom 57 (36.8%) reported no reflux symptoms at all (152). Conversely of 1307 consecutive patients referred for investigation of typical reflux symptoms, only 50% had pathological acid exposure (153). The possibility of confounding due to "misclassification" of participants based on a symptom based diagnosis within our study was addressed by a secondary analysis in which patients and controls were reallocated as GORD positive and GORD negative on the basis of the pH studies rather than symptoms.
- Technical factors such as body position and the use of sedation and analgesia during the endoscopic procedure may also impact on the biophysical properties of the OGJ and EndoFLIP® results.
- It may be that measurements of OGJ distensibility do not capture relevant pathophysiology. GORD is a multifactorial disease and the OGJ is a complex structure with the lower

oesophageal sphincter, crural diaphragm and clasp and sling fibers at the angle of His all contributing to the "reflux barrier".(154) Endo-luminal measurement of CSA and distensibility may not reflect the action or interaction of all of these parameters.

3.4.4 Demographic factors on EndoFLIP® results

Age and BMI were higher in the patient population compared to the healthy controls. Both of these demographic parameters are associated with increased acid exposure (153, 155). Univariate regression showed a positive correlation of age and OGJ CSA that was significant only at the 30ml distension level; however the effect was very weak. In contrast, the correlation between obesity and EndoFLIP® findings was consistent and relatively strong. High body mass index (BMI) was associated with reduced CSA and decreased distensibility. As a result the comparatively obese patient group had lower CSA and distensibility than the healthy controls and this effect appears to dominate any effect abnormal OGJ properties in GORD may have had on these measurements. The mechanism of this effect could be due to mechanical factors, in particular the effects of increased intra-abdominal pressure (156), that may not be fully corrected by baseline correction. An *in vivo* experimental model that may provide some insight into this effect is EndoFLIP® measurement during the introduction of

pneumoperitoneum. Nathanson *et al.* reported findings from 50 patients undergoing a variety of laparoscopic operations that had EndoFLIP® measurements after induction of anaesthesia, post-pneumoperitoneum and just prior to extubation (157). Note that insufflation pressure in this surgical study was 13mmHg of CO_2, which is similar to the intra-abdominal pressure seen in morbid obesity. The findings showed that intra-bag pressure (IBP) and CSA decreased following pneumoperitoneum, but distensibility increased (157). This result is not that expected from simple mechanical compression of the EndoFLIP® bag which would *increase* intra-bag pressure due to pneumoperitoneum and, therefore, decrease CSA *and* distensibility. For distensibility (=CSA/IBP) to increase during insufflation, intra-bag pressure must decrease disproportionally more than CSA due to changes in OGJ anatomy or, just as likely, OGJ relaxation triggered by stimulation. These findings highlight the complex relationship between obesity, intra-abdominal pressure, OGJ physiology and reflux that is reflected also in a recent clinical series of nearly 600 GORD patients. Anggiansah *et al* showed that, although obesity is associated with impaired OGJ function and prolonged oesophageal acid exposure on univariate regression, this association could not be explained by simple mechanical effects of obesity on the OGJ on multivariate analysis (155).

3.4.5 Histological analysis

The histological analysis was limited to the healthy volunteer cohort of this study and descriptive analysis. The changes reported by Zentilin *et al* (142) were reported more frequently in our healthy population and 7/18 had a reflux score greater than 7, reported to have greater specificity. However, these biopsies were reported by a single pathologist (although was blinded to endoscopy and pH results) limiting the value. However, in routine clinical practice oesophageal biopsies would only be reported by a single pathologist, suggesting there are limitations to using histology alone for diagnosis of reflux disease without pH studies.

3.4.6 Limitations of study

In retrospect, a limitation of this study was that the healthy controls and GORD patients were not matched for demographic factors *including* age and obesity. It is well known that obesity is associated with an increased risk of GORD (158) and so any patient population is likely to have a greater BMI than a control cohort. However controlling for obesity is not possible in clinical practice and this further emphasizes that establishing usable "normal values" of OGJ CSA and distensibility for diagnosis of GORD in clinical practice would be very difficult as multiple parameters affect these measurements.

Histology was only measured in HV and ideally should have been completed in the patient cohort as well.

There were some cases of positive pH studies within the HV population. Expanding the number of healthy controls to have a greater number of "normal" results may have been useful.

The study was completed at two centres, one for HV and one for patients. This was to increase recruitment and reduce overall study time. Although both teams attended training at the same time for equipment use, differences inevitably occur. This is exemplified by no histological data being collected in the patient cohort. Ideally, both sets of participants would have been studied at the same site to minimise differences between the patient and volunteer populations, but it was not possible with time constraints within this study. Is also means there may have been differences in how the tests were carried out in each centre.

The EndoFLIP® device was placed through the mouth and to the level of the OGJ, after the endoscopy had taken place (therefore length to the OGJ known) but not with the endoscope present. This was under advice from the manufacturing team. The EndoFLIP® images then take on a classic "hourglass" shape which also confirmed position. A more robust method would have been to put the EndoFLIP® probe through a channel of the scope and directly view the OGJ to ensure exact placement. The EndoFLIP® probe is

designed to be used without the need for concurrent endoscopy, however, within a trial context it would have been a more vigorous process to use down a scope channel and minimised possible variability.

3.5 Conclusion

EndoFLIP® is a technically viable means of measuring OGJ CSA and distensibility at endoscopy; however, results from this feasibility study demonstrate that these measurements do not predict the presence or severity of reflux disease. This is due to wide variability of these parameters within the study groups and the presence of an important interaction between obesity and these measurements. Thus the EndoFLIP® technique is not suitable for GORD diagnosis. However, this technology may well be of use in other settings where patients have serial measurements (i.e. act as their own controls) and in which the complex interaction of structure and function is less problematic. For example increasing use of EndoFLIP® has been made as a "smart bougie" to guide anti-reflux and bariatric surgery (157, 159-161) and to monitor response to dilatation pre and post treatment in achalasia or eosinophilic oesophagitis(162).

4 Chapter 4 - Non-invasive methods for the measurement of gastric emptying in health and disease

4.1 Introduction

The principles of investigation and current status of gastric emptying studies has been described in the main introduction of this thesis. A more thorough explanation of the techniques used in the work in this MD and their relevance to clinical practice will be detailed, along with a comprehensive description of the functional dyspeptic population.

4.1.1 Gastric emptying and methods of assessing

Delayed gastric emptying, impaired accommodation and hypersensitivity have all been recognised as pathophysiologic mechanisms in functional dyspepsia (32). Current methodology for measuring these includes barostat studies, gamma scintigraphy gastric emptying studies, SPECT and MRI. A simpler method that is thought to provide a representative assessment for some of the parameters measured by more complex technologies is the nutrient drink test.

Gamma scintigraphy and the Nutrient Drink Test can be used in routine clinical practice but other technologies are confined to research unit settings either requiring specialist, expensive equipment (such as MRI) or invasive procedures poorly tolerated by patients (e.g. barostat studies). Invasive procedures also risk

interfering with normal physiology and in a population where visceral hypersensitivity is important, this is especially undesirable. Therefore, the development of non-invasive methods of assessing gastric function are required. This study was designed to develop such techniques.

4.1.2 Symptom scoring

Recording and documenting symptoms in functional dyspepsia has created challenges, to try and objectify them as much as possible and allow comparison between different individuals. A method used and validated by several different studies is the visual analogue score (VAS). In this, a 100mm line, with 0 at the bottom and 100 at the top is listed above each dyspeptic symptom. 0 indicates none of the symptom and 100 indicates maximum. Text descriptors indicating "none, mild, moderate, severe, maximum" serve to reduce individual variation. The patient then scores a line through the point on the line they feel represents the severity of their symptoms. This VAS has been used in nutrient drink tests and gastric barostat studies, for gastric hypersensitivity (54, 163) in both functional dyspepsia patients and healthy volunteers. Dyspeptic symptoms used included epigastric pain, heartburn, nausea, fullness, satiety and bloating.

4.1.3 Psychological state assessment – questionnaires

As discussed in the introduction, psychological state is implicated in functional dyspepsia. Methods of assessing this have always been difficult. Full psychological testing is time consuming and requires access to a dedicated psychiatrist/psychologist. Most commonly in studies on functional dyspepsia, questionnaires are used.

4.1.3.1 PHQ-15

The Patient Health Questionnaire 15 is 15 questions to assess somatization, the description of multiple symptoms that cannot be accounted for by medical or organic disease(164). The PHQ-15 uses criteria from the Diagnostic and Statistical Manuel of Mental disorders, version IV, produced by the American Psychiatric Association. Each symptom is scored from 0-not bothered at all to 2- bothered a lot. In a study of 6000 individuals in America PHQ-15 scores have been compared to functional status, sick days, clinic visits and difficulties related to symptoms (165). A score of 5 was associated with low somatic symptom severity, 10 with medium and 15 and above with high.

4.1.3.2 EuroQuol Health Questionnaire

The EuroQuol heath questionnaire asks participants to grade a total of 5 aspects of general health, such as mobility and self-care from three options, from no difficulties, some problems or unable to complete. It also includes a visual analogue scale for state of

heath on the day of completion, from 0-100. It is also known as EQ-5D. It has been validated in a dyspeptic cohort of 113 patients, of which 70% were functional dyspeptics (166). Its advantages over other questionnaires is that it is relatively short so easy for participants to complete and is generic health questions, so can be used easily in comparison to healthy volunteers and other disease cohorts.

4.1.3.3 The Hospital and Anxiety Depression scale (HADS)

The HADS scale has been used for over 30 years to assess depression and anxiety in a variety of diseases (167). It has fourteen questions asking regarding the frequency of symptoms relating to anxiety and depression, seven questions relating to each. A maximum score of 21 can be given for both depression and anxiety. 8-10 is associated with low level anxiety/depression, 11 and above shows significant depression/anxiety. It has been used and validated in the dyspeptic population (168).

4.1.4 Nutrient drink test

As one of the predominant symptoms in FD is that of post-prandial distress, drink tests (water and nutrient) have been suggested as an easy and accessible way of assessing symptoms. Early satiety has been associated with abnormal fundal accommodation and visceral hypersensitivity on barostat and, therefore, an indirect way of measuring these aspects of gastric function (38). A drink test

involves the patient drinking water or nutrient at a set rate, whilst scoring dyspeptic symptoms until a maximum tolerated volume is reached (when symptoms prevent further ingestion). However, there are many different options for nutrient drink tests. Drinking rate, nutrient or non-nutrient substrate and best outcome measure are examples. A Dutch group compared nutrient drinking and water drinking tests in FD patients, those with mild dyspeptic symptoms and healthy volunteers with the results of barostat studies(105). Although the FD group drank less water and nutrient than the two control groups, it was not associated with one particular symptom and did not predict an abnormal barostat study. It was noted that the rate of drinking was 100ml/min, a relatively high rate of ingestion. A different study by Tack *et al* showed that a slow drinking test (15ml/min) with calorific nutrient showed a correlation in functional dyspeptic patients with maximum tolerated volume and impaired accommodation in concurrent barostat studies(106). A possible explanation suggested for this was that rapid drink tests don't allow for adaptive gastric accommodation, which can take up to fifteen minutes to have full effect (38). Further non-nutrient drink tests don’t evoke the inhibition of gastric emptying from via negative feedback from lipid within the duodenum. Tack’s group have also shown that drink test results (in terms of maximum volume tolerated) are reproducible in both healthy volunteers and functional dyspeptics (169).

Although the nutrient drink test has reasonable sensitivity for functional dyspepsia, specificity is lower with overlap between health and disease. Tack's study from 2003 found in a multivariate analysis, gastric accommodation to a meal was the only independent factor related to maximum tolerated volume in the drink test, whereas gastric emptying rate and sensitivity not (106). However, other researchers using drink tests of a fixed volume found that visceral sensitivity was a key determinant of patient symptoms after a test meal. Further, the maximum tolerated volume measured by drinking test cannot differentiate between a small stomach with a small overall capacity and a normal stomach with decreased accommodation, from, due to simple biomechanics (170), although these are associations. Assessing the development of normal and dyspeptic symptoms as gastric volume increases could potentially help to discriminate these two possibilities. It should be noted also that in the study by Tack *et al* gastric emptying was assessed by a ^{13}C octanoic acid breath test. The authors acknowledge that the breath test will only give information about overall gastric emptying, rather than early and late phases, which may be important in functional dyspepsia.

Together these results suggest a slow rate, calorific drink test of a fixed volume is the most appropriate and practical to use as within a functional dyspepsia population, with symptom monitoring

alongside. Studies have shown that 100% of healthy subjects can ingest >400ml of liquid nutrient with female and male healthy volunteers averaging ~850ml and 1200ml respectively (169). The same study shows that at least 80% of FD patients can ingest >400ml of liquid nutrient, averaging ~500ml (169). These findings were repeated in pilot studies in our own institution (see results)

4.1.5 Gamma scintigraphy

Gamma scintigraphy gastric emptying studies have long been used in the investigation of functional dyspepsia and post-prandial symptoms (31, 171-173). These currently use small, solid meals labelled with a radioactive component (normally technetium-99m) which is then measured with a gamma camera, for approximately 3-5 hours. For each time point measured a 2D image of the radio-labelled stomach contents is produced. The principles of the technique are described below.

Gamma scintigraphy has been used many years now on the study of drug absorption and gastro-intestinal transit times. A substrate (commonly foodstuff or drug) is labelled with a gamma ray emitting radioisotope, commonly technetium -99 due to its half-life of 6 hours and pure gamma emitting nature(174)). These then pass through a gamma or Anger scintillation camera. Within the gamma camera these rays pass through a collimator, which only rays from a set direction are allowed to pass through, so images

can be created of organ studied, such as stomach, which would otherwise be indistinct. Collimators can be converging, diverging, pin hole or parallel hole. The gamma photons then hit a scintillation crystal, which releases a burst of light. This is detected by photomultiplier tubes. The resulting signal can be digitalised to produce quantative images. As peak photon energies vary depending on isotopes used, multiple isotopes to be used in the same study to label separate foodstuffs/drugs (175).

To calculate gastric emptying a region of interest (ROI) is drawn round the stomach area as counts are produced. These are corrected for radioactive decay, to ensure images from the start of the study can be compared to later images. As the stomach lies at an angle with the gastric antrum more anterior, counts from this area travel through less tissue for detection by the gamma camera, giving a larger number of counts from the antrum in comparison to the fundus unless corrected. To compensate for this anterior and posterior images are taken. Once both these images are taken the counts can be used to calculate the geometric mean for a given time point for a gastric ROI. This is the square root of the product of the counts in the stomach ROI on the anterior and on the posterior views [geometric mean = (anterior * posterior) $^{1/2}$]. This compensates for the variation in counts due to attenuation through tissues.

An international standard has been established for meal usage and time periods, with a 2 pancake meal commonly being used or batter based meal substrate(176) with imaging at 0, 1,2 and 4 hours (177). This recommendation has been endorsed by the American and European Neurogastroenterology and Motility societies. Gastric emptying is considered delayed if 60% of meal is retained at the 2 hour time point or more than 10% at the 4 hour time point or of the time taken for half the meal to empty (t50) is prolonged. However, in practice, the test meal applied and other aspects of the performance and analysis of gastric scintigraphy can vary between centres dramatically and this has proved a lack of uniformity between different centres and tests.

Every different test meal if significantly different in calorie load, physical structure and / or frequency of imaging, needs its own normal values establishing. Subject characteristics are also important. It has been reported also that age and sex affects gastric emptying. Tougas *et al* studied 123 healthy volunteers with gamma scintigraphy gastric emptying. They found that gastric emptying was increased with age and was initially faster in men, although was comparable between the sexes at 4 hours (176). Interestingly, body mass index had no effect in this study.

4.1.6 Magnetic Resonance Imaging

Another methodology increasingly being applied in gastric emptying studies is magnetic resonance imaging. Currently, it uses are limited to a research setting due to the requirements of dedicated MRI scanners and the expensive involved. Its main advantages are 3D images are gained and that no ionizing radiation is used, meaning repeated scans, multiple measurements and long study times can be performed in a wide population.

The principles of MRI involve the object to be studied is placed in a magnetic field. Hydrogen nuclei will align with this magnetic field (due to protons) and create a directional magnetic field. A radiofrequency pulse is then applied to the directional magnetic field, causing this to move away, via exciting the protons within the nucleus. The radiofrequency pulse is removed and the nuclei realign themselves with the original magnetic field. As they return to this position, they emit their own electromagnetic field, which is recorded by a coil (signal detection) and used to reconstruct a 3D image of the object being studied (99).

Initial studies with MRI have shown that it provides additional information such as two distinct early and late phases of gastric emptying (178). This has not been described with gamma scintigraphy because, by current convention, gastric volume is

normalized to 100% after ingestion of the meal. This automatically reduces sensitivity of the test to receptive accommodation which occurs during ingestion in response to the volume but not the calorie load of the meal. The measurement of gastric emptying by MRI has been validated by a study in healthy volunteers assessing the inter-observer error, at 12% for 200ml volumes and decreasing to 6% at larger volumes (600, 800ml). T50 measurements varied by less than 5% (100). Another advantage of MRI is that it can visualise gastric secretions. A 400ml chocolate meal was mixed with the contrast agent gadolinium. Meal volume was then assessed separately to total gastric contents volume (meal plus secretion) and secretion separately, in 14 healthy volunteers on rapid MRI scanning (179). The group found that meal volume decreased from the stomach over time, whilst gastric section increased, and this amount of gastric secretion was affected by the rate of meal volume emptying. There was significant variability between individuals however. A complex relationship obviously exists between the two parameters, which need further understanding. MRI has also been used to assess antral contraction waves and its potential to assess gastric motility remains promising (180)

MRI has been validated against scintigraphy, the conventional standard in assessment of gastric emptying. 8 healthy volunteers

underwent liquid and liquid and solid meals, with gastric emptying assessed by scintigraphy and MRI. Intra-class correlation was 0.988 for liquids and 0.917 for solids(181).

4.1.7 Pathophysiology in functional dyspepsia

Previous studies have reported that approximately 40% of FD patients have abnormalities on gastric emptying studies (32). However, there is a poor correlation between symptom severity and improvement in gastric emptying rates does not necessarily result in improved symptoms. Thus the results of existing gastric emptying studies do not necessarily establish diagnosis or guide effective treatment (34). Therefore, there is little in the way of tests to make a positive diagnosis of functional dyspepsia, with the majority of cases diagnosed when all other tests are negative.

Another group where similar difficulties lie is the population with symptoms suggestive of gastroparesis. Gastroparesis is characterised by symptoms of nausea, fullness and post-prandial vomiting with no structural abnormality to account for symptoms(182). Type 1 diabetes is commonly associated with these symptoms, but the condition can occur with autonomic disturbance, Parkinson's disease, collagen disorders and idiopathic associations have all been reported (183). As with functional dyspepsia, the degree of delay in gastric emptying does not necessarily correlate with severity of symptoms and objective

improvements in gastric emptying does not necessarily correlate with improvement in symptoms. A recent review in 2011 of the Gastroparesis Registry in America has found that up to 25% of those with gastroparetic symptoms have normal gastric emptying (173).

Similarities in symptoms and variability in gastric emptying between patients with functional dyspepsia and gastroparesis suggests that functional dyspepsia with post-prandial distress may be in fact part of the spectrum of gastric disorders. Conceptually gastroparesis can be considered as severe gastric motor dysfunction in which there is a loss of tone and contractility leading to severe delays in overall gastric emptying as assessed by gastric emptying half time, whereas functional dyspepsia is characterized by less marked motor dysfunction that have little or no impact on overall gastric emptying (although the dynamics of early and late emptying may be altered)(184). In both cases the severity of symptoms will be related to the severity of gastric motor dysfunction but also visceral sensitivity. Indeed in 40% of patients with functional dyspepsia heightened visceral sensitivity is the only abnormality detected on current physiological investigations(32).

4.1.8 Treatment options in functional dyspepsia

Currently treatment strategies for FD and gastroparesis are not very successful (185, 186). This is partly due to the heterogeneous

nature of patients and lack of one single pathophysiology mechanism to target with drug therapy. Current strategies for treatment options are listed below.

4.1.8.1 Histamine-2 receptor anatognists and proton pump inhibitors

Histamine-2 receptor antagonists and proton pump inhibitors are frequently used initially when patients are complaining of dyspepsia. The benefits of this in functional dyspepsia are limited with therapeutic gain of 7-10% (186). The majority of trials with H2RA were completed those with non-ulcer dyspepsia and did not use the ROME criteria for functional dyspepsia. Trials of PPI's have been completed in those with functional dyspepsia. A meta-analysis of randomised controlled trials of PPI in functional dyspepsia found there was an improvement against placebo, but required a number needed to treat of 14.6(187). They also found those with symptoms suggestive of dysmotility rather than reflux-like symptoms as a predominance, did not respond.

4.1.8.2 Prokinetic agents

Prokinetics can provide symptomatic relief in a small proportion of patients and are often tried as one the first line therapies in primary care. However, study results have shown variable response. A meta-analysis of pro-kinetics agents including metoclopramide, domperidone (both peripheral dopamine antagonists) trimebutine (an agonist for opiate receptors and

possesses anti-serotonin activity) and , cisapride, itopride, mosapride, - all 5-HT_4 agonists, was completed in 2007 (188). All trials included were randomised controlled trials and placebo controlled. They found a 30% excess probability that FD patients would respond to prokinetics.). Thus, a large proportion of FD patient do not respond and moreover, the most consistently effective medication, cisapride, has been withdrawn due to cardiovascular side effects.

4.1.8.3 Tri-cyclic antidepressants and other anti-depressants

Tri-cyclic antidepressants (TCA) have been used to reduce visceral hypersensitivity but have been effective in irritable bowel syndrome. Trials with FD patients have been relatively small. Amitriptyline has been shown to have superior response to placebo in 27 FD patients (70% vs 20).(189) Despite such small patient numbers this is a commonly used medication in this patient group. A study comparing venlafaxine and placebo found no benefit in FD patients (190). Paroxetine has also been seen to improve gastric emptying but studies in FD haven't been completed yet(191). There is currently a lack of large, prospective studies looking at TCA's in FD.

4.1.8.4 Alternative therapies

The herbal remedy iberogast has been used in a number of patients to provide symptom relief. It has been shown in healthy

volunteers to increased proximal gastric volume and antral motility (192), which is how it is thought to help in functional dyspepsia. . It is commonly sold as a combination, as STW 5. A randomised, double blind, controlled trial of 315 patient had a 8 week treatment of either the STW 5 preparation or placebo (193). They found a significant improvement in symptoms in the medication group based on symptom scores completed by the investigator. However, the absolute differences between the two groups in scores was small and there was a high responder rate in both groups.

4.1.8.5 New drug agents in functional dyspepsia

One possible new agent that is being considered is sildenafil, a phosphodiesterase inhibitor that increases availability of nitric oxide. This medication facilitates nitric oxide related relaxation of the proximal stomach and improved accommodation (impaired in functional dyspepsia). A study of 10 healthy volunteers measured fundal barostat measurements following placebo and sildenafil and liquid and solid gastric emptying rates(194). Sildenafil increased fasting intra-gastric volumes and delayed liquid gastric emptying. It also increased volumes of first perception. No randomised trials have been completed to date in the functional dyspeptic population but it remains a promising future therapy.

The lack of successful pharmacological therapy in functional dyspepsia means that identifying the underlying mechanisms of

symptoms is important as a means of targeting appropriate therapy in this difficult to manage population of patients. Such investigations would be invaluable also in the investigation of novel pharmacological products.

4.1.9 Strategies for investigating pathophysiology in functional dyspepsia

The above section summarised what is currently known regarding the mechanism of functional dyspepsia (and methods used to elicit this) and attempted treatment strategies. Changes are seen within the patient population but not consistently throughout. Why is there no correlation between symptoms and conventional measurements of gastric emptying in functional dyspepsia with post-prandial distress, or delays not seen in a larger proportion of the population? Two important possible reasons exist for this and central questions as to why are raised below.

- Is the size of the current test meal (approximately 200ml) not large enough to trigger symptoms and dysfunction on patient group
- Are parameters that are currently recorded (emptying time for half of meal, T50 or contents retention at set time periods, such as 2 or 4 hours) reflective of pathological processes?

There are many factors which make the development of gamma scintigraphy gastric emptying studies in FD desirable. As it is a technology already in use and readily available to most clinicians, adapting the current process would be rapidly turned out to the everyday gastroenterologist. It is non-invasive and well tolerated by patients. It requires only a small dose of radiation (much less than that of a computed tomography (CT) or barium study). Therefore if current protocols for gamma scintigraphy gastric emptying studies could be adapted with the above points taken into consideration, could reliable differences be established between health and functional dyspepsia and a new method for making a positive diagnosis of functional dyspepsia established?

In developing new gastric emptying studies by gamma scintigraphy it would be attractive to compare results with MRI studies of gastric function to validate that similar results were reached and assess whether any additional, clinically relevant information could be obtained by more detailed assessment of gastric structure and function (e.g. tonic relaxation, contractility, acid secretion).

Both of the above tests require specialist referral and hospital based investigation. The spectrum of individuals seen with functional dyspepsia within this setting tend to be those with more severe symptoms, often with alarm symptoms (e.g. weight loss) that demand investigation. A large proportion of patients with less

severe symptoms remain within the primary care setting. In patients with no alarm symptoms that do not require investigation, a technique to obtain a positive diagnosis within a primary care setting is desirable. The simple, nutrient drink test is one such technique. Individuals simply ingest a nutrient at a set rate until a maximum tolerated volume is reached. Dyspeptic symptoms are recorded throughout the test. This could be potentially used as a screening test and for the diagnosis of functional dyspepsia. If completed in the same individuals alongside more accurate measurements of gamma scintigraphy and MRI potentially drink tests could be used in primary care to confirm the diagnosis of FD without secondary care referral and avoiding further more expensive tests. It does have important limitations, most obviously it does not provide insight into the pathophysiology behind symptoms nor any detail regarding gastric emptying, which is why this would be regarded as a simple initial test in the patient population.

Evaluating the three different methodologies alongside one another has the advantages of providing options for the investigation and positive diagnosis of functional dyspepsia in multiple health care settings. The drink test being able to be used in the community setting, gamma scintigraphy being used in most secondary care

settings and MRI potentially provides additional, new markers of gastric function in specialist centres.

4.1.10 Meal choice in gastric emptying studies

When developing any new methodology to study gastric emptying, meal size, content and physical form are all important factors to be considered.

4.1.10.1 Calorific content and size of meal

In a study by Kwiatek *et al* in 16 healthy volunteers, a variety of meal volumes (200, 400, 600, 800ml) and calorific load (200, 300, 400kcal) were studied by MRI and intra-gastric pressure measurements by a minimally invasive fiber-optic recording system (FORS) (178). They found that (i) gastric emptying began during delivery of the meal (ii) larger meal volumes produced larger gastric volumes initially, regardless of calorific load, however the relative magnitude of this effect decreased with increasing meal volume due to more rapid "early" gastric emptying at high filling volumes, (iii) total gastric contents volume could increase after meal delivery due to the rate of gastric secretion being greater than the rate of gastric emptying, (iv) "late" gastric emptying was modulated by meal volume and calorie load .

A study using SPECT to assess liquid emptying (via 300ml labelled Ensure nutrient drink) and gamma scintigraphy labelled eggs, looked at FD patients (195). They assessed gastric emptying at 1,

2 and 4 hours. In the FD population initial gastric emptying at 1 hour was increased but overall gastric emptying delayed (at 4hours).

These studies support the contention that a larger meal than the conventional eggbeater meal may be required to reveal pathophysiology and also that there the traditional measurements of T50 gastric emptying are not sufficient to describe gastric dysfunction and the cause of symptoms in FD.

4.1.10.2 Liquid versus solid meal in gamma scintigraphy

Currently, in gamma scintigraphy gastric emptying studies, solid food is used in routine clinical practice to assess emptying. This is because of previous findings from dual-isotope simultaneous studies of liquid and solid emptying show significant differences in gastric emptying time (as measured by T50 and constant emptying times) in solids between patients and controls, but not on liquid emptying (196, 197). However, there is conflict within the literature with some studies suggesting delays in liquid only meals area predictive of delays in liquid-solid meals, although participant numbers are small (198). Liquid only studies have traditionally been reserved for those unable to take solid foods. There were also concerns about the effects of liquid and solid emptying on one another. Previous work has shown that liquids are more rapidly emptied than solids, liquids follow an exponential

pattern of emptying and liquid emptying is slowed by the presence of solids, but not vice versa (199). Ziessman *et al* published a study in 2009 where a 30 min clear liquid only gastric emptying study was performed just prior to the standard solid phase of the study in 101 patients and 30 healthy controls (200). In the patient studies, 16 had delayed solid emptying and 36 in liquid emptying (at least 3 standard deviations from the mean). The authors also completed imaging at minute intervals in the liquid phase of the study. They suggest that the infrequent imaging of traditional solid studies assesses only antral contractility and emptying and does not assess fundal emptying. They propose this may explain the lack of abnormalities seen in the patient population, if fundal dysfunction is the primary cause of symptoms, then other methods and modalities may be more suited to evaluate this. These are complex data as this study has chosen to separate the phases; however, although dual phase studies are more complex, they are obviously more representative of "real life" as the majority of symptomatic patients eat a combined liquid/solid diet.

One potential technical issue with dual isotope studies is the downscatter from the first ingested isotope into the second ingested isotope activity. In the Ziessman study discussed above there was no significant downscatter when using 10:1 ^{99m}Tc (technetium 99m) (solid) to ^{111}In (liquid) (Indium 111) (200). This

has also been confirmed in other studies, using doses of ^{99m}Tc 5 -6 times greater than that of ^{111}In (201, 202)

4.1.11 Development strategy of Nottingham Test Meal

The current practice in gastric emptying studies has been discussed and the difficulties in correlating this to pathophysiology and symptoms have been described. The modular Nottingham Test Meal was designed to address these challenges. It is applicable for all three investigations (drink test, GS, MRI) and settings. Its features include:

- A relatively large, liquid meal to trigger symptoms in the majority of patients with functional dyspepsia. The presence of symptoms is thought to reflect gastric motor or sensory dysfunction. Thus, if a meal is large enough to cause symptoms then it will also be more sensitive to gastric dysfunction. However, the meal needs to be of a size that will realistically be taken by patients with significant post-prandial symptoms.
- Addition of a solid component to assess gastric trituration. The rate of break down (MRI) and / or emptying (GS) represents an objective assessment of the mechanical work done by the stomach. Ideally this solid component would be non-nutrient, therefore assessing mechanical work only.

- Both liquid and solid components need homogenous and stable labelling to ensure that scintigraphic measurements are representative of the whole meal
- Volume and calorie load selected to allow assessment of liquid gastric emptying within 2 hours in most individuals
- Gastric parameters measured should be reflective of changes in underlying pathophysiology.

In developing a new technique such as this, normal ranges within healthy volunteers with no history of gastro-intestinal disease must be established. This provides normal ranges but also ensures the test is feasible in "real life" and time points and procedures can be completed as planned. (55)

Functional dyspepsia is a heterogeneous patient group. Whilst developing this new method of gastric emptying assessment, functional dyspepsia with post-prandial distress will be assessed, as underlying fundal +/- antral dysfunction is more likely to be present, that in the epigastric pain subgroup. Once this has been assessed, a more heterogeneous group of functional dyspepsia can be assessed, along with a disease control, such as gastro-oesophageal reflux disease. This latter disease control is important to establish if any abnormal pattern of gastric emptying clearly distinguishes patients with functional dyspepsia from patients with GORD; the most common differential diagnosis of

dyspepsia. Establishing this would allow treatment to be directed at specific therapeutic targets (e.g. reflux suppression, prokinetics).

In summary, the main aims of this study were:

- Assess the maximum tolerated volume ingested at nutrient drink test in health, FD and GORD.
- Assess whether a 400ml liquid test meal is a feasible test meal in health and establish normal values in a HV cohort with MRI and GS
- Assess whether differences can be established between FD and HV using new gastric emptying parameters, Gastric contents volume at time 0 (GCV0), gastric emptying rate at the time taken for half the stomach to empty (GErate@T50) and the established gastric emptying parameter, time taken for 50% of emptying to occur, (T50).
- In a smaller subset, add in 12 non-nutrient agar beads to the liquid test meal to assess differences between FD, GORD and HV with the above mentioned gastric emptying parameters.

4.2 Method

The principles behind development and reasoning for methods chosen have been described earlier. This section details methods used in the studies.

4.2.1 Liquid only study- Establishing normal values

4.2.1.1 Subjects – Healthy volunteers

Healthy volunteers with no functional dyspepsia as defined by the Rome Questionnaire and no more than mild symptoms on a maximum of 1 day a week on the GSRS, that met inclusion and exclusion criteria were recruited. Eligible subjects were block randomized by sex and age. Men and women in each age group (<40, 41-60, >60) were recruited. The aim was to recruit 10 in each group.

Healthy volunteers repeated the study days with (i) the same liquid test meal (ii) the liquid and mixed liquid / solid test meal, to assess whether results were reproducible and affected by the solid component of the Nottingham Test Meal (small, non-nutrient agar beads) respectively.

4.2.1.1.1 Inclusion Criteria

For inclusion into the study, subjects had to:

1. Be an adult patient above 18 years old
2. Meet the block randomization criteria for age and sex
3. Have a body mass index of >18 and <30kg.m^2 and not exceed a waist circumference of 99cm at 5cm above ileal crest
4. Be able to give voluntary informed consent and from whom written consent to participate has been obtained.

5. Be able to understand the study, willing to co-operate with the study procedures and able to attend all study assessments.
6. Be willing to abstain from alcohol for 24 hours before and during the imaging appointment
7. Be willing to fast from midnight prior to the screening and imaging appointment
8. Be able to ingest at least 400ml nutrient liquid (0.75kcal/ml at 40ml/min) during a Nutrient Drinking Test without experiencing more than moderate dyspeptic symptoms
9. Be willing to consent to their General Practitioner (GP) being informed of their participation.

4.2.1.1.2 Exclusion Criteria

Subjects must not:

1. Have a history of gastrointestinal disease or surgery (other than appendicitis or hysterectomy)
2. Have ongoing disease requiring active management
3. Have a documented history of alcohol or drug abuse
4. Fail to satisfy the investigator's assessment of fitness to participate based on a survey of inclusion and exclusion criteria
5. Have consumed alcohol within 24 hours of start of study

6. Have participated in a similar study involving the use of radioisotopes in the previous 3 months such that participating in the current study would exceed the recommended yearly exposure limit (5mSv)
7. Take any medication which may affect oesophageal or gastric motility for a minimum 7 days
8. Have had previous history of gastric surgery
9. Have active upper gastrointestinal diseases
10. Have an active Eating Disorder
11. Have an allergy to milk protein (milk based, lactose free test meal)
12. Be a vegan
13. Be pregnant or breastfeeding
14. Have any contraindication to MRI scanning according to local guidelines

4.2.2 Recruitment

Posters were placed on Nottingham University Hospital NHS Trust site and the University of Nottingham sites. Volunteers then contacted the investigators directly, if interested in participating in the trial, via telephone or e-mail. An information sheet and copy of the consent form was then sent to the participant. A screening visit was then arranged to assess suitability for the trial, at least 24 hours after the information sheet had been sent.

4.2.3 Study Schedule

During the study, each subject was required to attend the unit on three occasions as follows:

Screening Visit with Nutrient Drink Test,

Scintigraphy and MRI Imaging Appointments

Each visit was separated by a minimum 2 days and maximum 28 days

For participants taking part in repeat studies to assess test-retest reproducibility, each of these investigations were separated by more than 2 days but less than 4 months

This study was sponsored by Nottingham University Hospitals NHS Trust. The study was conducted at (i) the Research Unit, Department of Medical Physics, Queen's Medical Centre, Nottingham, UK (scintigraphy study days). (ii) the Nottingham Digestive Diseases Centre and Biomedical Research Unit, Queens Medical Centre, Nottingham, UK (screening visit and nutrient drink test) and (iii) the Sir Peter Mansfield Centre for Magnetic Resonance Imaging (MRI study day).

4.2.4 Screening visit

Age, sex, height, weight and waist circumference 5cm above the iliac crest was recorded for all prospective participants. For women menstrual state was noted. The presence of functional GI

symptoms was assessed by the Rome III Adult Questionnaire, Psychologic state documented by the Hospital Anxiety and Depression Score (HADS) and the Perceived Health Questionnaire (PHQ15), and GI health related quality of life assessed by the EuroQual.

Subjects attended the screening visit time between 08.00 and 11.00 (to ensure no diurnal variation in gastric function), having abstained from strenuous exercise and alcohol for at least 24 hours and fasted from midnight.

Each subject was evaluated according to the inclusion/exclusion criteria, and a survey of demographics, medical history, ongoing conditions, and concomitant medications. A brief physical examination was performed including height, weight and waist circumference 5 am above the iliac crest, heart rate, blood pressure and abdominal examination. Before admission into the study, each subject was given a verbal explanation of the study and supplied with a copy of the Informed Consent Form. Written informed consent was then obtained. He/She was then allocated a study number.

The Nutrient Drinking Test was performed as part of the screening visit to ensure that all participants that consented to participate in the full study were able to complete the imaging studies that

require ingestion of 400ml liquid nutrient (Fortisip Vanilla (Nutricia Clinical) diluted 1:1 with water to 0.75kcal/ml, 4.5g fat/100ml).

Subjects drank from a series of beakers containing 40ml liquid nutrient every minute. Compliance was confirmed by the investigator. During the drinking test, subjects scored satiety, fullness, bloating, heartburn, nausea and epigastric pain at 5-min intervals using the 100mm visual analogue scale. Participants were instructed to cease intake when they reported maximal satiety or very severe dyspeptic symptoms (defined as VAS score of >90 mm). The total volume ingested was recorded. Symptoms were then assessed again 15 and 30 min after cessation of intake.

If the participant satisfied inclusion criteria and completed the nutrient drink test, imaging appointments were planned in a randomized sequence and took place between 2 days and 4 months after the screening visit. Each imaging appointment was also separated by 2 days and occurred within 4 months of the initial screening visit.

4.2.5 Imaging appointments

The subject attended the Unit between 08.00 and 11.00, and again abstained from strenuous exercise and alcohol for at least 24 hours and fasted from midnight the previous evening. Each subject was questioned to ensure no changes in his/her health had occurred that may affect eligibility. In the case of pre-menopausal female, a

urinary β-HCG pregnancy test was completed on the morning of the imaging appointment. Participants were randomly allocated to either GS/MRI or vice versa using a randomising sequence.

4.2.5.1 Gamma scintigraphy

4.2.5.1.1 Radiolabelling of the investigational product

Dispensing was performed in the Radiopharmacy unit at Queen's Medical Centre Nottingham. 12MBq Technetium-99m-DTPA (diethylene-triamine-pentaacetate) was added as a non-absorbable marker incorporated into liquid nutrient drink Fortisip Vanilla (Nutricia Clinical). The radiolabelling was performed by the study staff under Good Manufacturing Practice (GMP) conditions.

4.2.5.1.2 Radiation Dosimetry

The total effective radiation dose to each subject who consumes the entire liquid drink for one investigation will be 0.3 milliSievert (mSv) and 0.6 mSv for those undergoing reproducibility studies.

4.2.5.1.3 Investigational Product Accountability

The radio-labelled test meal was produced in the radiopharmacy at Queen's Medical Centre Nottingham. All documentation recording the production procedure is be stored according to GMP and is available for the study monitor to audit as required.

4.2.5.1.4 Study day - GS

After eligibility was confirmed the subjects ingested the radiolabelled liquid nutrient test meal according to a standardized

protocol. Subjects drank one from a series of eight beakers containing 50ml liquid nutrient every 30 seconds. Thus, the 400ml test meal was ingested in 10 minutes. During the test liquid meal, the subjects scored satiety, fullness, bloating, heartburn, nausea and epigastric pain at baseline, 5 minutes (following first 200ml of liquid meal) and 10 minutes (following full 400ml of liquid test meal) using a visual analogue scale (VAS 0–100 mm). These measurements were repeated at following every image taken and 15 minutes and 30 minutes following the end of the imaging scans.

The time of dosing was recorded. Radioactive markers were affixed to the subject at the right costal margin, both anteriorly and posteriorly, for accurate image position. Subjects stood in front of the gamma camera, and acquisition of anterior and posterior images recorded using a Mediso Gamma Camera (Nucline X-Ring-R, Budapest, Hungary). The time of the imaging was recorded. Gastric imaging was performed at baseline, after 200ml ingestion and 400ml ingestion. The imaging procedure was repeated at the following times after the meal: 5, 10, 15, 30, 45, 60, 75, 90, 115 and 120and at 30 minute intervals until isotope had reached the caecum. Symptoms were recorded with each imaging procedure on the VAS chart.

Light refreshments were provided after the study and the subject was be free to leave the Unit. The subject was not permitted to

eat, drink or smoke during the study period until all imaging had been completed.

4.2.5.2 Magnetic resonance imaging

Subjects ingested the paramagnetic contrast (0.5 mmol/l Gd-DOTA (gadolinium and 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid; Dotarem®, Laboratorie Guerbet, Aulnay-sous-Bois, France)) labelled liquid nutrient test meal according to a standardized protocol, as in the gamma scintigraphy imaging day described above, with the same symptom recording.

Studies were performed using a 1.5T whole MRI system (Intera, Philips, Best, The Netherlands). Six rectangular surface coils (height = 20 cm, width = 10 cm), fixed around the abdomen and connected to independent receive channels and were used for signal detection.

The time of the imaging was recorded case report file. Baseline fasting gastric scan was completed before meal ingestion. Gastric imaging was then performed after 200ml ingestion and 400ml ingestion. The imaging procedure was repeated at the following times: -5, 0, 5, 10, 15, 30, 45, 60, 75, 90, 115 and 120 minutes. Symptoms were recorded with each imaging procedure.

Gastric volumes were calculated in the same way for both the liquid and mixed studies by semi-automatic outlining of the

contents and air on each image slice using an intensity based method to define both high signal intensity gastric content volume (GCV) and low signal intensity air volumes using custom-written software (IDL version 6.4,Research Systems Inc., Boulder, CO, USA),. The total gastric volume (TGV) was calculated from the sum of the air and content regions. The segmented area on each slice was multiplied by the slice thickness and summed over all contoured slices to measure the different stomach volumes (TGV and GCV).

Gastric and duodenal motility scans were performed after each volume scan. Motility scans were obtained from three oblique coronal images slices covering luminal wall. Planning the optimal imaging plane for the complex 3D (3-dimensional) duodenal morphology was facilitated by "three-point plan scan". Contraction Waves were recorded using a dynamic bFFE (balanced fast field echo) sequence accelerated with the parallel imaging technique sensitivity encoding (TR/TE = 3.0/1.48msec; flip angle = 60°; SENSE (SENSitivity Encoding) reduction factor 2.0). A total of 177 dynamics will be acquired over a period of 124 seconds. Sequence parameters were as follows: slice thickness = 8 mm, FOV (field of view) = 360 mm, and matrix size = 180* 142 (spatial resolution = 2.00 *2.03 * 8.0 mm3).

Light refreshments were provided after the study. The subject was not be permitted to eat, drink or smoke during the study period until all imaging was completed.

4.2.6 Data analysis

Gastric motor function was assessed by objective endpoints specific for each measurement technique. Gastric sensation during filling is, however, similar during each procedure and is considered separately and via symptoms.

4.2.6.1 Nutrient Drink Test and Assessment of Sensation during Gastric Filling

In addition to the maximum volume ingested sensation will be assessed for satiation / fullness, bloating, nausea, heartburn and epigastric pain in terms of threshold (i.e. volume at which each symptom is first recorded), sensation at 400ml (for direct comparison with imaging studies) and sensation at maximal volume ingested.

4.2.6.2 Gamma scintigraphy

Gastric scintigraphic images were acquired until contents reached the small bowel. Measurements: time taken for 50% gastric emptying (T50), gastric contents volume at time 0 (which is after ingestion of 400ml meal) and gastric emptying rate @ T50 (GE rate @T50).

Liquid gastric empting begins during ingestion before completion of the test meal. To measure this "early gastric emptying" two regions of interest (ROI) were defined around the labelled meal on the 0 min scan immediately after completion of the test meal (1) around the stomach only representing the volume of the test meal in the stomach after completion of the meal (2) around the stomach and small bowel representing the total volume of the test meal (i.e. 400 ml). The same process was repeated for all subsequent scans from 5-120 min. This analysis allows volume of the meal in the stomach to be expressed as a volume (ml) and also as a proportion (%) of the total meal volume at every point in time. All counts were corrected for background radiation and isotope decay.

4.2.6.3 Magnetic Resonance Imaging

Gastric volume data for GS were fitted to a previously described and validated three-parameter model of gastric emptying (eqn 1) using Matlab® (The Mathworks Inc), to characterise gastric emptying(178, 180).

$$V(t) = V_0 \left(1 + \frac{\kappa t}{t_{empt}}\right) exp\left(\frac{-t}{t_{empt}}\right) \qquad \text{Eqn 1}$$

The model had a constraint that the kappa coefficient (κ) could not exceed "1";A value above 1 indicates an increase in gastric content

volume after completion of the meal. Any such increase is related to secretion and thus applicable only to MRI data. To improve the reliability of parameters derived from the fitted data only time points up to 80 mins were used in the fit, provided that by 80 mins the volume had reduced to below 50 % of the ingested volume (200 ml). If this was not the case, additional time points were included until such a time where the volume fell below 200ml. .

For the MRI data, which has the additional complexity of secretions included in the gastric contents measured, a more complex 5-parameter model was used (Eqn 2). This model was adapted from Eqn 1 with a linear term added to better describe the later phase of emptying. All data acquired over the 120 mins (liquid), 115 mins(mixed meal) were included for fitting. Both TGV and GCV data were fitted to Eqn 2.

$$V(t) = V_0 f\left(1 + \frac{kt}{t_{empt}}\right) exp\left(\frac{-t}{t_{empt}}\right) + (1-f)(1-Gt)$$

Eqn 2

Key parameters during emptying were derived from a least-square fit of each volume-time curve to the models described above. These included; T50, the time at which the meal volume had dropped to 50 % of V_0 (Eqn 1 and 2) in mins; GCV0, the modelled initial volume at t=0 in ml (V_0 from eqn 1 and 2); GErateT50, the

rate of change of volume at the calculated T50 time point in ml/min.

4.2.7 Primary and Secondary Outcome Parameters

4.2.8 Nutrient Drink Test

Primary outcome:

Maximum tolerated volume in nutrient drink test.

Secondary outcome:

Sensation threshold volume for fullness, bloating, nausea, heartburn, epigastric pain and sensation at 200ml and 400ml ingested.

4.2.9 Imaging Studies (MRI and GS)

Primary outcome:

Estimates of early gastric emptying – gastric contents volume at time 0 (assessed following full 400ml meal ingestion), gastric emptying rate after 50% meal emptying (ml/min) and time taken for 50% gastric emptying (T50) derived from the model.

Secondary outcome:

Sensation threshold volume at 400ml ingested.

4.2.10 Methods of analysis

4.2.10.1 Data summaries:

Demographic and baseline characteristics are summarised.

The gastric emptying curve, and the T50 summary parameter, was calculated for each subject and for each imaging study. A statistical analysis compared the primary outcome parameters between diagnostic tests to assess agreement between techniques.

4.2.10.2 Determination of Sample Size

(This advice was provided by a statistician at Trent Research & Development Support Unit).

It is assumed that the percentile reference ranges can be calculated from an approximate Normal Distribution, possibly after a suitable transformation such as a Box-Cox transformation.

A general criterion for sample size for reference ranges is given by Harris and Boyd (1995), based on the 90% confidence interval for the reference limit being "small" compared with the 95% reference range for the population.

The width of the 95% reference range is 2x1.96*s = 3.92s where s is the estimated standard deviation. The 97.5^{th} percentile is estimated as $\bar{x}$ + 1.96s/√N and its standard error is approximately √ {(s^2/N){1+(1.96^2)/2}} = √(2.92s^2/N), so the width of the 90%

CI (confidence interval) for the percentile is $2x1.64x\sqrt{(2.92s^2/N)} = 5.62s/\sqrt{N}$

The target relative variation $R = 5.62/(3.92\sqrt{N})$, which, using a medium-sized value for R of 0.2 as a criterion for "small" yields a required sample size of 52 studied.

The Box-Cox family of transformations may be expressed as follows:

$y = \left(\frac{x^{\rho} - 1}{\rho} \right)$ where ρ is a parameter to be estimated.

If $\rho = 1$ then the data are essentially untransformed apart from a location shift, but as ρ approaches zero, the transformation approaches a logarithmic form. This approach addresses the common problem of skewness, but not distributions in which the tails are heavier or lighter than a Normal distribution. The value of ρ that optimises the fit to a Normal distribution may be found using maximum likelihood (as in the Stata *boxcox* routine).

It has been suggested that to allow for sampling variation in this parameter the sample size should be increased by as much as

56%, but in practice this seems to be not needed in many real applications.

If there is evidence that the data do not fit a Normal distribution even after the Box-Cox transformation then efficient estimation of reference limits may be performed using quantile regression (again available in Stata) – this does not require transformation of data. (203)

The initial liquid study has been conducted as a pilot to assess feasibility in a patient group and 8 FD patients were planned to be recruited.

The clinical and physiologic measurements from patients with functional dyspepsia (N=8) represents pilot data; however previous studies have shown that relevant differences in gastric function can be detected between this size of patient and control groups. At approximately the same rate of calorie delivery to be provided in the NDT (nutrient drink test), previous studies have shown good reproducibility and that maximal satiety was reached at a lower volume in dyspeptic patients (489 +/- 276 and 503 +/- 248 mL for first and second test respectively) than controls (937 +/- 428 and 1048 +/- 421 mL, $P < 0.0001$).(169) There was good separation between the two groups with >95% of patients but only ~40% of patients able to ingest >600ml liquid nutrient (>80% of patients were able to drink 400ml, the volume at which initial

imaging will be acquired (a secondary outcome measurement)). Based on these figures, power calculations show that 12 patients and controls provide a 90% chance of detecting a significant and clinically relevant (200ml) difference on maximal drinking volume.

It was planned to complete MRI and GS studies in 12 FD and GORD patients in the mixed meal study.

Concerning imaging, recent publications have shown that the accuracy and reproducibility of MRI measurements of gastric volumes and emptying is very good both in health and dyspeptic patients.(184) Power calculations show that comparing 12 patients and controls before and after intervention provides an 80% chance of detecting a 20% difference in gastric emptying rate at $p < 0.05$.

4.2.11 Development of solid component

The inclusion of 12 solid agar beads of known breaking strength will allow calculation of the time taken to break down solids and for these to empty from the stomach.(204)

These beads were composed of food grade agar as non-nutrient so represents mechanical component of gastric emptying only. Barium was added to ensure the beads sank. They beads are 11.5 mm in diameter so easily swallowed but unable to pass through the pylorus whole. Therefore this allows assessment of mechanical

work of antrum as the beads need to be broken down before being able to leave stomach.

To ensure the agar beads maintained their own radiolabelling and the radiolabelling of the liquid component of the meal did not leak into the beads within the scintigraphy part of the study. To do this 12 non-labelled agar beads were placed in a solution of fortisip with 0.5 Mbq In-111 Cl. The addition of 0.1M HCl, heating to 37^{o}C and mixing were completed to recreate the stomach environment. The agar beads were removed after 4 h Instant Thin Layer Chromatography (ITLC) documented that only 0.002% of In-111 Cl had permeated the solid component. 12 agar beads labelled with Tc-99 MAA (microaggregated albulim) were submerged in non-labelled fortisip, again heated to 37^{o}C and mixed. Samples were taken from this solution over 4 hours and counts measured. These showed that less than 10% of Tc-99 MAA leaked out into fortisip solution. The beads also remained uniformly labeled.

The end composition of the 12 agar beads was food grade agar (1% Agar-Agar; Cuisine-innovation, Dijon, France), 7.0 g barium sulphate (E-Z-Paque: Buckinghamshire, UK Ph Eur 96% w/w) with 5 Mbq Technetium-99m-MAA (Technescan® LyoMAA (DRN4378), Mallinckrodt Medical B.V.,The Netherlands). The breaking strength was 0.8 N/mm^2 as calculated by a tablet hardness tester (Erweka THB100, Heussentamm, Germany).

Within the MRI section, the beads required no separate labeling as the fortisip was had the contrast agent gadolinium-DOTA (0.5 mmol/l Gadolinium-DOTA; Dotarem®, Guerbet, Aulnay-sous-Bois, France). This means the fortisip appears bright within MRI images while the barium within the beads causes them to appear dark, therefore providing contrast between the liquid and solid components of the meal within MRI images.

4.2.12 Mixed meal study – Healthy volunteers

After the development of the solid component for the test meal, 12 food grade solid agar beads of known breaking strength have been added.

The participants will follow the same procedure as for the liquid only test meal, with the following changes:

4.2.12.1 Screening visit and nutrient drink test

The screening visit had no changes. The nutrient drink test proceeded as previously, but at the end the test, following all symptom recording, one test agar bead was ingested whole by the participant, to ensure there would be no difficulty in swallowing the beads on imaging study days.

4.2.12.2 Imaging study days

The randomisation sequence was taken out of the protocol. This was predominantly done to enable easier allocation of imaging days in the patient population, to minimise the time spent off

medication. There was no difference in the liquid only results depending on the order of MRI or GS.

Subjects drank the full 400ml test meal and ingest the 12 agar beads within 10 minutes. The first 200ml liquid test meal were ingested within 2 min, 50ml every 30 seconds (100ml/min). The subject was then imaged (GS/MRI depending on study day). The second 200ml of the nutrient drink was then given, 50ml every 30 seconds, with 3 agar bead swallowed whole alongside every 50ml of liquid meal (100ml/min, 12 agar beads in total). Imaging sequences then proceeded as per liquid study.

4.2.12.3 Additions to liquid meal method for solid component;

4.2.12.3.1 Gamma scintigraphy

Technetium-99m was added as a non-absorbable marker incorporated into the agar beads. The agar beads were weighted with barium sulphate and were 11.5mm in diameter. The participant swallowed 12 beads. The total amount of radiation in all of the beads equalled 5MBq (mega Becquerel).

The liquid nutrient drink Fortisip Vanilla (Nutricia Clinical) will be radiolabelled with 0.5 MBq of the non-absorbable marker Indium-111.

The total effective radiation dose to each subject who consumes the entire liquid drink and agar beads for one investigation will be 0.3 mSv.

Liquid and solid gastric emptying were measured in the same way as the liquid only study. The same ROIs were used to calculate the volumes and percentage of liquid and solid meal in the stomach. The In-111 overlap onto the Tc99 channel was estimated from the first 200 ml of fortisip administered to the subject. The numbers of counts were then converted to a percentage of the total test meal volume. Due to the low count produced by the 0.5 Mbq In-111 label in the mixed meal, the counts were corrected also for background radiation (average of anterior and posterior images taken separately assessed at 0 min). The number of beads present in the stomach at 1 h and 2 h calculated as a percentage of counts.

4.2.12.3.2 Magnetic resonance imaging

Apart from the addition of the agar beads, as detailed in imaging study days sections above, there were no additional procedures during MRI study days from the liquid meal study.

The volume of the agar beads included in the GCV and TGV of the mixed meal study was small (9.6 ml). The number of intact agar beads left in the stomach at 1 h and 2 h was counted directly from

volume and coronal scans. Counting was aided by use of custom written software (IDL 6.4) which allowed semi-automatic tracking of beads through the different slices.

4.2.12.4 Outcome measures and sample size calculation

The primary outcome measurement is gastric emptying time T50 and the number of participants to be recruited will provide an 80% power to detect a 20% difference in this parameter between healthy volunteers and patients with functional dyspepsia. T50 was used to generate power calculations because this is the most widely published summary assessment of gastric function. Previous studies suggest that 40% of FD patients have delayed gastric emptying using gastric scintigraphy (205). The normal range of T1/2 was 129+/88 min (mean+/2SD (standard deviation)) among the control group. The mean T1/2 of the patient group was 160+/96 min ($P<0.01$). Twelve of 22 male patients and 11 of 13 female patients had prolonged T1/2. Based on these results at least 13 participants are required to show a significant difference between the healthy and FD patient groups.

4.2.12.5 Statistical analysis

Data analysis was completed using Graphpad Prism 6 (GraphPad Software Inc., La Jolla, CA, USA). Student's t-test and Mann-Whitney test were used to compare quantitative parametric and non-parametric variables respectively. Significance was set at $p <$

0.05. Paired student's t-test (Wilcoxon signed rant test in non-parametric data). Inter individual variation in gastric emptying was analysed using Bland-Altman plots. The bias and the standard deviation of the bias are presented. Inter observer correlation coefficients (ICC) were calculated with SPSS version 16.0 (SPSS, Chicago, Illnois, USA).

4.2.13 Functional dyspepsia patient recruitment

4.2.13.1 Patient identification

Patients were identified through the specialist upper gastro-intestinal functional clinic of Dr Mark Fox (consultant Gastroenterologist and MD supervisor) at Queens Medical Centre, Nottingham University Hospitals NHS Trust, Nottingham. They were given an information sheet and copy of consent form and allowed at least 24 hours before being contacted to arrange a screening visit for the study. Patients were also recruited from endoscopy lists where upper gastro-intestinal endoscopy was normal and symptoms of functional dyspepsia present. In this instance, permission was always sought first from the referring consultant before any information sheet was given to the patient.

4.2.13.2 Inclusion and exclusion criteria in patients

Inclusion/exclusion criteria were as for healthy volunteers for the liquid and mixed meal, with the additional points:

1. Symptoms consistent with functional dyspepsia with postprandial distress syndrome as defined by the Rome IV Questionnaire and at least moderate symptom severity on at least 3 days a week
2. Have a normal upper gastro-intestinal endoscopy
3. Have a negative 24 hour ambulatory pH-impedance study, if any symptoms suggestive of possible gastro-intestinal reflux disease
4. Be able to stop all medications that affect upper gastro-intestinal motility and sensory function for one week prior to the screening visit and for the duration of the study. If these were given for important medical conditions e.g. calcium channel blockers for hypertension, the patient would be excluded from entering the study. Prohibited medications included:
    a. Proton-pump inhibitors
    b. Pro-kinetic agents
    c. Calcium channel blockers
    d. Tricyclic antidepressants
    e. Nitrates
    f. Opiates

4.2.14 Ethical and other required approvals for the studies

Both the liquid and mixed studies were given ethical approval from the National Research Ethics Service East Midlands – Derby Committee.

The Research and Innovation Department approval from the Nottingham University Hospitals NHS Trust was given for both studies.

The Administration of Radioactive Substances Advisory Committee (ARSAC) from the Health Protection Agency approved certificates of administration for all radioactive medicinal products given in the studies.

4.3 Study results

This study was split into two main sections, summarised in the methods section.

1. Liquid Nottingham Test Meal in HV and FD patients.
2. Liquid Nottingham Test Meal with 12 solid agar non-nutrient beads in HV, FD and GORD patients.

In each case GS and MRI measurements were acquired with concurrent assessment of sensation. After review of the initial results of the mixed meal study, there was concern the presence of dual isotopes in the GS section were causing relatively poor spatial

resolution for the liquid component and overlap between the measured counts for the liquid and solid component in some cases. Due to this a major amendment was granted to complete further studies within the patient population with no beads in the GS section. The higher counts acquired with technetium labelled meal after the amendment reduced the effects of decay and background counts on measured activity. The beads were kept in the MR section as there was no issue with dual isotopes within that modality.

Thus, the healthy volunteers and the initial 8 FD patients in the mixed meal were studied with liquid and beads labelled with indium and technetium isotopes respectively. However, the subsequent FD patients and **all** GORD patients were studied after the major amendment (i.e. without beads in GS). The methodology was identical for the MRI studies in all cases.

As this section of the thesis is a development of a new gastric emptying study, after the demographic of different groups are described, the normal values will be presented for HV, initially for the liquid part and then mixed. Following this, the validation of the new meal with comparison of results for HV who repeated the liquid meal twice and HV who completed both liquid and mixed meal. The results of three observers are compared for both GS and MRI analysis. This will be followed by FD patients compared to HV

in the liquid meal and FD and GORD patients and HV in the mixed meal.

In summary, the main processes of the trial

- Participants recruited, complete questionnaires screening visit
- All participants complete nutrient drink test as part of screening visit

Liquid study:

- HV and FD patients studied
- 400ml ingested at MRI study day and GS study day
- Gastric emptying parameters measured

Solid study:

- HV, FD and GORD patients studied
- 400ml ingested with 12 non-nutrient agar beads for all MRI study days in HV, FD and GORD
- 400ml ingested with 12 beads in all HV and 8 FD patients at GS
- Further FD and all GORD patients had NO beads in GS studies due to problems with dual isotope crossover

4.3.1 Liquid study participants

Healthy volunteers were recruited prospectively in stratified age/sex blocks. These were separated in to men< 40 years, women <40 years, men 41-60 years, women 41-60 years, men> 60 years and women > 60 years. The aim was to recruit 10 individuals into each block. 8 men were recruited into the 41-60 block, 9 into men > 60 and 6 women into > 60 block. The remaining blocks had 10 in each. Therefore 53 HV were recruited in total. Mean age was 44.6 years (range 18.2 – 78.2 years). A total of 59 HV were screened. 1 was taking medications that precluded them from the study (calcium channel blocker), 3 failed to come to their second study day, 1 vomited following the nutrient drink test and 1 had reflux type symptoms.

Each patient was matched to three randomly selected healthy volunteers from the block age/sex group stratifications.

10 FD patients were screened, 1 vomiting following drinking the minimum 400ml at nutrient drink test and 1 patient declined to have the medication washout required for the trial. 8 were included in total.

4.3.2 Demographics

There were 7 female patients and 1 male with a mean age of 50.2 years (range 23.7-72.1 years). There was no significant difference

between height and weight between the patients and healthy volunteers, (p=0.628 and p=0.06 respectively) although weight had a trend to be lower, with a mean of 57.5 kg for patients and 63.9kg for HV. Patients BMI was significantly lower than their matched controls (p=0.026).

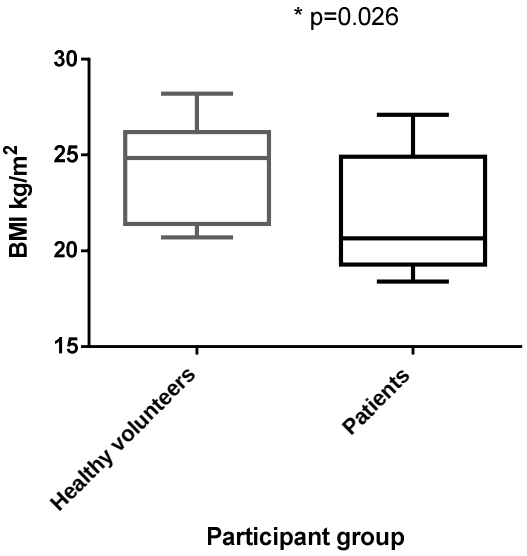


Figure 23. BMI in patient and healthy volunteer groups in liquid study

4.3.3 Mixed meal participants

Only participants who had both MRI and GS results were included in analysis.

Healthy volunteers were collected prospectively in the same age/sex blocks again. 24 were collected in total; 3 men <40, 4 women <40, 5 men 41-60, 2 women 41-60, 5 men > 60 and 5 women > 60. Mean age was 47.7 years (range 19.1-69.0). 27

were screened but 1 could not attend available dates for study days, 1 withdrew and 1 scored too highly on the ROME III questionnaire.

26 FD patients were screened, 5 did not complete MRI study day – 1 felt her tattoo heated up and the study day was aborted, 2 vomited during the scans, 1 felt too claustrophobic in the MRI scanner and 2 did not attend their MRI appointment. 1 patient did not attend for GS as went back on her medication. Of the 19 remaining, 2 had several scans missing from MRI study day, therefore their results could not be used. A total of 17 FD patients had usable results from GS and MRI.

14 GORD patients were screened. 2 declined to stop their medication and 1 did not attend MRI study day. Therefore 11 were included in final analysis.

4.3.4 Demographics

12/17 FD patients were female while 9/11 GORD patients were male. The mean age for FD patient was 40.5 years (range 20-71 years) and 40.7 years (range 23-56 years) for GORD patients.

The mean height for HV, FD and GORD patients were 1.71m, 1.70m and 1.73 respectively. There was no significant difference between any of the groups.

Mean weight was HV=73.9kg, FD=66.9kg and 81.2kg. There was no significant difference between the HV and FD or GORD and HV (although a trend for lower in FD), but was between FD and GORD (p=0.002).

Median BMI was HV=24 kg/m^2, FD = 22.8 kg/m^2 and GORD = 27 kg/m^2. FD patients had a significantly lower BMI (p=0.041) compared to HV and to GORD patients (p=0.0008). There was no significant difference between the HV and GORD patients.

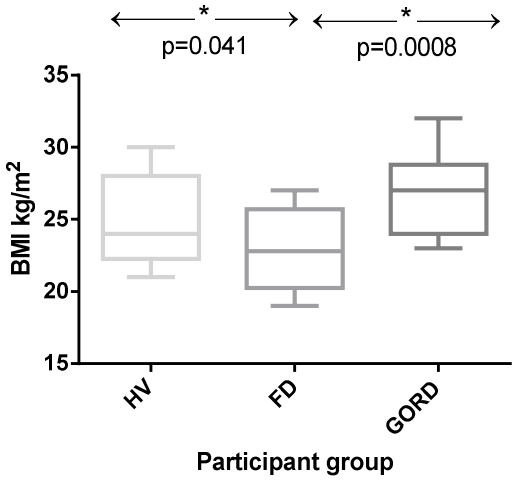


Figure 24. BMI and participant group in mixed study

4.3.5 Normal values

This study was the development and validation of a liquid and mixed meal with new gastric emptying parameters measured. As

such, normal values are first described, along with validation before the presentation of the patient data.

4.3.5.1 Normal values – liquid study

Table 9. Values for healthy volunteer gastric emptying parameters in liquid study

Healthy volunteers	GS liquid N=53	MRI liquid N=53	N=53
	GCV	GCV	TCV
GCVO (ml)			
Median	353.6	401.7	491.1
IQR	340.6 365.1	379.7 443.4	453.3 550.1
T50 (min)			
Median	45.10	70.90	68.15
IQR	37.60 55.75	52.50 85.80	47.90 87.30
GErate@T50 (ml/min)			
Median	3.742	2.438	3.328
IQR	3.034 4.487	1.679 2.917	2.315 4.302

The table shows the normal values. Parameters measured are; GCV – gastric contents volume, TGV – total gastric volume (gastric contents plus air), GCV0 - gastric contents volume at time 0 (completion of meal), T50 – emptying time for half gastric meal,

GE rate @ T50 – gastric empting rate @ emptying time for half gastric meal.

The data used from gastric emptying measurements for participants in the trial were modelled, as detailed in the methods section. This produced the values for GCV0, T50 and GE rate @ T50. An R^2 above 0.9 indicated a good fit of the model. The majority of healthy volunteers had a value above this for the liquid study. Only 1 HV in GS had an R^2 less than 0.9, 1 HV in MRI GCV and 2 HV in MRI TGV. The two sets of data (all data versus only that with R^2 above 0.9) were statistically compared for each gastric emptying parameter in both MRI and GS. No significant difference was found between any of the groups in the liquid study for the two data sets. Therefore, for further analysis, data is used regardless of R^2 result.

One healthy volunteer didn't have a R^2 above 0.9 (0.85) for GS. On reviewing his gastric emptying curve, initial emptying (represented by GCV0) was close to the median but GErate@T50 (representative of later emptying) was slow, below the 25th percentile, although not the minimum value.

Within the MR data, one healthy volunteer had a low R^2 for both gastric contents volume (labelled fortisip and secretions) and total gastric volume (labelled fortisip plus gastric secretions and air), and one further healthy volunteer had a low R^2 for total gastric

volume for MR. The participant with low R^2 for both MR measurements had slow emptying throughout but more pronounced in the later phase (GEirate@T50) and was interestingly the oldest healthy volunteer (male, 78 years) in the study. The participant with low R^2 for total gastric volume in MR was slow emptying throughout, again particularly pronounced in the later phase. All patients had an R^2above 0.9.

The results were compared between GS, MRI GCV and MRI TGV.

- GCVO was significantly lower in GS than MRI GCV and TGV (p>0.0001in both)
- T50 was significantly lower in GS than MRI GCV and TGV (p>0.0001 in both)
- GE rate @ T50 was significantly higher in GS than MRI GCV and non-significantly so in MRI TGV (p>0.001 and p=0.125 respectively)

4.3.5.2 Demographic affect on gastric emptying parameters – healthy volunteers

The effects of demographic variables were assessed on GCV0 results. Linear regression was performed for height, weight, BMI and age on GCV0 in both GS and MRI.

Table 10. Demographic affects on gastric emptying parameters

	GCV0 GS	GCV0 MRI GCV	GCV0 MR TGV
Height	N	N	N
Weight	Y -ve	N	N
BMI	Y -ve	N	Y +ve
Age	N	N	Y +ve

4.3.5.3 VAS scores – normal values

The normal values from the whole HV cohort were recorded for fullness at 400 ml during MRI and GS.

Table 11. Normal values for fullness VAS scores at 400ml in test meal

HV	GS	MRI
Fullness @ 400 ml		
Median	40	30
IQR	18-63	16-45

Normal values – mixed study; liquid component

Table 12. Values for healthy volunteer gastric emptying parameters in mixed study

Healthy volunteers		GS mixed all N=24	MR mixed N=24	
			GCV	TCV
GCV0 (ml)				
	Median	368.8	437.1	530.9
	IQR	348.4 - 388.0	416.4 - 466.1	483.9 - 569.4
T50 (min)				
	Median	52.45	68.15	60.50
	IQR	37.55 - 72.88	56.03 - 77.08	50.03 - 79.40
GE rate @T50ml/min				
	Median	3.108	2.985	3.760
	IQR	2.068 - 4.073	2.634 - 3.282	3.015 - 4.210

18 of the 24 HV in GS had a $R^2 < 0.9$ while all MRI data had an $R^2 > 0.9$. The GS data was compared for that with $R^2 > 0.9$ and all data. As with the liquid study, no significant difference was found between the 2 groups for each of the gastric emptying parameters dependent on R^2 results.

- GCV0 p=0.252,
- T50 p=0.334
- GE rate @ T50 p=0.395

The results were compared between GS, MRI GCV and MRI TGV.

- GCVO was significantly lower in GS than MRI GCV and TGV (p>0.0001in both)

- T50 was non-significantly lower in GS than MRI GCV and TGV (p=0.114 and p=0.584)
- GE rate @ T50 was not significantly different between GS and MRI GCV or TGV

4.3.5.4 Solid emptying rates

Solid emptying retention rates were recorded for time points 60 minutes (T60) and 120 minutes (T120). MRI records the number of intact beads seen within the stomach (this can be clearly seen on MRI image slices). GS records the retention of beads within the stomach (as a of number of counts), these can be intact or broken beads. GS is unable to differentiate between intact and broken beads.

4.3.5.4.1 GS – solid emptying

Table 13. GS solid retention rate

HV	T60 (%)	T120 (%)
Median	80	65
IQR	74-86.5	42-79.3

4.3.5.4.2 MR – solid emptying

Table 14. MR solid retention rate

HV	T60 (%)	T120 (%)
Median	58.3	20.8
IQR	41.7-83.3	2.1-50

4.3.5.5 VAS scores – mixed meal

Fullness at 400ml during the test meal was measured for HV

Table 15. Normal values for VAS scores at 400ml in mixed meal

HV	GS	MRI
Fullness @ 400 ml		
Median	28	30
IQR	15-56	12-50

4.3.6 Validation of test meals

The liquid and mixed studies have been used to compare HV's to patient groups. However, should this type of study be used widely in common clinical practice, validation within the HV population must be completed. The following section describes this. HV's who underwent both the liquid and mixed study were compared.

4.3.6.1 Participants

Of 27 HVs screened, 24 HVs (13 male, 19-69 years; mean age 49 years +/- 19.3 years) successfully completed both MRI and GS study days for the mixed meal. 9 Subjects (5 male, 21-78 years) completed the liquid test meal study twice. 11 (of the 24) HVs (9 male, 20-68 years) completed both the liquid test meal study and the mixed meal study.

All subjects tolerated both the liquid and mixed 400ml test meal. More than moderate fullness (>70mm VAS) was reported by <20% HVs during GS and MRI studies. More than mild dyspeptic symptoms (>30mm VAS for bloating, nausea or pain) were reported by only one HV on one occasion. There were no significant differences in sensation of fullness or tolerance of the test meal in the upright and supine positions.

4.3.6.2 Baseline MRI volumes

For the MRI data the fluid and air present in the fasted stomach could be measured before the study began. These baseline volumes of GCV before meal (liquid or mixed) ingestion were small (median 19 ml (IQR 12-33 ml, maximum 39 ml) and median TGV 46 ml (IQR 24-69ml, maximum 161 ml).

Baseline volume scans were completed on two separate occasions for 20 subjects that attended for MR studies on more than one occasion (11 HVs 2 meal types, 9 HVs 2 repeats of same meal).

Variation was small (<20ml GCV, <60ml TGV) with no evidence of a sequence effect (P=0.314 and P=0.648) as shown below.

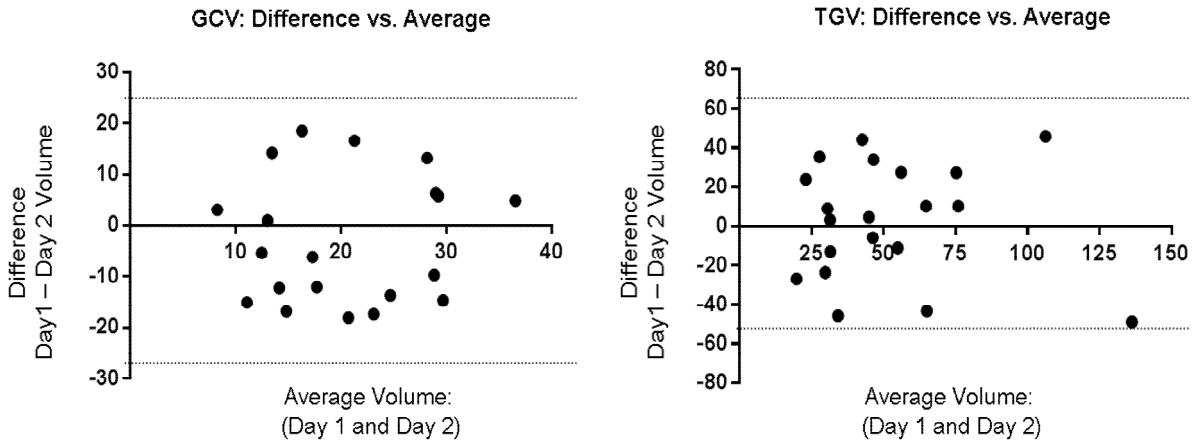


Figure 25. Bland-Altman Plot of baseline (residual) volumes in HVs measured by MRI prior to ingestion of test meal. The 95% C.I. are represented by the dotted line in each case (GCV: upper 22 ml, lower -27ml, TGV: upper 61 ml, lower -55ml).

4.3.6.3 Liquid study: Reproducibility

GS and MRI study days for the liquid study were repeated in 9 HVs as shown in Figure 26. Bland Altman plots compart GS and MRI GCV in the 9 HV. The HV's repeated both GS study days within a mean 87 days SD 85 days (95% C.I. Upper 152 days, lower 21 days.). Both MRI study days were carried out between mean 74 days SD 58 days (95% C.I. Upper 118 days, lower 29 days).

The Bland-Altman plots demonstrated for GS GCV0 bias of 9 ml (95% C.I. upper 34 ml, lower -16ml), T50 bias 8 min (95% C.I. upper 29 min, lower -14 min) and the GErateT50 bias -0.9 ml/min (95% C.I. upper 1.1 ml/min, lower 1.1 ml/min). For MRI (GCV)

the GCV0 bias was -25 ml (95% C.I. upper 41 ml, lower -91 ml), T50 13 min (95% C.I. Upper 39 min, lower -13 min) and GErateT50 bias 0.2 ml/min (95% C.I. upper 2.3 ml/min, lower -1.8 ml/min). Similarly MRI (TGV) followed in the same pattern GCVO – bias 4.0 (95% upper 47 ml, lower -55 ml), T50 bias 6 min (95% C.I. Upper 49, lower -37 min) and GErateT50 bias 0.03 (95% C.I. Upper 3.3 ml/min, lower -3.3 ml/min)

Early and late GE for GS tended to be faster on the second test day than the first. The average difference in GE between study days for GS was GCV0 7.5 ml, T50 9 min and GErateT50 was -0.9 ml/min. In MRI (GCV and TGV) the opposite occurred with early and late GE tending to be faster on the first test day than the second. The average difference between study days for MR (GCV) was GCV0 25 ml, T50 9 min and GErateT50 0.2 ml/min. The average difference for MR (TGV) was GCV0 -4 ml, T50 6 min and GErateT50 0.03 ml/min. The absolute differences are relatively small between parameters.

In GS only the GErateT50 was shown to vary significantly between the two study days (GS: GCV0 P=0.070, T50 P=0.077, GErateT50 P=0.036). This effect on the GErateT50 was not seen with MRI (GCV or TGV). However, MRI (GCV) at GCV0 was shown to differ significantly between study days (GCV0 P=0.050, T50 P=0.019, GerateT50 P=0.496. There was no significant sequence effect for

MRI (TGV) (GCV0 P=0.652, T50 0.460, GErateT50 P=0.956). However, between the two modalities there was no order effect of MRI or GS.

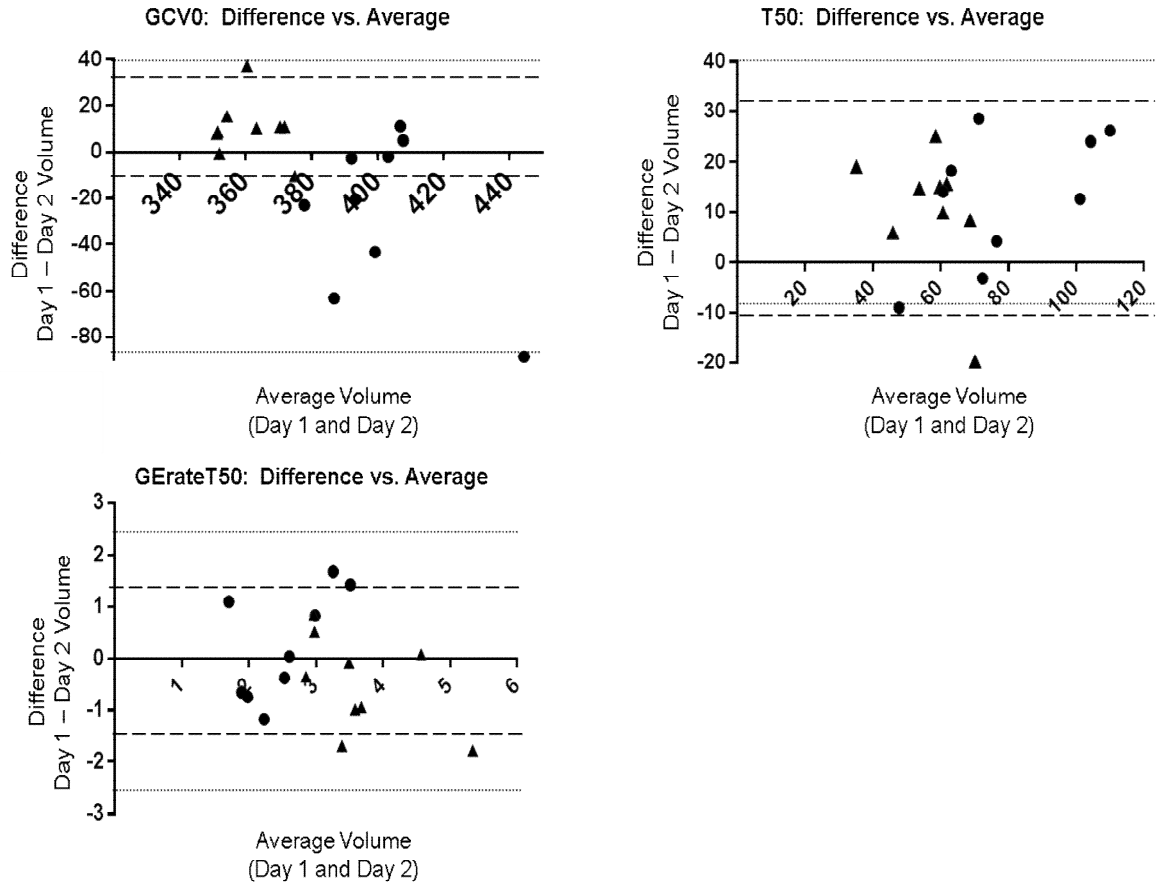


Figure 26. Bland-Altman Plots showing GS and MRI GCV results from 9 HV imaged on two separate occasions after ingestion of liquid test meal; Panel A**: GCV0,** Panel B**: T50** Panel C**: GErateT50. MRI volumes are represented by • with the 95% C.I. represented by the dotted line. GS volumes are represented by the ▲ and the 95% C.I. by the dashed line.**

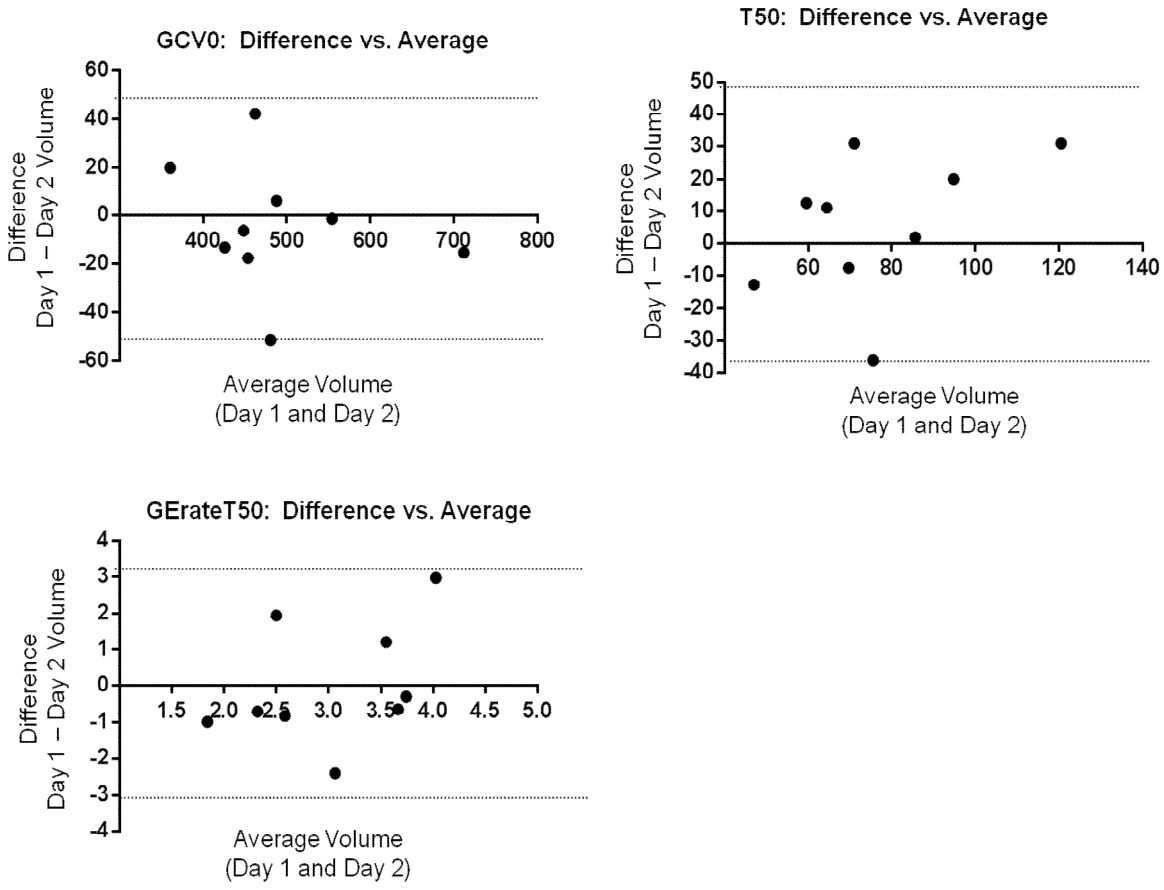


Figure 27. Bland-Altman Plots showing MRI TGV results from 9 HV imaged on two separate occasions after ingestion of liquid test meal; Panel A: GCV0, Panel B: T50 Panel C: GErateT50. MRI volumes are represented by • with the 95% C.I. represented by the dotted line.

4.3.6.4 Effect of adding solids to test meal on liquid gastric emptying

GS and MRI study days were repeated for the liquid and mixed solid/liquid test meal in 11 HVs. The mixed test meal was always performed after the liquid meal. For GS the study days were separated by an average of 334 days SD 77 days (95% C.I. Upper 385 days, lower 282 days). The MRI study days were separated by

an average 358 days SD 76 days (95% C.I. Upper 385 days, lower 282 days).

Bland-Altman plots demonstrate more rapid gastric emptying for the liquid component of the mixed meal if 12 agar beads without nutrient value are added to the NTM. GS GCV0 bias was -29 ml (95% C.I. Upper 46 ml, lower -103 ml), T50 bias -5.0 min (95% C.I. Upper 38, Lower -48 min) and the GErateT50 bias was -0.1 ml/min (95% C.I. Upper 3.5 ml/min, Lower -3.6 ml/min). There was a significant difference in GCV0 between the liquid meal and mixed meal (P=0.032). However, there was no significant difference between the late phase emptying T50 and GErateT50 (P=0.474 and P=0.903 respectively).

The MRI (GCV) GCV0 bias was -31 ml (95% C.I. Upper 60 ml, Lower -122 ml), T50 9 min (95% C.I. Upper 49 min, Lower -30 min) and the GErateT50 bias was 0.06 ml/min (95% C.I. Upper 1.9 ml/min, Lower -2.0 ml/min). There was significant difference between the liquid and mixed meal emptying with MRI GCV for GCV0 but not T50 or GE rate @ T50 (GCV0: P= 0.050, T50: 0.152, GErateT50 0.834)

The MRI (TGV) bias was -45 ml (95% C.I. Upper 138.6 ml, Lower -228.7 ml), T50 8 min (95% C.I. Upper 75 min, Lower -58 min) and the GErateT50 bias was 0.27 ml/min (95% C.I. Upper 2.5 ml/min,

Lower -3.0 ml/min). There were no significant difference between the liquid and mixed meal emptying with MRI TGV (GCV0: P=0.142, T50: P=0.440, GErateT50: P=0.540).

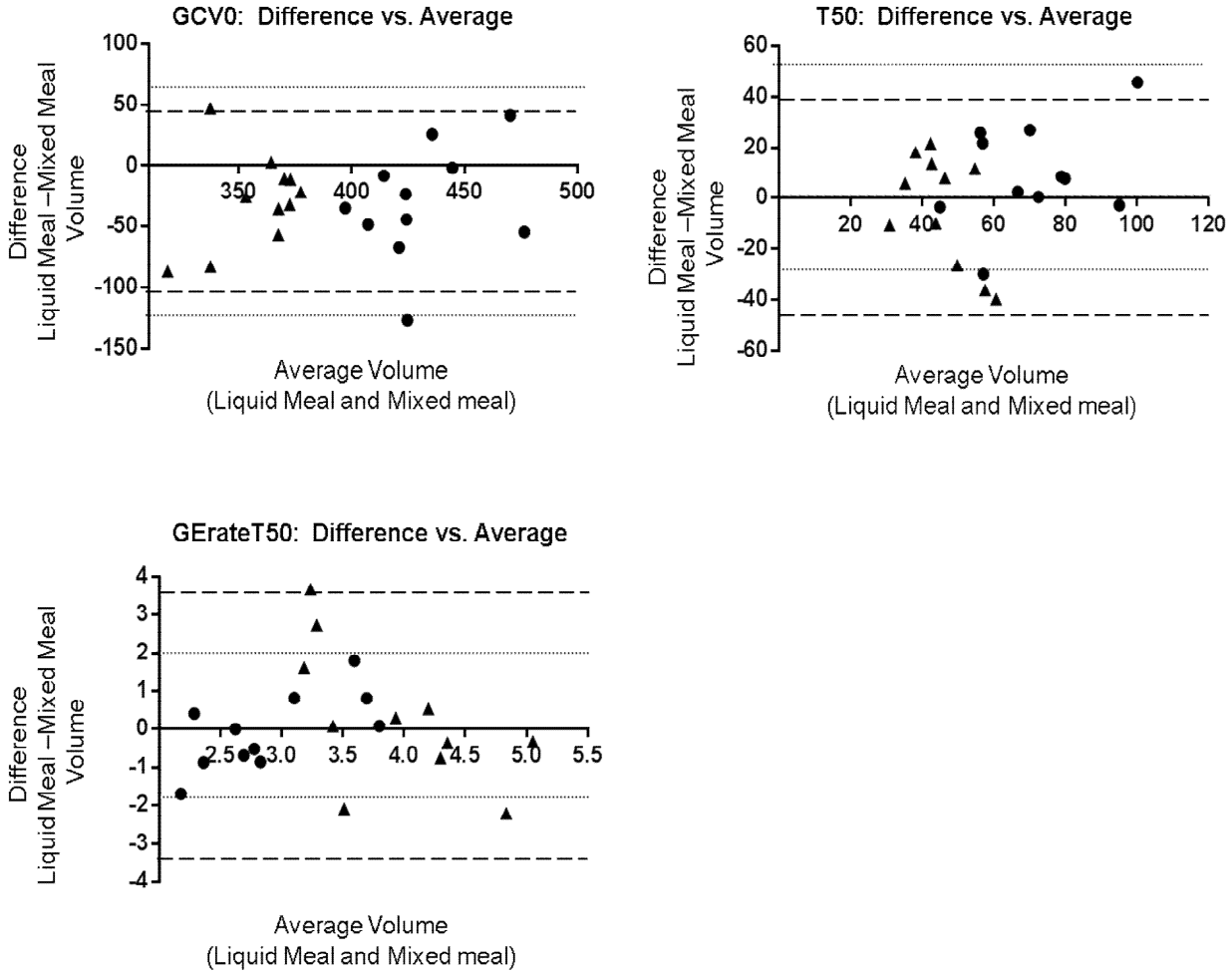


Figure 28. Bland-Altman Plots showing GS results and GCV for MRI with 11 HV imaged on two separate occasions after ingestion of liquid test meal and mixed test meal; Panel A**: GCV0,** Panel B**: T50** Panel C**: GErateT50. MRI volumes are represented by • with the 95% C.I. represented by the dotted line. GS volumes are represented by the ▲ and the 95% C.I. by the dashed line.**

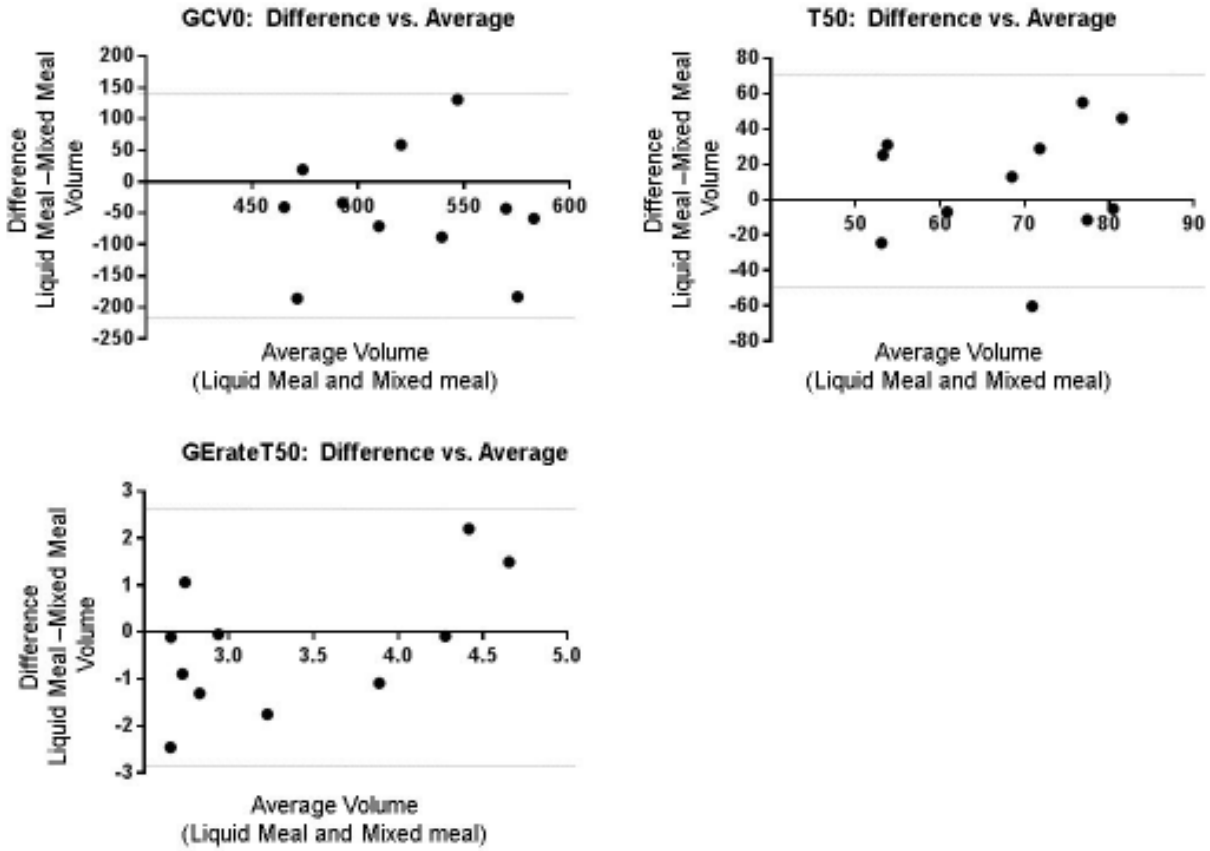


Figure 29. Bland-Altman Plots showing TGV MRI results from 11 HV imaged on two separate occasions after ingestion of liquid test meal and mixed test meal; Panel A**: GCV0,** Panel B**: T50** Panel C**: GErateT50. MRI volumes are represented by • with the 95% C.I. represented by the dotted line.**

4.3.6.5 Inter-observer agreement

Original image data of 10 HVs were analysed after ingestion of the liquid meal by three independent observers for both MR and GS study days. Results from the volume data fitted to the gastric emptying models were utilised to calculate the inter-observer agreement for the three key parameters of liquid gastric emptying.

For GS the raw data (percentage count at GCV0) was also calculated (Table 16)

The data comparing the number of beads counted by the observers is shown in Table 17 and it can be seen that the semi-automatic tracking program increased inter-observer agreement.

Table 16. Intra-class correlation between observers

		Intra-class Correlation	95% Confidence Interval	
			Lower Bound	Upper bound
	GCV0	0.830	0.501	0.954
	T50	0.990	0.970	0.997
MR	**GErate@T50**	0.977	0.932	0.994
	GCV0 (% Count)	0.764	0.308	0.936
	GCV0 (model)	0.687	0.084	0.915
	T50	0.960	0.884	0.989
GS	**GErate@T50**	0.897	0.700	0.972

Table 17. Intraclass correlation for bead counting

Agar bead count	Intraclass Correlation	95% Confidence Interval	
		Lower Bound	Upper bound
60 min Manual	0.727	-0.101	0.935
60 min Semi-automated	0.982	0.856	0.985
120 min Manual	0.976	0.904	0.994
120 min Semi-automated	0.999	0.997	1.000

4.3.7 Nutrient drink test vs test meal

The nutrient drink test was compared for fullness at 400ml to the same parameters in GS and MR for the liquid meal.

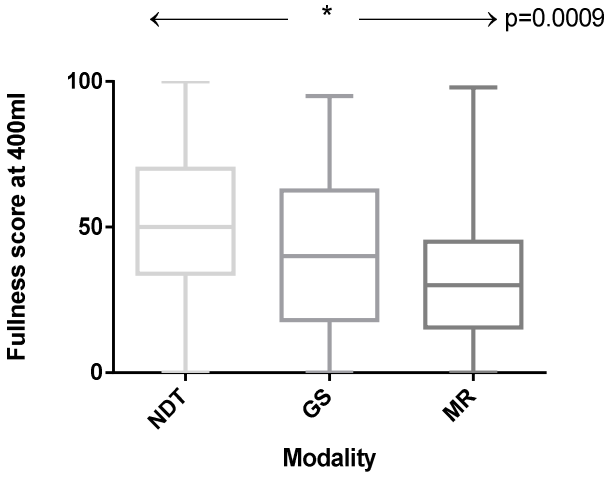


Figure 30. Nutrient drink test vs liquid meal for fullness at 400ml

Fullness scores were significantly lower for MRI in comparison to the NDT, and non-significantly lower in GS. The reasons for this are unclear. GS and MR were randomised as to order, but NDT was always first, as completed as part of the screening visit to ensure participants could drink at least 400ml required for the test meal. This could contribute to the higher scores. Most participants went on to drink far more at the NDT.

4.3.8 Comparison between health and disease

The above work has demonstrated the normal values and validation of the gastric emptying study, test meal and its

measured parameters. The healthy volunteer group have now been compared to patient groups.

4.3.8.1 Liquid study

4.3.8.1.1 Gastric emptying parameters – FD patients

Table 18. Values for functional dyspeptic patients gastric emptying parameters in liquid study

FD patients	MRI liquid		GS liquid N=8
	N=8	N=8	
	GCV	TCV	GCV
GCV0 (ml)			
Median	417.6	506.0	335.0
IQR	393.5-441.1	481.2-576.9	325.2-352.4
T50 (min)			
Median	75.45	74.10	48.75
IQR	59.25-96.77	59.08-95.55	40.03-52.28
GErate@T50 (ml/min)			
Median	2.012	2.868	2.960
IQR	1.809-2.652	2.173-3.611	2.664-3.050

One patient with a low R^2 (0.72) had values for all three parameters within the interquartile ranges.

4.3.8.1.2 Gamma scintigraphy

The 8 FD patients were compared to the same healthy volunteers as in demographics group. The symbol * denotes significance.

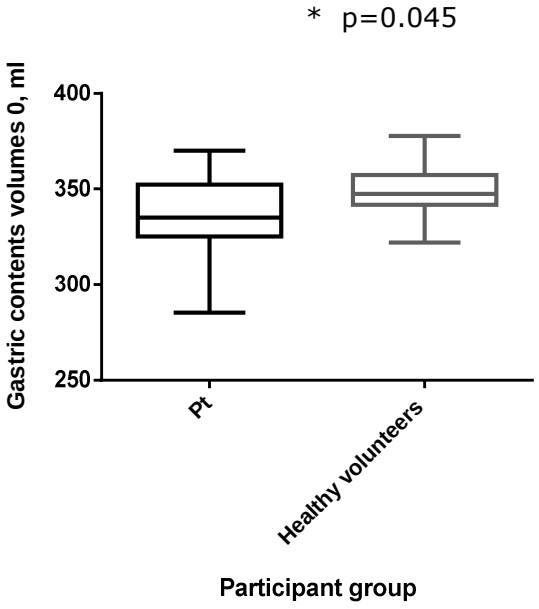


Figure 31. GCV0 in GS between participant groups.

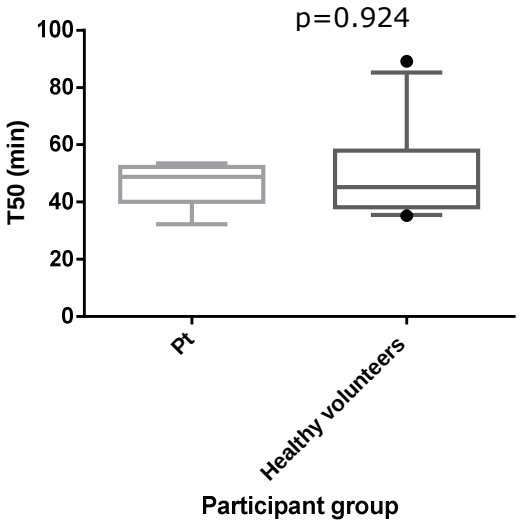


Figure 32. T50 in GS between participant groups.

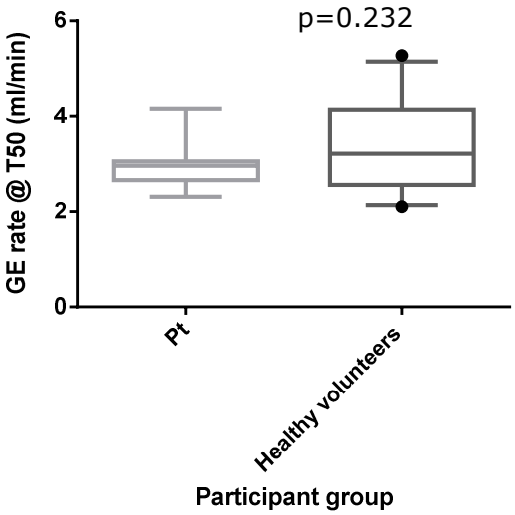


Figure 33. GE rate @ T50.

As can be seen in the graphs above, GCV0 was significantly lower in the patient group. T50 was similar. GE rate @ T50 was lower in the patient group but did not reach significance. This suggests significantly faster earlier emptying in the patient group with a trend towards slower later emptying. T50 provided no discrimination between groups. GE rate maximum was compared between the 2 groups, median was 4.325 ml/min and 4.578 ml/min in the patient and healthy volunteer group respectively, with no significant difference (p=0.640).

4.3.8.1.3 MRI

4.3.8.1.3.1 MRI gastric content volume (GCV)

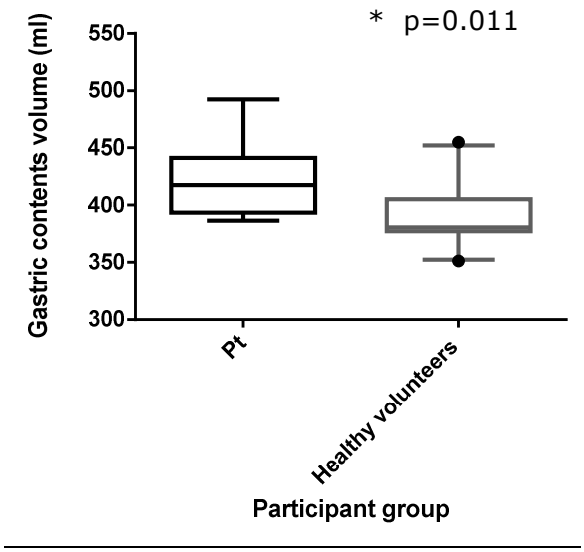


Figure 34. Gastric contents volume for MRI

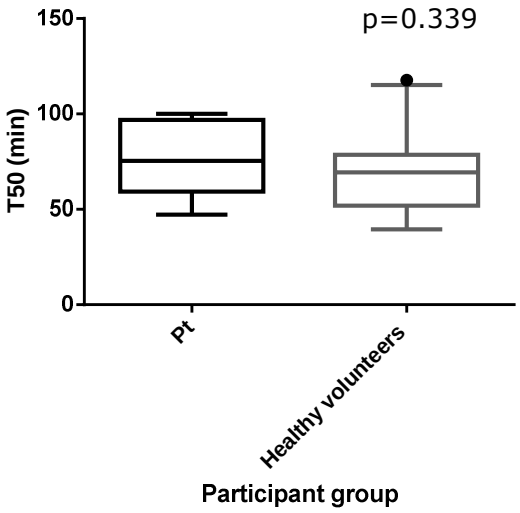


Figure 35. T50 for MRI contents

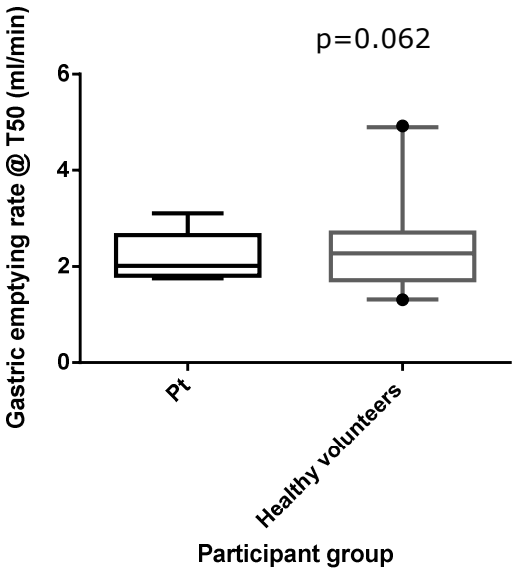


Figure 36. GE rate @ T50 for MRI contents.

GCV0 was significantly higher in the patient group (p=0.011). There was no difference between the patient and healthy volunteer group for T50 (p=0.339). There was no difference between groups for GErate @ T50 (p=0.062). There was no difference between groups for GE rate maximum (p=0.0351).

4.3.8.1.3.2 MRI total gastric volume

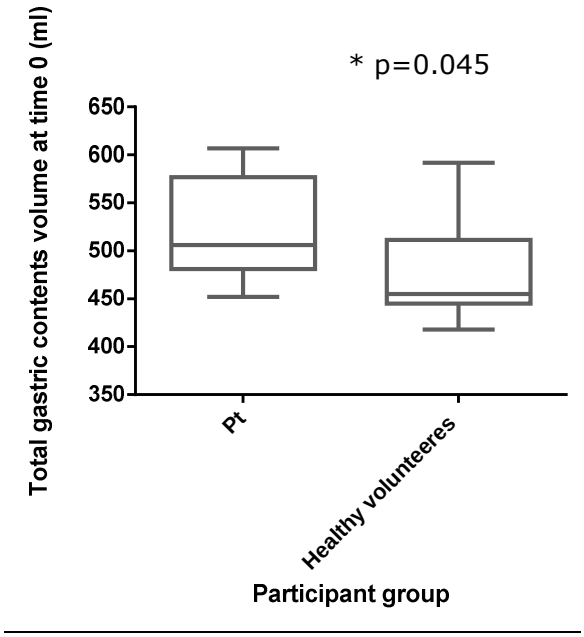


Figure 37. Total gastric contents volume for time 0 in MRI total gastric contents.

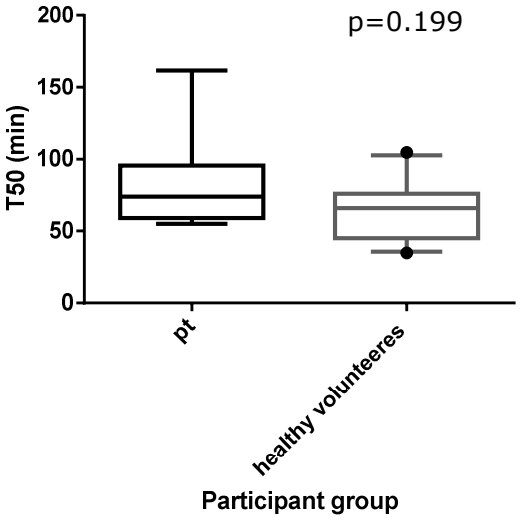


Figure 38. T50 in MRI total gastric volume

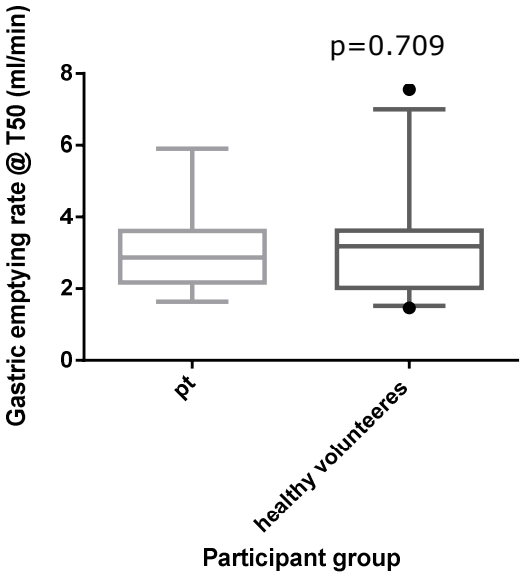


Figure 39. Gastric emptying rate @ T50 in MRI total contents

Total gastric contents volume at time zero was significantly higher in the patient group (p=0.045). T50, GE rate @ T50 and GE rate maximum were all similar between groups (p=0.199, p=0.709 and p=0.557 respectively).

The MRI results for gastric volume and total gastric contents volume followed the same trends within each group, with GCV0 being significantly higher in patients for both. However, GCV0 was significantly lower in patients in GS. The predominant difference between the two modalities is that GS can only measure the ingested labelled nucleotide, while MRI can measure additional features such as surrounding soft tissue and other gastric contents, such as secretions and air. When analysis of the MRI data took place for gastric contents volume only, the appropriate area of liquid within the stomach (ingested fortisip plus secretions) is

drawn around manually. The fortisip is highlighted by the labelling agent gadolinium. The whole of the liquid stomach contents is then highlighted for total gastric volume (liquid plus air). The addition of secretions may account for some of the differences in results between the 2 modalities. Separating the secretions from the fortisip to try and establish this is complex. Simple observation shows secretions can either be a separate layer on top or mixed within the meal. Therefore simple observation is not sufficient to differentiate the secretion and meal.

This is supported by 5/8 (63%) patients have GCV0 in MRI contents above 400ml (400ml was the ingested volume of labelled fortisip) suggesting secretions are being counted within the MRI gastric contents volume, while it is not possible to have a volume greater than 400ml in GS measurements.

However, this does not explain why GCV0 is greater in the patient population in MR but lower in GS unless the volume of secretions is greater in the patient population. As noted above, GCV0 is above the volume of ingested fortisip in 63% of patients. Of the matched healthy volunteers, 7/24 (29%) had GCV0 above 400ml in MRI contents. This does support the possibility that patients are producing more secretions.

The gastric contents volume of gastric contents and total gastric contents were compared between patients for MRI and healthy volunteers.

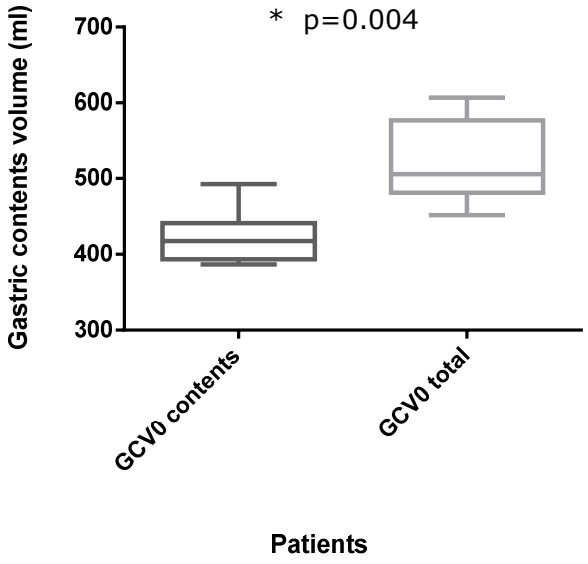


Figure 40. GCV0 in MRI contents and MRI total contents in patients

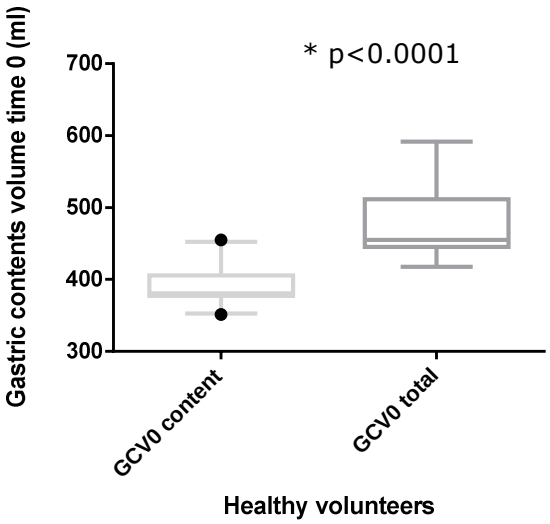


Figure 41. GCV0 in MRI contents and MRI total contents for HV

GCV0 is significantly higher in total gastric contents within the patient population, indicating higher values are recorded with the second measurement. This is also seen in the HV.

The patient GCV0 went up by 23% from GCV0 contents to GCV0 total, and increased by 22% in the matched healthy volunteers. It is interesting these are similar to one another. It suggests increase in *total* gastric volume is proportional to volume present at time 0. This is supported by previous MRI work in FD and HV. (178, 184)

The GCV0 in GS and MRI compared for the patient population. This is significantly greater in MRI.

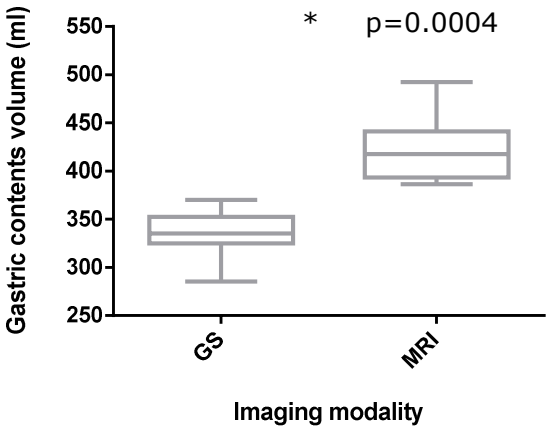


Figure 42. GCV0 for MRI contents and GS in patient population

The GCV0 was then compared for the same volunteers in GS and MRI contents who were used in the matched analysis with patients. GCV0 was significantly greater in MRI contents supporting secretions being the difference between modalities.

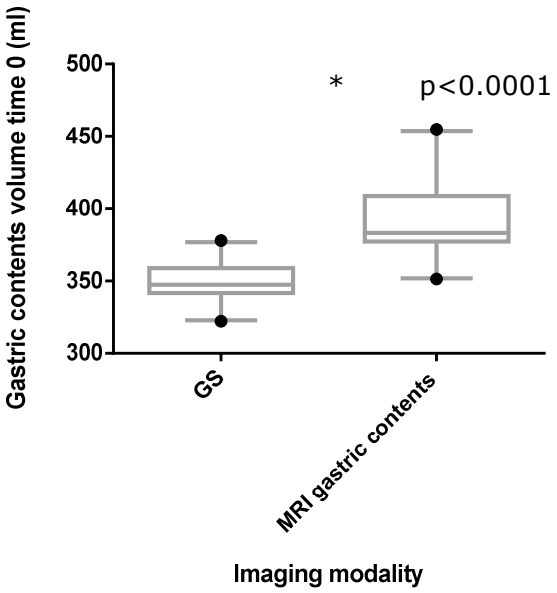


Figure 43. GCV0 for MRI contents and GS in Healthy volunteers

Baseline MRI scans were recorded for all subjects as part of the protocol. This provides a measurement of secretions for all participants before meal ingestion. The median value of baseline volume for HV (for the MR contents) was 11.76 ml and 19.35ml for the patients. There was no significant difference between the two groups baseline volumes, p=0.278. The difference between the median values for GCV0 for GS and MR in HV was 35.8ml and 82.6ml for patients. Therefore baseline volumes do not account for all the differences seen.

4.3.8.2 Mixed meal

4.3.8.2.1 Patient results

The mixed meal results have been split into 2 sections. An initial 8 FD patients who had full original protocol for mixed meal, followed by the further FD patients and GORD patients with the amendments described at the start of the results section.

4.3.8.2.1.1 Pilot FD patient results

Table 19. Values for functional dyspeptics gastric emptying parameters in mixed study

FD patients		GS mixed N=8	MR mixed N=8	
			GCV	TCV
GCV0 (ml)				
	Median	366.3	419.0	496.5
	IQR	343.5 - 382.7	370.2 - 443.7	464.3 - 674.3
T50 (min)				
	Median	72.30	77.45	69.65
	IQR	42.30 - 88.80	71.80 - 82.15	59.23 - 96.03
GE rate @T50 (ml/min)				
	Median	2.335	2.286	2.547
	IQR	1.767 - 2.955	1.391 - 2.667	1.845 - 3.845

2/8 FD patients had R^2 <0.9 in GS but none in MRI.

There was no significant difference between group in GS depending on R^2 value (GCV0; p=0.252, T50; p=0.395, GE rate @ T50; p=0.334).

4.3.8.2.2 Pilot FD patients versus healthy volunteers

4.3.8.2.2.1 GS

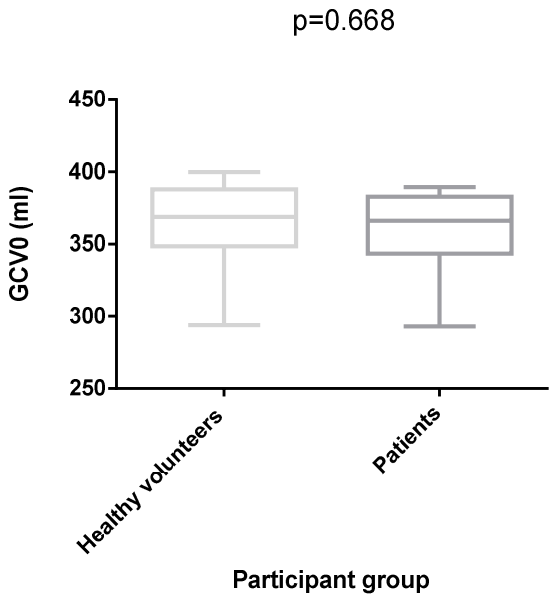


Figure 44. Healthy volunteers compared to patients in GS mixed meal; GCV0

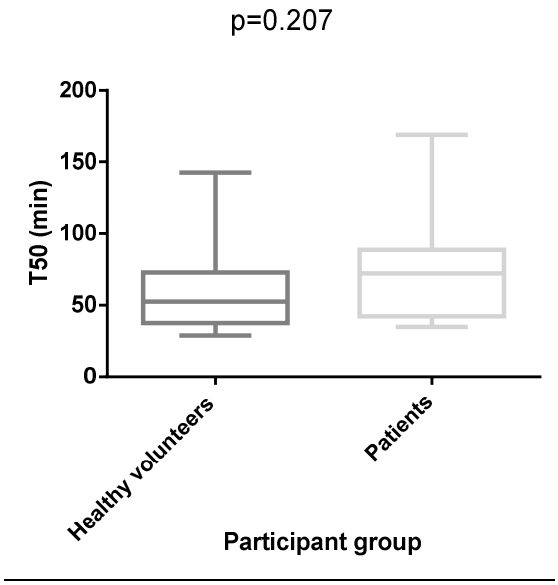


Figure 45. Healthy volunteers compared to patients in GS mixed meal; T50

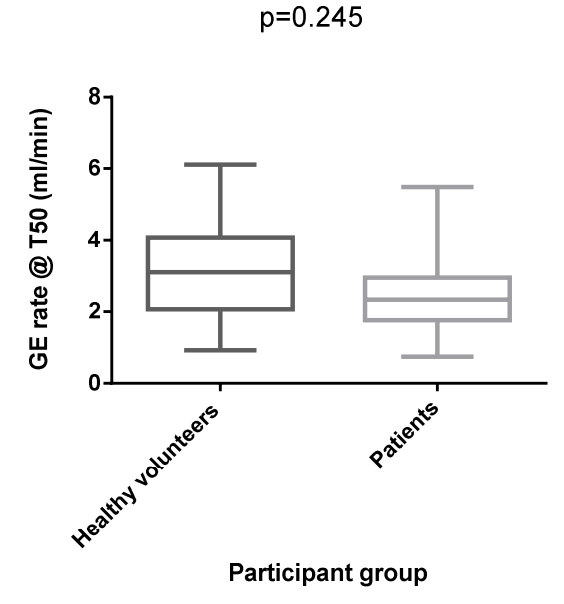


Figure 46. Healthy volunteers compared to patients in GS mixed meal; Ge rate @ T50

Comparisons between the FD patient and healthy volunteer group showed no significant difference between the two groups, GCV0

was very similar in the two groups, T50 non-significantly higher and GE rate @ T50 non-significantly lower in the patient group.

4.3.8.2.2.2 MR – gastric contents volume

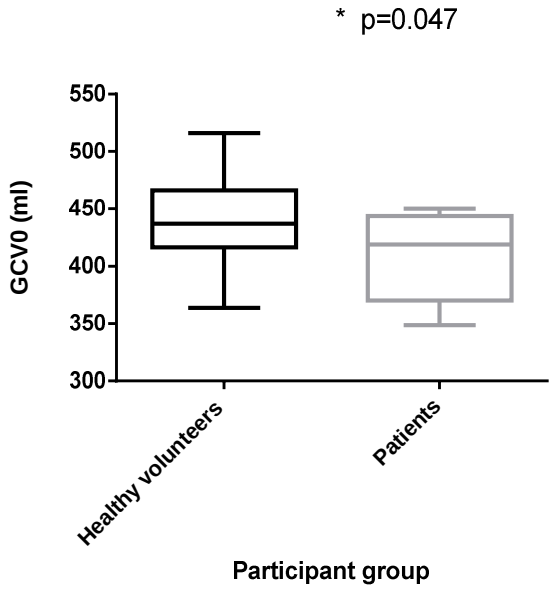


Figure 47. Healthy volunteers compared to patients in MR content; GCV0

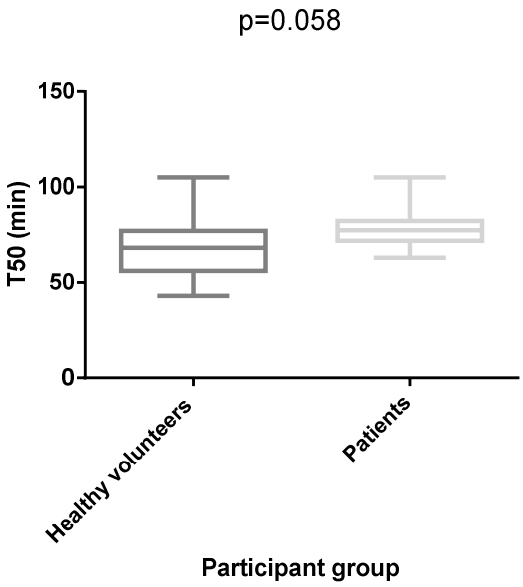


Figure 48. Healthy volunteers compared to patients in MR contents; T50

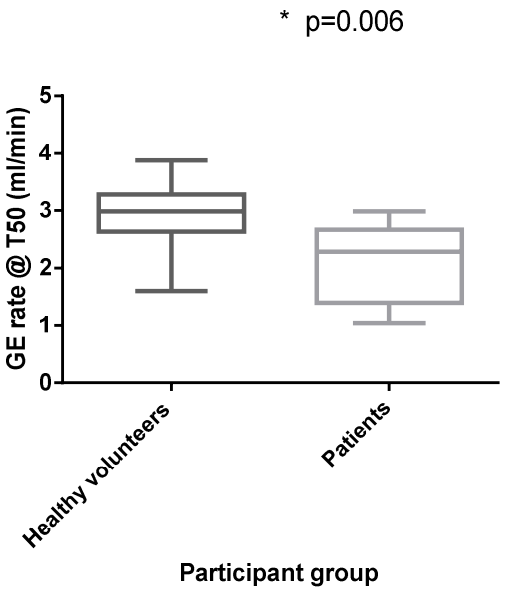


Figure 49. Healthy volunteers compared to patients in MR contents; GE rate @T50

GCV0 is significantly lower in the patient group, T50 non-significantly higher and GE rate @ T50 significantly lower in the patient group.

4.3.8.2.2.3 MR – total gastric volume

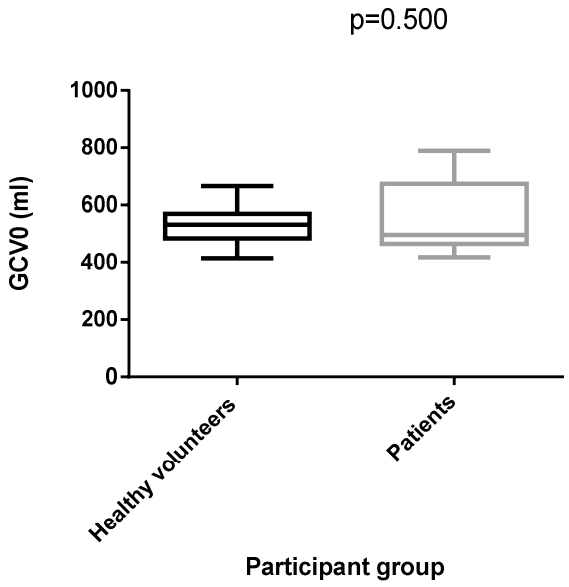


Figure 50. Healthy volunteers compared to patients in MR total; GCV0

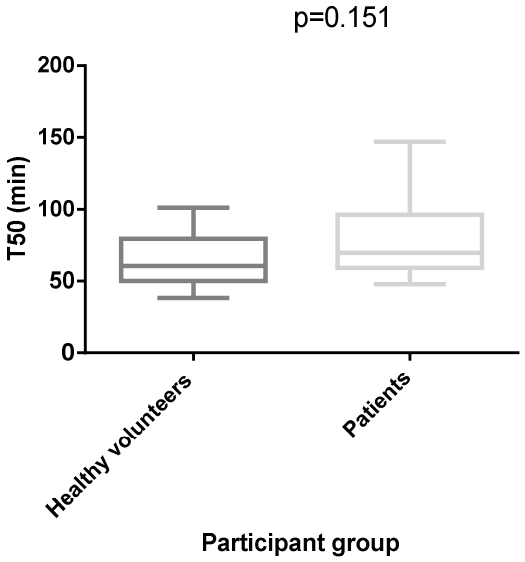


Figure 51. Healthy volunteers compared to patients in MR total; T50

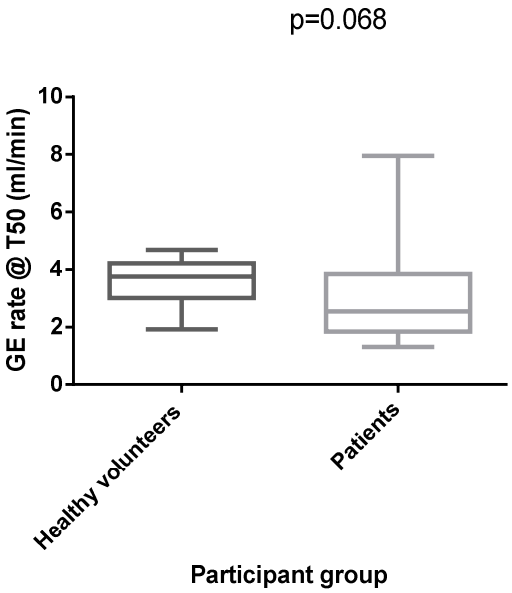


Figure 52. Healthy volunteers compared to patients in MR total; GE rate @ T50

There were no significant differences between the two groups in MR total but GCV0 trended to lower values in patients, T50 higher and GE rate @ T50 lower.

4.3.8.3 Comparison between liquid and mixed meal results

Table 20. Comparison of gastric emptying parameters in patient's vs HV in liquid and mixed meal

	Patient – liquid meal (n=8)	Patients – mixed meal (n=8)
GS		
GCVO	↓*	↓
T50	↑	↑
GE rate @ T50	↓	↓
MR content		
GCV0	↑*	↓*
T50	↑	↑
GE rate @ T50	↓	↓*
MR total		
GCV0	↑*	↓
T50	↑	↑
GE rate @ T50	↓	↓

*- denotes significance

The results for the patients versus healthy volunteer groups have been compared between the liquid and mixed meal. The arrows indicate whether the patients results are higher or lower than the HV group and * indicates significance.

GS follows the same trends for both meals. The only parameter that varies between MR for both contents and total volume is GCV0. GVCO is higher in the patient group for the liquid meal (when compared to the HV). This is inconsistent with the liquid meal. To further examine this result, the two sets of 8 patients were compared for GCV0 MR content in the mixed and liquid meal. There was no significant difference between the two patient groups, p=0.511. However the two sets of 24 HV volunteers were compared for GCV0 MR contents liquid versus mixed and a significant difference was found, p>0.001. The mean (±s.d.) of GCV0 for the HV liquid meal was 390.2 (±27.24) and 442.2 (±37.68) for the HV mixed. That's a difference between the two means of 52ml.

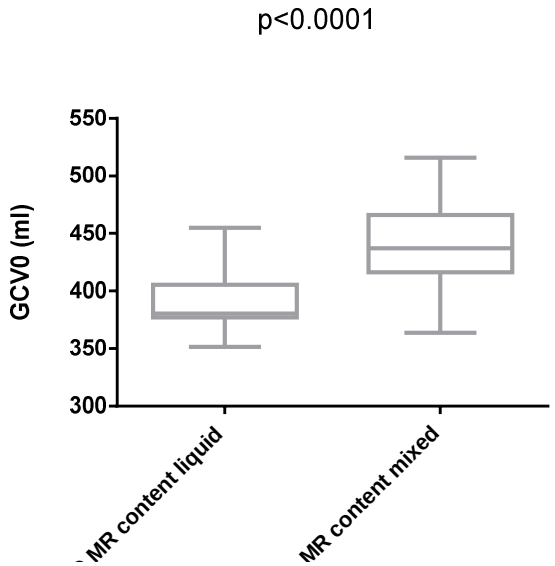


Figure 53. HV compared for GCVO MR content between liquid and mixed meal

Because of the above results, the GCV0 were compared for the HV in MR total gastric volume. The results were significantly higher in the mixed meal group, mean 529ml (±62.9ml) compared to 476ml (±50.0), p=0.002. The effect was still seen when all the HV from MR content from the liquid study were compared to those in the mixed study (p=0.008). This excluded a sampling error from the matched volunteers in the liquid study as a cause for the differences.

The most obvious possible explanation for this is the presence of the beads. This would be more prominent in the MR as the beads in GS are labelled with an alternative isotope so shouldn't be counted in liquid part of study. There are 12 beads present in the mixed meal of 11.5mm diameter. The volume of one bead is ~0.8 cm^3, a total of 9.6cm^3 for all beads. This equates to approximately 9.6ml. This could potentially account for some of the differences seen between the healthy volunteer groups. However, the simple volume of the beads present does not account for all. The beads are less likely to affect the parameters measured in GS as they are labelled with a separate isotope, so measured differently to the liquid part of emptying. This also supports the beads are the cause of the differences seen between the two meals.

Interestingly, its effect is not seen on the emptying parameters T50 and GE rate @ T50. This could be that the beads presence

have most effect on early emptying and have been titrated at the later point of emptying.

The same pattern was not seen in the patients – this could be due to different meal distribution within the patient population or the lower number of patients within the study groups.

4.3.8.4 Mixed meal – additional patient groups

As previously described, further FD patients and GORD patients were studied, under the amendments as described at the start of the results section.

4.3.8.4.1 Gamma scintigraphy

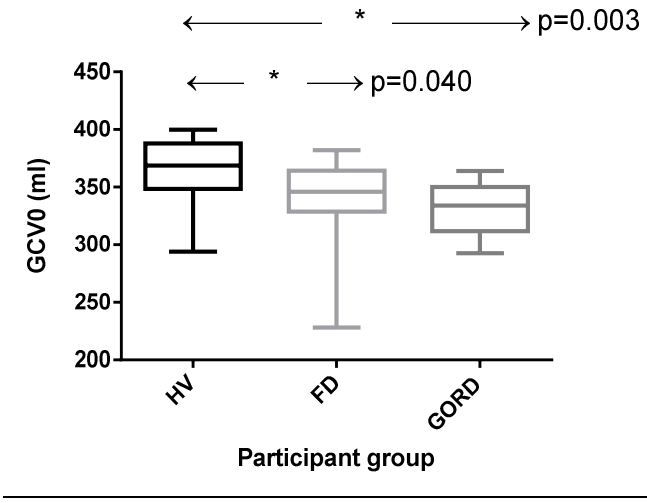


Figure 54. Comparison of healthy volunteers, FD patients and GORD patients for GCV0 in mixed meal with no beads in patients groups

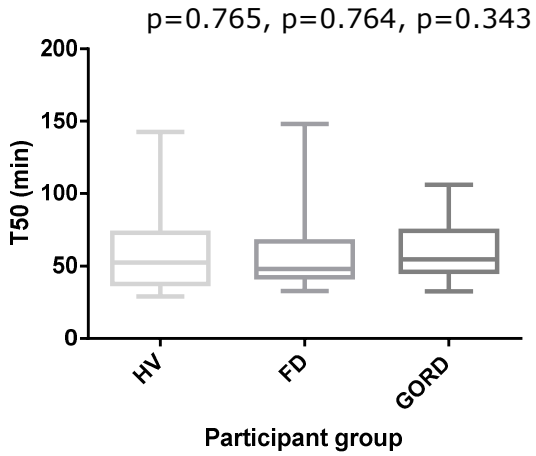


Figure 55. Comparison of healthy volunteers, FD patients and GORD patients for T50 in mixed meal with no beads in patients groups

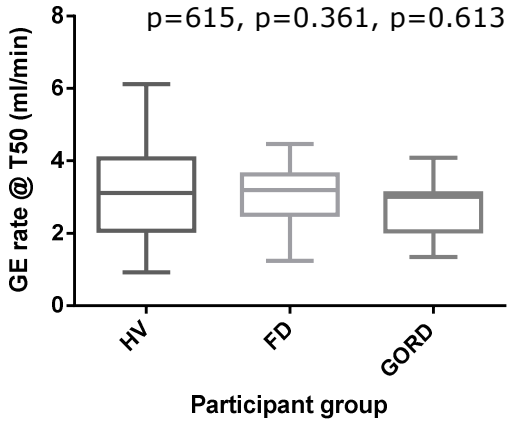


Figure 56. Comparison of healthy volunteers, FD patients and GORD patients for GE rate @ T50 in mixed meal with no beads in patients groups

GCV0 is significantly lower in HV vs FD and HV vs GORD. GCV0 is lower in FD patients and lower again in GORD patients although there is no significance difference between the two patient groups.

4.3.8.4.2 MR – gastric contents volume

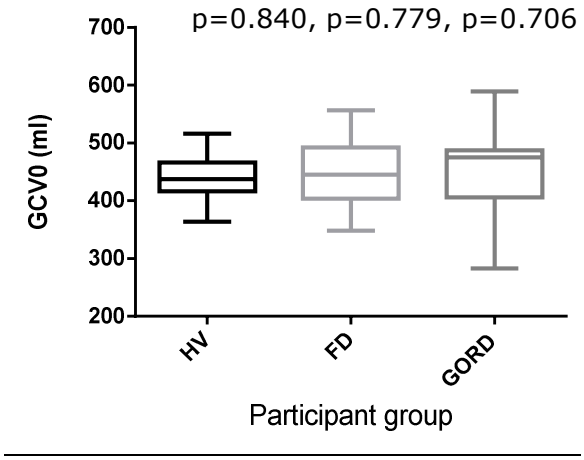


Figure 57. Comparison of healthy volunteers, FD patients and GORD patients for GCV0 in MR contents

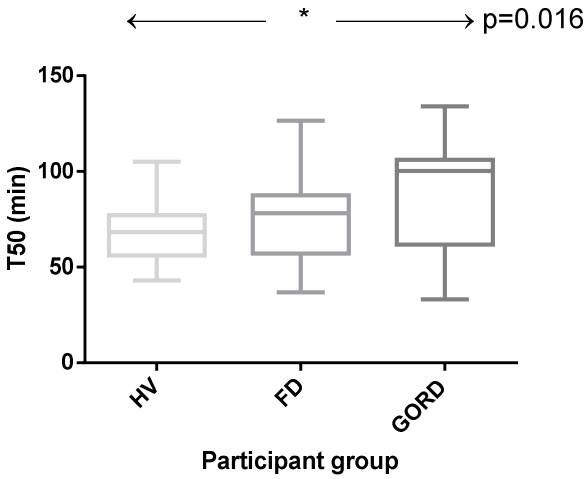


Figure 58. Comparison of healthy volunteers, FD patients and GORD patients for T50 in MR contents

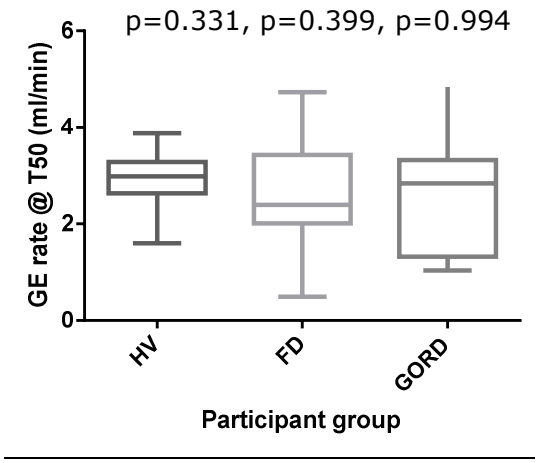


Figure 59. Comparison of healthy volunteers, FD patients and GORD patients for GE rate @ T50 in MR contents

The only significant difference is between the HV and GORD patients for T50, with T50 significantly higher in the GORD patients.

4.3.8.4.3 MR – total gastric volume

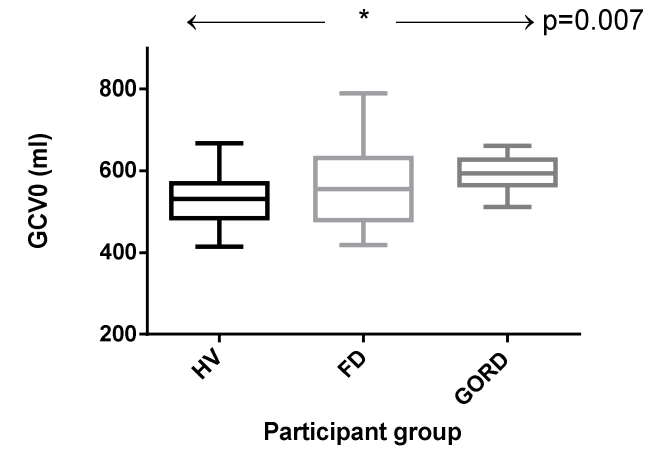


Figure 60. Comparison of healthy volunteers, FD patients and GORD patients for GCV0 in MR total gastric contents

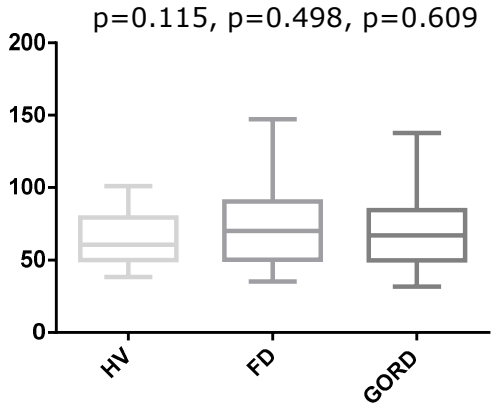


Figure 61. Comparison of healthy volunteers, FD patients and GORD patients for T50 in MR total gastric contents

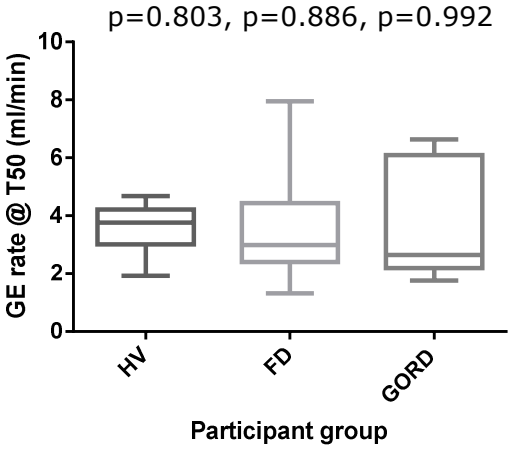


Figure 62. Comparison of healthy volunteers, FD patients and GORD patients for GE rate @ T50 in MR total gastric contents

The only significant difference is between the HV and GORD patients for GCV0, with GCV0 being significantly higher in the GORD patient group.

4.3.8.5 Solid emptying rates

Solid emptying retention rates were compared for the patients and HV who has beads present, at time points 60 minutes (T60) and

120 minutes (T120). For GS this was 8 patients due to the previously mentioned amendment and concerns regarding dual isotope study. The full MR cohort had beads present (including the GORD patients) as there are no issues with isotope counts. Results are displayed as % of beads retained. Again, this is intact beads for MRI and any beads (fragment+whole) in GS.

4.3.8.5.1 GS – solid emptying

Table 21. GS solid retention rate

		T60 (%)	T120 (%)
HV	Median	80	65
	IQR	74-86.5	42-79.3
FD	Median	69	59
	IQR	47.5-85.3	31.5-82.3

There was no significant difference between the groups for T60 ($p=0.215$) or T120 ($p=0.772$).

4.3.8.5.2 MR – solid emptying

Table 22. MR solid emptying retention rate

		T60 (%)	T120 (%)
HV	Median	58.3	20.8
	IQR	41.7-83.3	2.1-50
FD	Median	83.3	41.7
	IQR	58.3-100	12.5-83.3
GORD	Median	75	41.7
	IQR	50-100	25-75

There was again no significant difference between the groups.

4.3.8.6 Nutrient drink test results

At each screening visit a nutrient drink test was performed with maximum tolerated volume recorded. This was the same for both the liquid and mixed study.

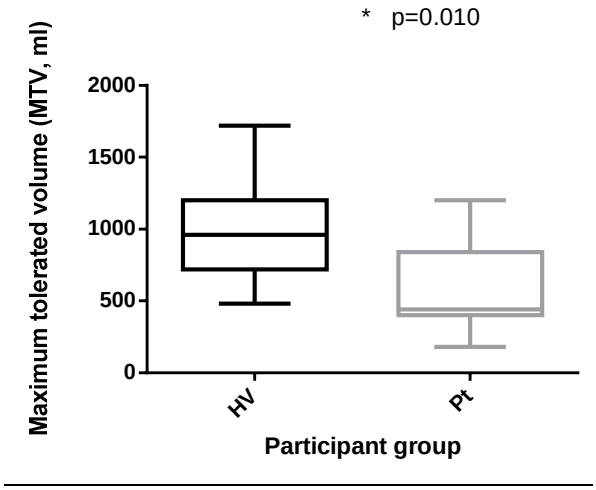


Figure 63. Maximum tolerated volume at nutrient drink test between healthy volunteers and FD in liquid study.

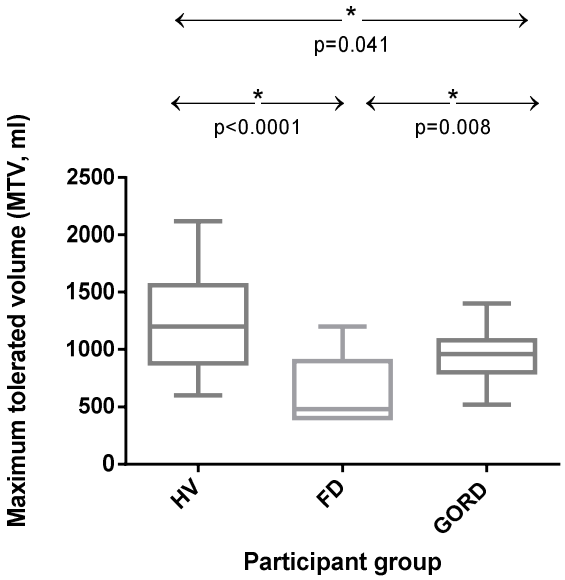


Figure 64. Maximum tolerated volume at nutrient drink test between healthy volunteers, FD and GORD patients in mixed study.

As can be seen, the FD patients drank significantly less than the healthy volunteer and GORD groups. The GORD patients also drank significantly less than the HV, suggesting some overlap between the two conditions.

4.3.8.7 VAS scores

Fullness at 400ml (i.e. completion of the test meal) was compared for the patients and HV's. Fullness score in GS was significantly higher in the FD in comparison to both the HV (p=0.0008) and GORD (p=0.001). There was no difference between the HV and GORD patients (p=0.594). The results followed the same trends for MR (HV:FD p= 0.028, HV:GORD p=0.418) but there was no significant difference between the GORD and FD (p=0.229) although the FD's tended to have higher scores.

Table 23. Vales for fullness at 400ml in mixed meal

HV	GS	MRI
Median	28	30
IQR	15-56	12-50
FD		
Median	70	70
IQR	48-100	25-93
GORD		
Median	40	40
IQR	30-46	30-50

4.4 Discussion

This research describes the development, validation and application of a gastric emptying study in a healthy volunteer population with pilot clinical data in patient groups with functional

dyspepsia (FD and gastro-oesophageal reflux disease (GORD). Both a liquid (nutrient drink) and an optional solid (non-nutrient agar bead) component are tested to provide a modular test of gastric motor function. Patient symptoms were assessed to test gastric sensory function as well.

4.4.1 Demographics

Healthy volunteers were collected in age/sex stratified blocks. A total of 27 men were recruited and 26 women were recruited and the average age was 44.6 years (min 18.3 – max 78.4) in the liquid study. In the mixed meal there were 13 men and 11 women with a mean age of 47.7 years (min 20.1 and max 69.2). Within the patient population, the majority (7/8 in liquid study, 12/17 of mixed,) of FD patients were female consistent with current literature (206). Conversely the majority (9/11) GORD patients were men, consistent with reflux being more common in men(207). Mean age of the FD patients was 50.2 in the liquid study and 40.5 years in the mixed. Mean age of the GORD patients was 40.7 years.

FD patients had a significantly lower BMI than the HV As we studied a population of FD with post-prandial distress this was expected (208). Conversely GORD patients tended to have higher BMI, consistent with literature that GORD is associated with obesity(207)

4.4.1.1 Liquid study

The normal values of gastric emptying for both liquid and mixed meal were established and validated within the healthy volunteer population. Normal values were provided for both GS (gamma scintigraphy) (meal volume) and MRI (gastric contents volume (GCV) and total gastric volume(TGV)).

Gastric contents volume at time 0 (GCV0) was significantly lower in GCV and TGV in MRI than GS, as was emptying at time taken for half meal to empty (T50). Gastric emptying rate @ T50 (GE rate @ T50) was significantly higher in GS for MRI GCV and non-significantly higher in TGV. It should be noted that the residual volume of secretion observed in the stomach on MRI prior to meal ingestion was small (median >20ml) and not sufficient to explain the difference in volume recorded by the two modalities. The dynamic change in gastric volume over time was different for GS and MRI studies. Typically, as described by previous authors (209), gastric emptying of a liquid nutrient meal documented by GS is linear or exponential and the mathematical model that we applied to describe this data was designed to fit such data. Conversely gastric emptying of a liquid nutrient meal documented by MRI shows a characteristic rise in volume or apparent lag phase (i.e.no change in volume) after ingestion due to the rapid production of gastric secretions (178). This is then followed by a linear or

exponential reduction in volume as the contents leave the stomach. This pattern of gastric volume change after meal ingestion required an additional term (kappa, see methods) to be added to the model of gastric emptying that we used to describe the data and generate measurements.

It follows that the volume in the stomach immediately after completing the meal (GCV0) is lower in GS (75^{th} percentile of GS lower than the 25^{th} percentile of both MRI GCV) because GCV measured by MRI includes meal and secretion volume (plus some residual). For the same reason the gastric emptying half time T50 is faster in GS than MRI since only emptying of the meal and not meal and secretion is documented. The GE rate @ T50 is remains slightly faster in GS than MR. This again is most likely due to the ongoing production of secretions.

Fullness at 400ml on VAS score was slightly lower in MRI than GS, although this was non-significant. It is well known from previous MRI studies that fullness closely follows changes in GCV in healthy subjects (210, 211). Obviously although the measured gastric volume by GS was lower than that for MRI this has no effect on the actual gastric content volume (meal and secretion) or total gastric volume. Thus, no difference in fullness would be expected based only on imaging modality.

4.4.1.2 Mixed study: liquid component

Overall comparison of measurements obtained by the two modalities followed the same pattern as for the liquid meal. GCV0 as significantly lower in GS than MRI but other parameters were non-significantly different.

Comparing the fit results for liquid and solid, the fit for GS in the mixed meal liquid component for HV is not as good as for the liquid only, with 18/24 having a r^2>0.9 compared to 52/53. This was not, however, directly related to the presence of the agar beads, but rather due to dual-labelling of liquid and solid components. In the liquid study 12MBq of ^{99m}Tc DTPA were used. In the mixed meal 0.5 Mbq of In-lll was used to label the liquid component (the beads were labelled with 5MBq of ^{99m}Tc MAA). The relatively low dose of the liquid in the mixed meal was an attempt to minimise the radioactive exposure to participants (dual isotope study) but lower recorded counts are more susceptible to measurement error as small changes in the absolute counts have a greater effect on the estimated volume. For further studies it was decided to focus on liquid emptying for two reason i) because we wanted to keep exposure to radiation to a minimum in our often young and female FD patients ii) because, in contrast to liquid emptying, we had no difference in gastric emptying of solids between groups.

4.4.1.3 Solid component

Solid emptying retention rate appeared to be lower in MRI than in GS. This is because MRI measures presence of intact beads in the stomach and, therefore, the work done by the stomach in breaking down solid beads over time. In contrast GS measures the rate at which the solid material leaves the stomach which is a two stage process.

The agar beads were 11.5 mm in size and it is known that particles larger than 3mm are unlikely to pass the pylorus and leave the stomach intact (gastric sieving) (212). Thus MRI measures work done to break down the beads and GS measures the time is takes for the beads to break down and leave the stomach. This two-step process will clearly last longer being an integrated function of the time taken to break down the beads into tiny fragments and for these to be emptied into the small bowel.

4.4.1.4 VAS scores

Vas scores are similar for the two modalities. This is not surprising as the combined volume of the agar beads was only about 10ml and this is not sufficient to alter gastric stretch or tension.

4.4.2 Validation of test meals

4.4.2.1 Reproducibility – liquid study

A number of healthy volunteers underwent the liquid meal twice (n=9) and the mixed liquid and solid meal twice (n=11). GS and MRI were performed in randomised order on both occasions.

GCV0 and GE rate @ T50 were both faster on the second day for GS, but these differences were small with an average difference 7.5ml for GS in GCV0 (not significant) and 0.9ml/min for GE rate @T50 (p=0.036). The opposite was observed for MRI with an average difference of -25ml for GCV0 (p=0.050) and -0.2ml/min for GE rate @ T50 (not significant). These findings are within the documented day-to-day variation and are at the limit of resolution for individual studies. Further no sequence effect was seen when the order of study days (GS, MRI) was considered.

Although the effects are small and could be due to random variation it is interesting that the comparison between the repeated study days showed opposite effects on day 1 and day 2 in GS and MRI. It has previously been recognised that stress delays gastric emptying (213) but increases gastric secretion (214). One could speculate that an interaction between the imaging modality and these effects could explain the unexpected findings. You would anticipate that participants would feel more nervous before their first imaging day as unsure of the day's events, consistent with the

findings for GS. However, this did not occur for MRI. It may be that MRI is a more difficult and a less "physiologically normal" process for the individuals. It is already well known that MRI is poorly tolerated in some individuals (215). Consequently, the anticipation of MRI on the second visit may have been greater leading to the differences between MRI and GS study days.

4.4.2.2 Liquid – mixed meal differences

Bland Altman between 11 HV showed more rapid early emptying (GCV0O of the liquid component of the mixed meal (GS -29ml, MRI GCV -31ml and MRI TGV -45ml). No difference was seen in T50 and GErate @ T50.

The 24 HV who underwent the mixed meal were compared to the 24 matched HV in the liquid study. GCV0 was significantly greater in mixed meal results for MRI GCV and GS (t-test/Mann Whitney U test).

Comparisons are difficult to be drawn between two different populations who had different statistical tests.

4.4.2.3 Inter-observer variability

Intra-class correlation was 0.83 and above for all three parameters that describe liquid gastric emptying in MRI. Agreement was lower in GS with intra-class correlation varying between 0.687 – 0.960 for the gastric emptying parameters.

MR has less variability between observers than GS for all three gastric emptying parameters. Both methods require the individual analysing results to draw round the ingested labelled fortisip. As MRI also visualised the surrounded tissue (unlike GS), it is easier to recognise a distinct outline of the ingested meal. A comparison of GS and MR that demonstrates the spatial resolution available from both modalities is included below.

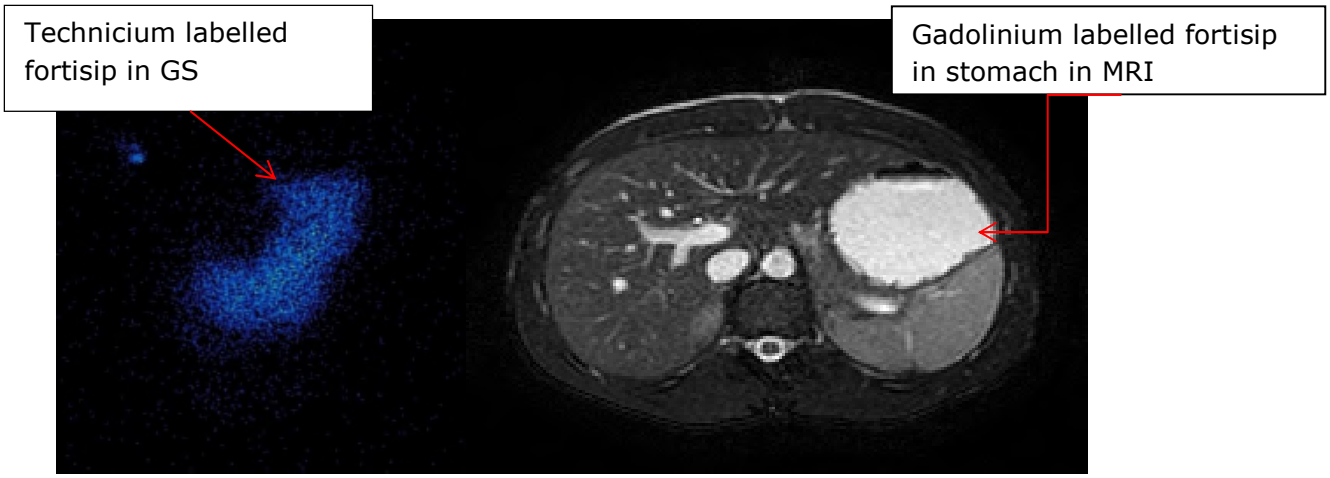


Figure 65. GS image (left) and MRI slice right.

A semi-automated tracking program was used within the MRI model for the beads. This improved the intra-class correlation between the observers. Agar bead count at 60 minutes correlation increased from 0.727 to 0.982 and from 0.976 to 0.999 at 120 minutes with the additional program.

4.4.2.4 Sensation within modalities

Fullness was non-significantly higher in GS than MRI and was significantly higher in the NDT than MRI. The NDT was always the first performed, as part of the screening visit. Stress is known to delay gastric emptying (213) and the participants were taking part in the study for the first time during the NDT. It is conceivable that they may be more nervous/anxious about the first visit. They frequently went on to drink much more than 400ml. However, it is surprising that MRI fullness was the lowest. The MRI study day is potentially considered the more "stressful" of MRI/GS and the least physiological as MRI is performed lying down. It may be the altered position and therefore altered position of fluid within the stomach affects fullness. Previous studies have shown in health and FD that fullness is more closely related to antral distention (103, 216). The gastric contents may lie more proximally when lying in comparison to upright and could explain some of the differences seen.

4.4.3 Health versus disease: liquid study

4.4.3.1 GS

GCV0 was significantly lower in the FD population with no significant difference between T50 and GE rate @ T50 indicating more rapid early gastric emptying. As this is phase of emptying

occurs during ingestion of the meal itself (takes place over 10 minutes), this is thought to be representative of receptive accommodation of the proximal stomach.

As discussed earlier, the primary responsibility of the fundus is to act as a reservoir when food is delivered into the stomach, allowing initial mixing of contents with pepsin and hydrochloric acid. Delivery of the meal into the stomach leads to reflex relaxation (reduction in tone known as accommodation) which then gradually recovers. Liquid emptying is driven by a gastro-duodenal pressure gradient, generated by tonic fundal contraction, with the rate of emptying regulated by the pylorus (217). Initially, during early gastric emptying, the process is driven by mechanical factors (i.e. volume ingested). Later, during the majority of gastric emptying, fundic tone and emptying rate are also modulated by nutrient feedback (following nutrient delivery into the duodenum). Thus in the early phase, non-nutrient and nutrient liquids empty at the same rate; however, in the later phase non-nutrient empty faster than nutrient liquids (21).

Previous work in functional dyspepsia has identified impaired accommodation in at least 40% of patients (37). The lower GCV0 on our patient group within is most likely representative of impaired receptive accommodation. FD patients have also been shown to be hypersensitive to antral distension and exhibit reduced

fundic relaxation in response to antral distension when compared to healthy volunteers (216). The results of this study are consistent with the published hypothesis that impaired accommodation of the proximal stomach leads to relative distension of the antrum and rapid early emptying of liquids into the duodenum. This could account for many of the post-prandial symptoms in the FD population. Also it provides a non-invasive biomarker of gastric dysfunction that could be amenable to specific therapy.

The physiology of gastric accommodation is complex. Gastric accommodation and tone are vagally mediated. Both nitric oxide (NO) and vasoactive intestinal polypeptide (VIP) inhibit smooth muscle tone, leading to reduced gastric fundal tone (218, 219). NO produces these effects by causing the formation of cyclic guanosine-3',5'-monophopshate (cGMP) via soluble guanylate cyclase (220, 221). This has been supported by nitric oxide inhibitors leading to reduced fundal relaxation post-prandially in healthy volunteers (221). Also, the cGMP phosphodisesterase inhibitor, sildenafil, which reduces degradation of cGMP has been shown to increase fasting intra-gastric volumes and reduce liquid emptying (194). This supports the findings in this study that the FD population had faster early liquid emptying due to impaired accommodation. Sildenafil's potential therapeutic activity in gastric

accommodation and emptying has only been completed in a small group of healthy volunteers with no FD patient studies to date.

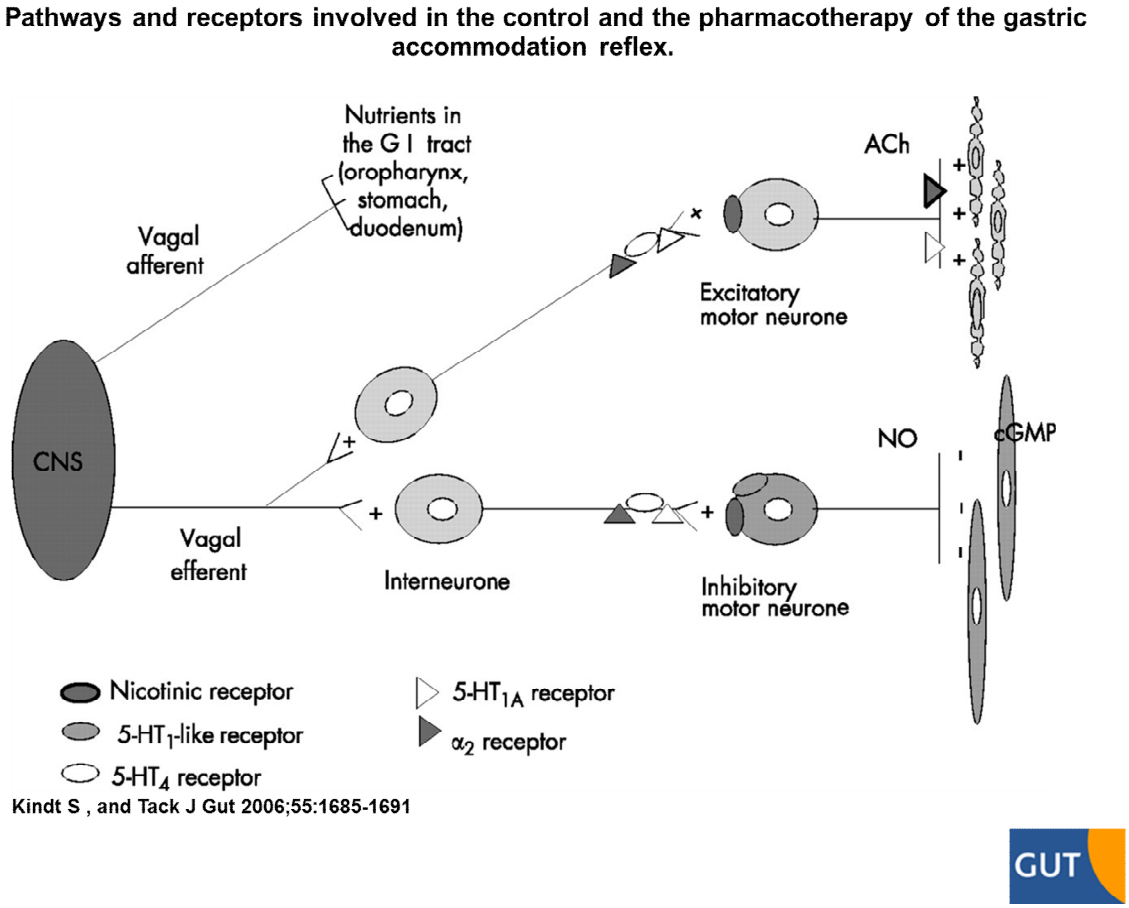


Figure 66. The gastric accommodation reflex – reproduced from Kindt S and Tack J. Gut. 2006: 55: 1685-1691

Excitory neurones are also involved in accommodation via their effects on cholinergic pathways. α_2-adrenorecpetors and 5-HT_{1A} receptors (which are inhibitory) have been found on these neurones (222). Studies using clonidine (223) (a α_2-adrenorecpetors agonist) and buspirone (a 5-HT_{1A} receptor agonist) have both been found to improve accommodation to a meal (224). Buspirone was given to 17 FD patients, in a blinded cross-over

design with placebo, for a 2 week period. It increased gastric accommodation and delayed emptying time of liquids (although not solids). These results support the hypothesis that the lower GCV0 seen in our FD population is due to impaired accommodation which subsequently causes increased initial liquid emptying.

If initial emptying is faster in FD, this can result in the early nutrient delivery into the small bowel, which exerts a negative feedback on further gastric emptying and accommodation. This mechanism has been described previously and is sometimes referred to as the "duodenal brake" (23). Neuro-hormonal and mechanical factors are important in activating this. The digestive hormone CCK seems to play a significant role. In studies looking at this, a non-nutrient water load was given to healthy volunteers 1 hour after a meal and was found to stimulate CCK release and decrease antral motility (225). The authors hypothesised that this is due to the remaining fatty chyme in the stomach being flushed through into the duodenum by the water. This is supported by other work showing that a selection of lipids infused directly into the duodenum in healthy volunteers were found to cause CCK release and increase gastric volume (226). The effect on CCK release was most pronounced with long-chain triglycerides, as was the reduction in the sensation of hunger. Medium chain triglycerides did not cause a release of CCK. In the same study the

proximal stomach was also distended 30 minutes following the triglyceride infusions. Although both medium and long chain triglycerides increased gastric volumes, levels of CCK did not rise during this period, as it had done in the initial triglyceride infusion. This suggests that although CCK is plays a role in the duodenal brake, it is not the only contributing factor.

4.4.3.2 MRI

GCV0 was significantly higher in the FD population in comparison to the HV. Several factors could be important in this. Stress (known to often be higher in FD) (168) impairs gastric distension, reducing accommodation (54) but would be expected to produce rapid earlier emptying, therefore a lower GCV0. However, it also increases secretion production (214). MRI can measure secretion/other gastric contents as well as ingested meal. If secretion production was greater in FD's, then this would produce the higher GCV0. Secretion production would have to exceed any early emptying for this to occur.

There was no difference between T50 and GE rate @ T50. This was the same in GCV and TGV. MRI showed similar baseline volumes between HV and FD in MRI.

4.4.4 Comparison of GS and MRI

One of the most interesting results for the liquid study is that GCV0 was lower in the patient population in GS but greater in MRI. As discussed in the results section, MRI also measures secretion while GS cannot. Fasting scans do not show a greater level of secretions. Thus any difference is most likely due to the secretory response to the Fortisip ingestion and greater volumes of secretions are produced (therefore the higher GCV0 values in MRI). 63% of patients had a GCV0 greater than 400ml (the ingested volume) in MRI. Due to this difference in measurement abilities between the 2 modalities, it is perhaps not surprising that the differences were seen. MRI will always be able to potentially measure more than GS, meaning trying to compare the 2 modalities within one test may not appropriate unless some attempt is made to correct for secretion.

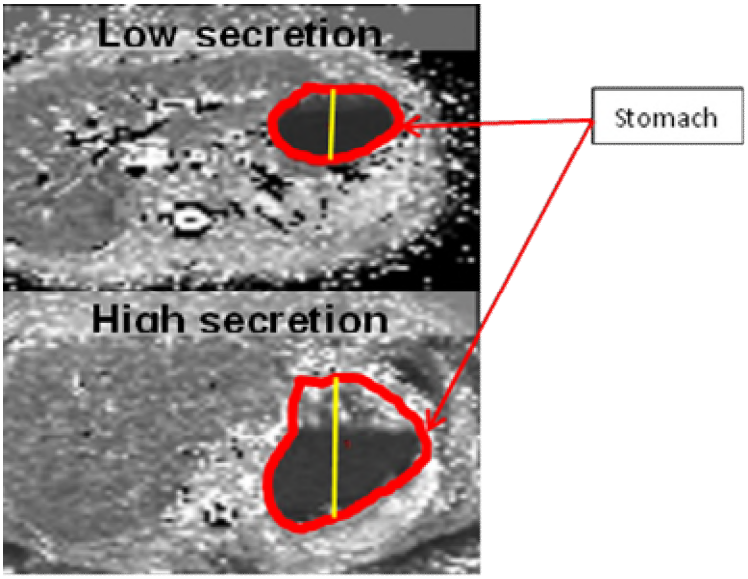


Figure 67. Secretions in MRI (T1 image) – reproduced with permission from Dr Caroline Hoad, MRI Research Fellow

Some work has been completed in secretion volume production in MRI. The MRI labelling agent gadolinium reduces the T1 relaxation time (time taken for protons to return to longitudinal axis). As the ingested meal is diluted with secretions, the change in T1 can be measured and is reflective of secretion volume (227, 228). A recent study has looked at secretion volume production in 14 HV studied by MRI (179). Meal volume, secretion volume and gastric contents volume were all measured. Secretion volume was measured after meal ingestion and was 35ml+/- 30ml. There was large variability between individuals. When the gastric emptying was modelled in this group, the secretion rate constant showed correlation with the meal emptying rate constant. They also found meal volume decreased in early emptying but gastric contents

volume (meal and secretions) decreased more slowly or even remained constant during the same time period. This suggests gastric secretions affect gastric emptying (particularly in the early phase) and that for MRI to be used in this manner, secretions must also be measured.

Our MRI department has been working to quantify these secretions using the above method. They have recently provided preliminary data on this, completed by Dr Caroline Hoad, Research Fellow within the MRI unit. It is included here to support the hypothesis for different volumes recorded between the two modalities being due to secretions.

16 HV from the mixed meal have had secretion volume assessed at time points 15 and 75 minutes. Mean secretion volume at 15 minutes was 64 ml (standard deviation 51ml) and 110 (40) at 75 min. Secretion increased between the two time points in all but one individual. Increase of secretions was a mean of 52 (29) ml. The results for GCV as then compared between GS and MRI i) meal and secretions ii) meal only.

15 min GS 310ml (32)

MRI (meal only) 338ml (50).

MRI (meal and secretion) 402ml (58)

75 min GS 112ml (61)

MRI (meal only) 92ml (36)

MRI (meal and secretion) 202(50)

This initial work shows that meal volume in MRI is similar to GS when secretion volume is accounted for.

4.4.5 Mixed meal

Following initial development and analysis there were several amendments to the mixed meal study during the course of the study. Due to this and a more heterogeneous method within the patient population, an initial 8 pilot FD patients were compared to the HV before extending this to further FD and GORD patients.

4.4.5.1 Pilot 8 FD versus HV

GS showed no significant differences between groups. In MRI GCV, GCV0 was significantly lower in the patients. A significantly higher MRI GCV GCV0 was seen in HV between liquid and mixed meal, while such a difference was not seen between the patients.

4.4.5.2 Additional patient groups

When the study was expanded to additional FD patients and GORD patients, GS showed significantly lower GCV0 in HV>FD=GORD. T50 and GE rate @ T50 didn't have any significant results.

In MRI , T50 was greater in GORD group in MRI GCV and GCV0 MRI TGV was significantly higher in the GORD group versus HV. Thus the pattern of gastric emptying was different in the three groups

FD patients were compared to GORD and HV in the mixed meal. The most interesting parameter was GCV0. GCV0 was significantly HV>FD=GORD in GS and GORD>HV for GCV0 in MRI total contents (with the same trends in MRI contents). FD results were non-significantly higher than HV.

This may support the hypothesis that gastric secretions are the important variable between health and disease. Considerable work has been documented in gastric acid suppression in GORD, but less so on gastric acid secretions volume, although some studies have suggested this is higher in GORD (229, 230). One study determined the buffering capacity of a meal *in vitro* and then used the same meal to identify the time taken for gastric pH to drop to 2 in GORD patients and HV (231). This study found that meal stimulated gastric acid secretion and post-prandial gastric acidity was significantly higher in the GORD group. However, the total gastric acid secretion over 24 hours was the same in both groups. This provides an interesting insight, suggesting the speed of secretions in response to a meal differs between health and GORD. There are some similarities between GORD and FD (post-prandial

symptoms, pain) and overlap has been suggested between the two conditions (2, 232). Increased speed and volume of secretion production may have an effect in both diseases. The results follow the same trends in FD and GORD of decreased GCV0 in GS but higher GCV0 in MRI, supporting this. The mechanism is likely due to a combination of increased volume and altered composition of gastric contents.

The altered gastric contents (with more secretions) potentially result in a more acidic chyme being delivered into the small bowel. Acid infusion into the duodenum has been studied and has been shown to affect fundal activity and sensation in a group of healthy volunteers (233). Acid infusion versus saline infusion was completed in a randomised, blinded manner with barostat measurements within the fundus. Fundal compliance increased with reduced fasting fundal tone with the acid infusion. Meal induced accommodation was then assessed with the acid versus saline infusion. Fundal pressures required for discomfort and relaxation during the meal were lower in the acid infusion group. Duodenal acid exposure has also been known to delay gastric emptying (234), which has thought to be a protective mechanism for the duodenal mucosa. Its role in FD is unclear. One study showed that duodenal acid induced nausea in FD patients and not healthy volunteers (235) but other studies have shown that

duodenal acid can also induce nausea in healthy individuals along with other dyspeptic symptoms (236, 237). Duodenal acid exposure has also been shown to be increased in FD patients and that its clearance reduced (238). This may explain the subset of FD patients that report symptomatic benefit to PPI's, despite not having GORD. The mechanism by which duodenal acid induces symptoms still remains unclear though. It may be that an increased volume of secretion partly promotes this effect.

Another area where meal composition is important in FD is with lipids. Lipid infusion into FD patients results in increased dyspeptic symptoms of nausea and bloating in comparison to healthy controls (239) and lipid infusion can differentiate between FD patients and healthy controls (56). Glucose does not produce the same differentiation between patients and volunteers or symptom production (240). This may be partly due to CCK. It is consistent with the FD population describing symptoms following fatty foods and often stimulates gastro-oesophageal reflux symptoms as well (241). It may be the fortisip meal used in this study stimulated symptoms and even secretion production.

4.4.6 Nutrient drink test

Maximum tolerated volume was significantly lower in FD compared to HV for both liquid and mixed meal. GORD patients drank significantly less than HV but more than FD patients. This is

interesting and supports the theory that there are significant overlap between GORD (particularly non-erosive reflux disease) and FD (232). Symptom profile can be similar (e.g. post-prandial period affected). The population of GORD patients recruited are those who were referred for pH studies. These are commonly only completed if there is a lack of full response to PPI, if anti-reflux surgery is being considered or if the diagnosis is unclear. Therefore there is potential for significant crossover between the two patient populations.

4.4.7 VAS scores

FD patients reported significantly greater fullness at 400ml for both GS and MRI in comparison to HV. They also reported more fullness than the GORD patients in GS. Similar trends were followed in MRI but not significant. Asking participants to drink to 400ml is a more practical than asking them to drink to maximum tolerated volume.

4.4.8 Limitations

No study is without problems. Some limitations have been discussed earlier in the text. Given that FD and GORD are likely to be heterogeneous conditions the patient numbers are relatively small for both studies. One of the difficulties of recruiting was the need for both FD and GORD patients being required to come off any medications that may affect the gastric parameters or sensation. Obviously, many patients are reluctant to do. This is

especially important as gastric function does vary in individuals on a daily basis and increasing overall numbers would minimise this effect.

Differences were seen between MRI and GS, with GS GCV0 being consistently lower in GS than MRI in both the liquid and mixed meal. This has been discussed and due to the addition of gastric secretions.

The measurement of gastric secretions would seem to be pertinent to the results. This technique is being developed by the MRI section of the study team. Quantifying the amount and speed of gastric secretions in health and disease will hopefully elicit more differences between these groups. Preliminary work has been completed within the HV but this needs to be extended to the patient groups.

4.5 Conclusion

The study has sought to develop and validate a new test meal that assesses gastric function alongside sensory function in a non-invasive manner. A large group of age/sex stratified healthy volunteers have been assessed to establish normal values and validation work completed within this population. Once complete, FD and GORD patient groups were included. Results for these groups have confirmed pathophysiology, such as impaired accommodation but also highlighted less described differences,

such as increased gastric secretion in FD. The balance between these two factors may explain some of the variation in gastric emptying study results currently seen in FD patients. These results are important as can be used to make a positive diagnosis in FD, rather than the current diagnosis of exclusion often used. They can be completed with relatively accessible technology (GS) and MRI if further input is needed. Sensory function has also been recorded and shown that hypersensitivity is an important feature in FD in a practical manner at 400ml ingestion. Using studies like this to separate conditions dependent on underlying pathophysiology can only help guide effective treatment.

5 Overall conclusion

This thesis has detailed how new, emerging modalities can add to our understanding of gastro-oesophageal disease, potentially guide therapy and utilise current tests in a more clinically useful way.

High resolution oesophageal manometry is a minimally invasive test with few side effects that can be used to correctly identify the causes of and classify patients with rumination syndrome and recurrent belching. This can have direct clinical impact on care, by directing treatment to the stimulus for rumination while applying generic biofeedback therapy to the learned behaviour. In a case where reflux triggered this behaviour it identified an individual who benefited from fundoplication. This requires careful thought and consideration as in the wrong patient it will simply lead to the development of new behaviour.

EndoFLIP® technology was a promising prospect in the diagnosis if GORD. However, its measurement of the gastro-oesophageal junction and associated parameters (distensibility and CSA) have been shown to be highly variable within patients and healthy volunteers, limiting its use as a diagnostic aid. Obesity seems to be a particular confounding factor, one which is common in the presence of GORD. However, it could be that situations in which patients are their own control, it may prove more beneficial.

Gastric emptying studies have long been completed in functional dyspepsia with relatively limited clinical impact. This work has shown that the modalities GS and MRI produce different results for the gastric emptying parameters GCV0, T50 and GE rate at T50. GCV0, representative of early emptying is greater in MRI than GS due to the presence of gastric secretions. GS has advantages of accessibility, cost and ease of interpretation. Advantages of MRI is that additional components of gastric secretions, gastric size, air quantification within the stomach can all be measured, while GS can only measure in the ingested, labelled meal. Lower GCV0 in GS in liquid studies suggests that early gastric emptying is quicker in a FD population. But contrast with MRI indicates that this is offset by greater secretion production in these patients. When the study was extended to additional patient groups, the GORD population seems to follow trends seen in the FD population.

Ongoing work is needed to further subdivide the different results seen in the liquid and solid sections of the gastric emptying study. Pilot secretion work within a limited number of the healthy volunteers has been completed and needs to be further extended out to the patient population. MRI provided a wealth of information that provides the potential of relating symptoms to underlying pathophysiology

Although it remains a significant challenge, trying to find the right treatment for the right patient should be the aim for all doctors working with patients with so-called “functional” gastrointestinal diseases, in the same way as in other areas of medicine. Only by exploring and testing new devices and discovering the differences between health and disease can we hope to improve the care we deliver to our patients.

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